

Advances in mast cell biology



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Mast cells (MCs) contribute prominently to all allergic diseases, yet are still poorly understood owing to their exclusive residence in tissues. Recently, the use of RNA-sequencing, proteomics, and other technological advances have accelerated the acquisition of new knowledge. This includes an expanded definition of MC heterogeneity and developmental origins, previously unrecognized functions for MCs, discoveries of genetic causes of MC-related disorders, the introduction of new therapies for clonal MC disease, and the identification of new potential target for treatments. This issue of Advances addresses key studies from 2020 to 2021. (J Allergy Clin Immunol 2022;149:1919-25.)

Key words: Mast cells, systemic mastocytosis, AERD, CRSwNP, *Staphylococcus*, single cell genomics, Mas-related G-protein-coupled receptor 2

Mast cells (MCs) are histochemically distinctive hematopoietic effector cells that are sentinels of innate immunity and also central to the pathophysiology of allergic diseases.¹ Although best recognized for their role in IgE-dependent type 1 hypersensitivity that underlies anaphylaxis and other acute manifestations of atopy, recent studies indicate that MCs likely play a central role in orchestrating immune responses at barrier tissues. Moreover, MCs display substantial functional diversity that is further modified in the setting of inflammation and disease.^{2,3} Endogenous homeostatic mechanisms limit the extent of potential immunopathology induced by MC activation. Genetic factors may play a significant role in regulating MC function in humans,⁴ particularly in MC disorders, where understanding these factors has led to novel therapeutic strategies. This issue of Advances focuses on recent studies highlighting these features of MC biology, its relationship to disease and host defense, and the development of novel therapeutics focused on MCs and emerging from a deeper understanding of their biology.

Abbreviations used

AERD:	Aspirin-exacerbated respiratory disease
CRSwNP:	Chronic rhinosinusitis with nasal polyps
DARPin:	Designed ankyrin repeat protein
DC:	Dendritic cell
HAT:	Hereditary alpha tryptasemia
ILC2:	Group 2 innate lymphoid cell
MC:	Mast cell
PGE ₂ :	Prostaglandin E ₂
SCFA:	Short-chain fatty acid
SEB:	Staphylococcal enterotoxin B
SM:	Systemic mastocytosis

MCs IN HOST DEFENSE AND INTERACTIONS WITH MICROBES

The ability of MCs to serve as sentinels of antimicrobial innate immunity in animal models could also contribute to their role in allergic mucosal diseases.⁵ Hayes et al⁶ had previously identified *Staphylococcus aureus*, an organism frequently linked to the pathogenesis of nasal polyps,⁷ residing within MCs found in chronic rhinosinusitis with nasal polyp (CRSwNP) tissue. The investigators used a combination of *ex vivo* approaches, microscopy, and a tissue explant model to explore the potential importance of interactions between Staph and MCs,⁸ and the potential role of Staphylococcal enterotoxin B (SEB), a superantigen implicated in CRSwNP pathogenesis,⁹ on this process. The investigators found that an MC line engulfed Staph by phagocytosis *ex vivo*. Staph replicated within MCs, and ultimately produced lysis of MCs and release of intracellular contents. The uptake of Staph was facilitated by the addition of SEB. The presence of Staph and SEB facilitated the movement of MCs from the submucosal compartment to the epithelial compartment of sinonasal tissue in sinus tissue explants. These findings suggest a role for MCs as reservoirs of Staph replication in the sinonasal tissue, and also suggests a contribution from Staph and SEB in the redistribution of MCs to the epithelial surface in CRSwNP.^{8,10}

Although MCs can generate cytokines in response to challenge with bacterial products *ex vivo* and are involved in certain models of antibacterial host defense,¹¹ less is understood about their potential role in viral infections. Types 1 and 3 interferons are critical effectors of antiviral host responses.¹² Portales-Cervantes et al¹³ examined the impact of IL-4 and IL-13 on interferon production by human cultured MCs. When exposed to rheovirus or to respiratory syncytial virus, a critical inducer of wheezing illnesses in children, cultured human MCs released the type 1 interferon IFN- α 2 and the type 3 interferon IFN- λ 1. Priming of the MCs with IL-4, but not with IL-13, significantly potentiated the productions of both interferons. Pharmacological inhibition of signal transducer and activator of transcription 6, but not insulin receptor subset-2, eliminated the IL-4-induced potentiation of virally

