Mechanisms of Mast Cell Activation in Severe Asthma
Beyond IgE

Mast cells (MCs) are present in all heathy human tissues and exhibit marked heterogeneity with respect to their development, mediator content, ultrastructure, and function (1). They secrete a vast arsenal of biological substances including the classical autacoid mediators (histamine, prostaglandin D_2, and leukotriene C_4), proteases, and cytokines. They respond rapidly to a multitude of perceived tissue insults with the initiation of a coordinated program of inflammation and repair. Potentially beneficial MC roles include protection against infection (2), kidney injury (3), envenomation (4), and in some circumstances, cancer progression (5). However, when a tissue insult is repeated or continuous, the effects of MC mediators are potentially deleterious, and it is not surprising that MCs are implicated in the pathophysiology of many diverse diseases including asthma (1) and idiopathic pulmonary fibrosis (6).

MCs release their mediators in response to all stimuli considered important for the development of asthma, day-to-day symptoms, and exacerbations (reviewed in Reference 1). The biological activity of their mediators can account for all of the physiological and pathological abnormalities present in asthmatic airways. Importantly, there is unequivocal evidence of the ongoing release of MC mediators in the airways of people with both mild and severe asthma, and in both T2-high and T2-low severe asthma (1, 7). MCs are unique among leukocytes in that they infiltrate the airway submucosal glands and airway smooth muscle bundles in asthmatic airways, and infiltrate the airway epithelium, placing activated MCs within these dysfunctional airway elements (1).

For decades the investigation of MC signaling focused on allergen-driven IgE-dependent activation via the high-affinity IgE receptor FcεRI. However, human MCs respond to a wide variety of stimuli including cytokines/growth factors, lipids, nucleotides, complement, proteases, products of infection (human MCs express all Toll-like receptors), pollutants, physical stimuli, occupational agents, drugs, and cell–cell signals (1) (Figure 1). The historic work of Okumura using human adult peripheral blood progenitor–derived MCs showed that although there are core changes in MC gene expression after activation via FcεRI, LPS, and IFN-γ, there are also many stimulus-specific changes, and these are modified with combined stimulation (8). It is likely that many stimuli contribute to MC activation within the airways of an individual over time, but to date, there has been little information on the activity of IgE-independent pathways in asthmatic airways.

In this issue of the *Journal*, Tiotiu and colleagues (pp. 397–411) have used gene set variation analysis to interrogate MC activation signatures in the sputum of the U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes) asthma and healthy volunteer cohort, cross-validated with the ADEPT (Airways Disease Endotyping for Personalized Therapeutics) cohort (with which there was moderate agreement) (9). They looked at the relationship of these activation signatures with asthma severity and sputum inflammation and also mapped them to three molecular clusters of severe asthma they had identified previously, described as transcriptome-associated clusters (TACs) 1–3 (10). The gene sets used were obtained from previous studies using cultured human blood progenitor–derived MCs that were unstimulated, FcεRI-activated (single and multiple activation rounds), LPS-activated, IFN-γ–activated, or IL-33–activated. There was also a signature from MCs identified in mild asthma bronchial biopsies by single-cell sequencing. In severe asthma, the FcεRI-activated, single-cell sequencing signature from mild asthma, LPS-, and IL-33–dependent signatures were enriched compared with heathy controls. These signatures were also enriched in the subset of patients with severe asthma who were eosinophilic, but the LPS- and IL-33–dependent signatures were enriched further in people deemed neutrophilic or mixed granulocytic defined by sputum percentage cell counts. FcεR1-related signatures were enriched predominantly in people expressing the T-helper cell type 2 eosinophilic TAC1 molcular phenotype, whereas LPS- and IL-33–dependent signatures were enriched in the TAC2 neutrophil inflammasome–associated phenotype. These same MC signatures were similarly enriched in individuals with asthma with airflow obstruction compared with those without. The MC activation signatures correlated poorly with tissue MC numbers, supporting previous work indicating that MC activation and their tissue localization is more important than absolute numbers.

