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Non-Specific Airway Hyperreactivity in Asthma: A Clinical Correlate to What Exactly?

Larry Borish, MD [Professor]

Medicine and Microbiology, Carter Immunology Center, University of Virginia Health System, Charlottesville, VA 22908

Increased airway hyperreactivity (AHR) to non-specific stimuli is a feature of asthma and, indeed, the diagnosis of asthma is often defined on the basis of presence of such hyperreactivity. What has been surprisingly unclear is exactly what mechanism is driving the bronchospasm. AHR is routinely used as a virtuously synonymous term for airway inflammation, but exactly what components of inflammation are responsible remains enigmatic.

Traditionally, non-specific AHR has been elicited with many stimuli but currently these are most often performed with methacholine. Previous studies have suggested that the bronchospasm in response to methacholine reflects the expression of mast cells in airway smooth muscle (ASM) (1–6). This suggests that the ability of methacholine to elicit bronchospasm reflects the presence of mast cells in a location where their elicited release of histamine, cysteinyl leukotrienes, prostaglandin (Pg) D₂, and other vasoactive mediators drives the bronchospasm.

More recently mannitol has also been developed as a challenge agent and suggested to possibly have greater sensitivity than methacholine in diagnosing AHR (7). Previous studies have shown that hyperreactivity to mannitol correlated with markers of mast cell activation, including as the presence of urinary metabolites of PgD₂ and histamine (8). But these *correlations* are not the same as demonstrating that AHR predicts the presence of mast cells in the airway, their specific location, or that their activation is what is responsible for eliciting the bronchospasm. And, furthermore, it should be noted that these studies also correlated mannitol hyperreactivity with indirect markers of airway eosinophilia such as exhaled breath nitric oxide and urinary cysteinyl leukotriene (CysLT) metabolites (8, 9).

In the current issue of *Clinical & Experimental Allergy* Sverrild et al. advance our understanding of the mechanism of non-specific AHR to methacholine and mannitol and the nature of the underlying inflammation present in the airway (10). The authors use rigorous criteria to enroll clinically effected symptomatic asthmatics but without the confounding influences of inhaled corticosteroids, performing challenges followed by bronchoscopic biopsies. Exclusion of inhaled CCS is critical as these agents do influence MC expression and phenotype in the airway (11). Mast cells are divided into two main subtypes based upon

their granule content. Tryptase-containing mast cells (MC_T) are primarily expressed on mucosal surfaces such as nasal and lower airway epithelium and along the gastrointestinal tract. Chymase- and tryptase-containing mast cells (MC_{TC}) are most prominently expressed in connective tissue and, within the healthy lung, are expressed along pulmonary vessels, in the pleura. However, especially in severe and uncontrolled asthmatics, MC_{TC} are also present within airway smooth muscle and in the submucosa (7, 12).

The more striking results from these studies were within the submucosal biopsies. While no differences in total mast cell numbers were observed, the authors report increased expression of MC_{TC} in these submucosal biopsies, but only in asthmatics displaying AHR to methacholine and – to a somewhat lesser extent – mannitol. Asthmatics without AHR to either agent did not differ from healthy controls.

However, the ASM pathology results differed from previous studies. Again, no differences in total MC numbers were observed between asthmatics and healthy controls although the number of MC_{TC} was increased in asthmatics. However, there was no difference in MC number or phenotype between those displaying AHR to methacholine or mannitol and those without AHR. As such this study raises questions as to the previous concept that it is MC infiltration of the ASM bundles that is responsible for AHR. Although requiring confirmation, it is compelling that the current studies were performed in symptomatic yet inhaled steroid-naïve subjects and, equally compellingly, used unbiased automated technology to perform the immunohistochemical analyses. It is worth noting that studies using He³ diffusion technology show that reduced airflow in responses to methacholine inhalation is a focal process involving only a limited number of segments of the lung. As such, before excluding a role for ASM-infiltrating MCs it would also be important to consider focusing studies on those areas of the lung specifically demonstrated to react to the stimulus eliciting AHR (13).

So, if it is not the infiltration of the ASM bundle with mast cells that explains the AHR, what may be responsible? Bronchospasm – or at least diminished airflow – is certainly compatible with the authors' report of increased infiltration of the submucosa with MC_{TC}, reflecting either the diffusion of MC mediators to nearby ASM bundles or, more likely, direct influences of these vasoactive mediators in driving airway edema, mucus secretion, and, as such, the ASM-independent decrease in airflow. And, given this selective increase in MC_{TC} expression, it is intriguing that chymase is particularly associated with mucus hypersecretion and airway remodeling (14, 15).

Alternatively, this study also correlated increased AHR to mannitol – but not methacholine – with evidence of eosinophilic inflammation. That methacholine AHR fails to robustly correlate with eosinophilia has previously been reported (9), and it is totally reasonable that methacholine and mannitol could have distinct mechanisms of action. Mannitol-mediated eosinophil secretion of CysLTs and other eosinophilic vasoactive mediators could certainly explain elicitation of a reduced airflow response.

Finally, this study also demonstrated increased expression of carboxypeptidase A3 (CPA3) and the cytokine thymic stromal lymphopoietin (TSLP). Lung MCs include a more recently

described subtype characterized by this expression of CPA3 (16) and this differentiation reflects at least in part the action of TSLP (17). TSLP plays a prominent role in driving Th2 immune deviation. A role of TSLP in asthma, perhaps reflecting its activation and differentiation of mast cells, is supported by a recent report regarding the ability of an anti-TSLP to inhibit early and late responses to allergen challenges (18).

In summary, the current studies raise some doubts as to the specific role of ASM-infiltrating MCs in comprising the underlying basis for non-specific AHR but instead suggest roles for mucosal MC_{TC} or eosinophils. And, amongst those MC_{TC} this study also suggests a role for TSLP-induced expression of CPA3. But, ultimately these observations remain just correlations. And while still a central defining feature of asthma, we still do not know exactly what we are really learning about the airways of our patients in whom we demonstrate AHR.

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