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induced interferon production. Lastly, priming with IL-4 also markedly increased the quantities of IFN- α 2 and IFN- λ 1 induced by IgE receptor cross-linkage. These results suggest the potential for MCs to participate directly in host responses to RNA viruses, and that this potential may be enhanced in the setting of type 2 inflammation. Because asthma is associated with diminished production of type 1 interferons,¹⁴ the finding may imply a compensatory mechanism whose significance requires further investigation. It is also tempting to speculate that the potentiation of MC-derived IFN- α 2 and the type 3 interferon IFN- λ 1 production by IL-4 priming could contribute to the relatively low rate of severe acute respiratory syndrome coronavirus 2 infection observed in type 2 high asthma in some studies.¹⁵

INFERRING TISSUE MC HETEROGENEITY AND FUNCTION USING MOLECULAR TOOLS

Publicly available transcriptomic data sets have greatly aided immunology research by providing a framework for translational studies. Winter et al¹⁶ performed microarrays to identify transcripts expressed by cells isolated from the sputum obtained from 29 patients with eosinophilic asthma, 30 patients with non-eosinophilic asthma, and 18 healthy controls. Using a set of 134 MC and/or basophil-restricted candidate genes identified by the Immgen Consortium (<https://www.immgen.org/>), the investigators identified a subset of these transcripts that were expressed more strongly in microarrays from the asthma samples than in controls. They used PCR and flow cytometry in sputum from another 20 patients with asthma to validate 8 of these transcripts on the basis of significant correlations between their expression levels and the numbers of MCs and basophils in the corresponding sputum samples. The authors then measured the quantities of these transcripts in sputum collected from an additional 81 subjects with severe asthma. The expressions of *GATA2*, *TPSB2*, *CPA3*, *GPR56*, *HDC*, and *SOCS2* correlated with sputum eosinophil counts in this cohort, whereas expressions of *TPSB2*, *CPA3*, *GATA2*, *SOCS2*, *FCER1A*, and *HDC* correlated with blood eosinophil counts. *GATA2* and *SOCS2* correlated with exhaled nitric oxide levels, whereas decreased lung function (*KIT*, *ENO2*) and exacerbation history (*GATA2*, *SOCS2*) also tracked with characteristic gene signatures. This study provides a potential foundation for the development of MC/basophil-specific biomarkers in future clinical studies, and links features of asthma pathophysiology to MC and basophil function.

The use of single-cell genomics is a powerful tool for understanding the immunopathology of human disease. Dwyer et al² used an unsupervised single-cell genomic approach to characterize the transcriptional heterogeneity of sinonasal tissue MCs. Compared with MCs found in the tissues from patients with chronic rhinosinusitis without nasal polyps, CRSwNP MCs displayed marked phenotypic and transcriptomic complexity. CRSwNP tissue contained 4 distinct transcriptional MC clusters (compared with 1 dominant cluster in chronic rhinosinusitis without nasal polyp tissue). Clusters representing classical MC_T and MC_{TC} differed by approximately 300 genes. In addition, 2 previously unrecognized transcriptional clusters displayed intermediate levels of MC_T and MC_{TC}-related transcripts, suggesting that they were in a transitional stage of development. Importantly, 1 of these transitional clusters was strongly enriched for transcripts

associated with cell proliferation. Using candidate surface markers identified in the transcriptional data sets, the investigators confirmed that MC_T (Kit^{lo}, CD38^{hi}), MC_{TC} (Kit^{hi}, CD38^{lo}), and transitional MC (Kit^{hi}, CD38^{hi}) phenotypes could be distinguished by flow cytometry. Although proliferating (Ki67+) MCs were present in all 3 flow cytometric subtypes, they were especially enriched in the transitional population (Table 1). Proliferating MCs were highly enriched in polyps from patients with aspirin-exacerbated respiratory disease (AERD) and correlated with blood eosinophil counts. Moreover, polyp MC_{TC} differed markedly from skin MC_{TC} on a transcriptional level, and lacked the expressions of *CD88* and *MRGPRX2*, which are commonly used as MC_{TC} markers. These observations indicate that traditional histochemical characterization of MC phenotypes underestimates the true extent of their functional heterogeneity, especially in a setting of mucosal inflammation. Moreover, in situ proliferation may be an important mechanism driving the expansion of MCs seen in nasal polyposis, and potentially in other diseases associated with mucosal type 2 inflammation.