This is a comprehensive data analysis and the first study in severe asthma to examine MC gene expression signatures generated by different stimuli. The authors have uncovered several interesting associations between stimulus-specific MC gene signatures, clinical features of asthma, and molecular phenotypes, and this represents an important advance in our understanding of IgE-independent factors that may be driving MCs in severe asthma. The study also provides further evidence that MCs are activated across clinical and molecular severe asthma phenotypes.

There are some limitations to the study. Gene set variation analysis has problems because not all genes expressed are necessarily specific for one cell type, although the authors took steps to exclude genes that overlap with other leukocytes. The gene

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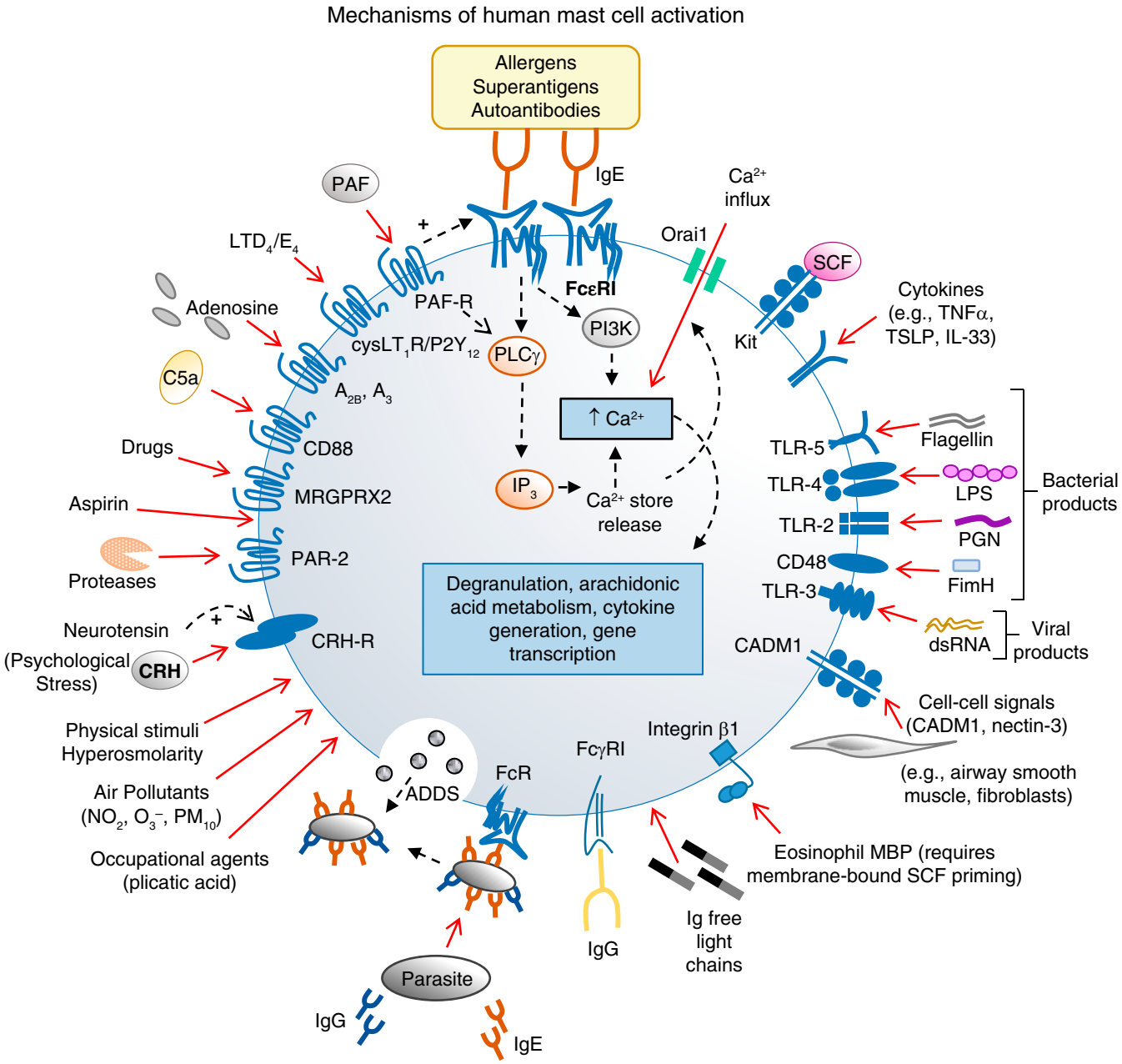