Proteomics provides an additional method of functional assessment, displaying all proteins expressed by a given cell type. Saha et al¹⁷ generated a comprehensive data set of surface proteins expressed by mouse bone marrow-derived MCs by enriching proteins for glycosylation before proteomic analysis by mass spectrometry. The authors identified 378 proteins localizing to the plasma membrane, including transporters, receptors, and small GTPases. They validated the presence of surface proteins not previously reported in MCs using real-time quantitative PCR and flow cytometry analysis of both bone marrow-derived MCs and peritoneal MCs. The authors targeted 1 cell surface protein, CD98hc, for gene disruption using CRISPR-associated protein 9 gene editing, and found a significant reduction in cell adhesion and proliferation. This study indicates the utility of proteomic approaches to identify previously unrecognized proteins that may control important features of MC function.

Plum et al¹⁸ used quantitative mass spectrometry to perform a comprehensive analysis of the proteins expressed by MCs isolated from human skin and fat, as well as MCs isolated from the mouse peritoneal cavity. Compared with PBMCs, MCs from human skin and fat displayed approximately 500 unique proteins, including proteases, surface receptors, and enzymes, with minimal differences between the cells from the 2 tissues. Comparison of mouse to human showed strong functional preservation across species. Comparison of the MC proteomes to those of other human leukocytes supported a unique lineage with only distant similarities to basophils and little resemblance to other cell types. Prominent among the MC-specific proteins were several with potential function for interactions with nerves, including adhesion receptors (CD171 and neurotrimin), transient receptor potential cation channel subfamily V member 2, receptors for neuropeptides (Mas-related G-protein-coupled receptor 2), and nucleotide signaling (P2RX1, CD203). Interestingly, Toll-like receptors were not detected in either human or mouse MC proteomes, in contrast to earlier studies that had reported functional Toll-like receptor 2 and Toll-like receptor 4 expression by cultured MCs.¹⁹ The investigators used a photosensitizing antibody against Mas-related G-protein-coupled receptor 2 and demonstrated that MCs could be selectively depleted from human skin on subsequent exposure to light. These findings suggest potential strategies for future therapeutic

TABLE I. Flow cytometric characteristics of sinonasal tissue MCs

Nasal polyp MCs	Kit (CD117)	FcεRIα	Chymase	Avidin	CD38	Proliferation
MC _T	Low	Low	Negative	Negative	High	+
MC _{TC}	High	Intermediate	Positive	Positive	Low	+
Intermediate	High	High	Bimodal	Intermediate	High	+++

Potentially differentially expressed markers were identified by single-cell RNA-sequencing. Chymase and avidin binding were measured in permeabilized cells. Proliferating cells were present in all 3 groups but enriched in the intermediate (CD117^{hi}CD38^{hi}) population.²

targeting of MCs. The applications of proteomic and RNA-sequencing to MC populations found in other organs and contexts will be necessary to understand the full scope of MC heterogeneity.

FACTORS CONTROLLING MC HOMEOSTASIS

The potential deleterious consequences of unchecked MC activation necessitates the existence of mechanisms that maintain MC homeostasis. One such mechanism may involve prostaglandin E₂ (PGE₂), the most ubiquitous of all arachidonic acid metabolites.²⁰ Although PGE₂ is thought to play a critical role in tenuously maintaining stabilization of airway MCs in patients with AERD,²¹ no previous studies examined its role in anaphylaxis. Using samples from patients enrolled in a European Anaphylaxis Registry, Rastogi et al²² demonstrated that serum levels of PGE₂ in subjects presenting with hymenoptera anaphylaxis were significantly lower than in healthy controls, and correlated inversely with severity of reaction. The investigators also used a model of passive systemic anaphylaxis to demonstrate that C57BL/6 mice displayed greater reductions in core body temperature and lower serum levels of PGE₂ than did BALB/c mice. The severity of anaphylaxis in C57BL/6 mice was attenuated by the administration of an inhibitor of 15-prostaglandin dehydrogenase, a PGE₂-metabolizing enzyme, as well as the administration of exogenous agonists of the E prostanoide (EP)₂ and EP₄ receptors (Fig 1). Exogenous PGE₂ inhibited mouse MC activation *ex vivo* by a mechanism involving EP₂ and EP₄ receptors, both of which stimulate the accumulation of cyclic adenosine monophosphate. These findings further validate the capacity of endogenous PGE₂ to dampen MC activation *in vivo*, and may help to explain some of the potentiating effects of COX inhibitors in anaphylaxis and some cases of urticaria.