Figure 1. Examples of ligands and receptors capable of activating human mast cells. Many of these ligands and activating stimuli are active in the airways of people with asthma and therefore likely to contribute to the ongoing mast cell activation evident in asthmatic airways. The downstream signaling pathways for many of these receptors in mast cells and how they interact with each other are not fully understood. Among the well-known signaling pathways are the PLC-γ and PI3K pathways, which mobilize intracellular Ca^{2+}, leading to Ca^{2+} influx via Orai channels. Modified from Reference 1. ADDS = antibody-dependent degranulatory synapse; CADM1 = cell adhesion molecule-1; CRH = corticotropin-releasing hormone; CRH-R = CRH receptor; FcR = Fc receptor; IP3 = inositol-1,4,5-triphosphate; LT = leukotriene; MBP = major basic protein; MRGPRX2 = Mas-related G protein–coupled receptor-X2; PAF = platelet-activating factor; PI3K = phosphoinositide 3-kinase; PAR = protease-activated receptor; PGN = peptidoglycan; PLC-γ = phospholipase Cγ; PM_{10} = particulate matter ⩽10 μm in aerodynamic diameter; R = receptor; SCF = stem cell factor; TLR = Toll-like receptor; TNF = tumor necrosis factor; TSLP = thymic stromal lymphopoietin.

sets studied were from cultured blood progenitor–derived MCs, which will not account for the heterogeneity likely present within an airway MC population. The study is cross-sectional, which is a strength in that it demonstrates significant MC activation in "steady-state" severe asthma but also a limitation as it is apparent that the TAC sputum molecular asthma phenotypes are unstable over time (11). In addition, true T2-low severe noneosinophilic asthma appears rare, with T2 biomarkers emerging as corticosteroid treatment is reduced (12). It would therefore be interesting to study MC expression signatures longitudinally and

with corticosteroid reduction and to explore whether airway immunosuppression due to high-dose inhaled corticosteroids promotes IL-33–dependent MC activation.

Studying sputum in asthma clearly provides important insight into disease pathophysiology, but MCs are rare in sputum compared with other granulocytes. There are also many further potential MC activators at play (Figure 1) with potential interactions between them. It is not known currently to what extent other relevant MC activators might overlap with the LPS and IL-33 signatures and, therefore, how much of the signal described here is due to these molecules exclusively. Thus, understanding the MC molecular response to multiple activators, ideally within human airway tissue stimulated *ex vivo*, and compared with tissue from various asthma phenotypes, using techniques such as single-cell sequencing and spatial transcriptomics/proteomics/lipidomics, would likely provide more granularity regarding the pathways driving MCs in any individual at the point in time they are studied. However, these may change from day to day, and particularly so during exacerbations, which highlights the challenge of targeting MCs effectively in asthma. A treatment for one set of pathways may be ineffective for another, exemplified by the partial efficacy of omalizumab in severe atopic asthma. Of note, a study targeting Kit signaling with masitinib to reduce MC survival looks promising in both eosinophilic and noneosinophilic asthma (13).

In summary, Tiotiu and colleagues provide insight into some IgE-independent pathways that are active in the airways of people with severe asthma. These data should be considered the tip of the iceberg in unraveling the multiple factors that might be driving MC activation in an individual. The challenge now is to delve deeper, taking into account the many other potential activators of MCs present in asthmatic airways, and find where there is commonality in the signaling pathways, so that multiple MC activation pathways can be targeted effectively at the same time pharmacologically. ■

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