Siglec 8 is a sialoglycan-binding member of the immunoglobulin superfamily containing an immunotyrosine inhibitory motif. MCs and eosinophils strongly express siglec 8, and siglec 8 engagement by antibodies induces eosinophil apoptosis and inhibits MC activation.²³ Although mAbs against siglec 8 hold promise for the treatment of allergic diseases,²⁴ identification of natural ligands for siglec 8 may accelerate the development of small-molecule alternatives. Gonzalez-Gil et al²⁵ isolated and characterized a high-affinity siglec 8 ligand from human cadaveric lung and surgically excised sinonasal tissue. Using size exclusion and affinity chromatography with proteomic mass spectrometry, the authors identified a siglec 8 ligand with an approximate 1000 kDa mass composed of deleted in malignant brain tumors 1, a large glycoprotein with multiple O-glycosylation repeats. The authors²⁵ confirmed that a deleted in malignant brain tumors 1 (DMBT1) carrying O-linked keratan sulfate side chains is the major high-affinity ligand for siglec 8 found in the human respiratory mucous layer, and subsequently demonstrated that its expression is strongly upregulated in the submucosal glands

in tissues from patients with eosinophilic CRSwNP.²⁶ Interestingly, MCs accumulate in these same submucosal glands,²⁷ and it is tempting to speculate that locally derived siglec 8 ligand could limit their activation at this site (Fig 1). Whether a soluble siglec 8 ligand could be developed as a therapeutic targeting MCs and eosinophils deserves further investigation.

MC DISORDERS

Systemic mastocytosis (SM) refers to a set of disorders characterized by the accumulation of clonal, neoplastic MCs in various tissues.⁴ Although most affected patients pursue an indolent course, some have severe, progressive disease. Most cases of mastocytosis are associated with the KIT D816V mutation causing a gain of function in the tyrosine kinase domain of Kit, the receptor for stem cell factor. Because this mutation renders the receptor resistant to tyrosine kinase inhibitors such as imatinib,²⁸ therapeutic options have been severely constrained until recently. The development of midostaurin, a multikinase inhibitor that targets the mutated Kit, is a major step forward in the development of therapies for severe SM. In a recent open-label clinical trial for patients with advanced SM, midostaurin induced marked cytological and clinical improvement²⁹ (ClinicalTrials.gov identifier, NCT00782067). Hartmann et al³⁰ sought to determine whether midostaurin improved health-related quality of life and MC mediator-related symptoms in this cohort. The investigators used 2 validated patient-reported questionnaires to assess changes in quality of life, and symptoms relating to MC-derived mediators were evaluated using a physician-reported survey. Patients experienced improvements based on all 3 sets of measurements, which were sustained for 36 months of follow-up. Midostaurin thus represents a breakthrough treatment for patients with advanced SM who previously would have had almost no effective alternatives. Additional inhibitors with greater specificity for mutated Kit may be on the horizon.

An increasing number of clinical disorders involving genetically induced aberrant MC development and/or function have been identified in recent years. Hereditary alpha tryptasemia (HAT) is characterized by elevated steady-state serum tryptase levels due to inherited duplications of the *TPSAB1* allele encoding alpha tryptase, affecting as much as 6% to 7% of the population³¹ and as many as 18% of patients with SM.³² Although the clinical significance and correlates of HAT are still under debate,³² a subset of such patients report symptoms that are potentially related to MC activation (including anaphylaxis).³³ Giannetti et al³⁴ performed a systematic analysis of bone marrow biopsies from a cohort of 43 patients who presented elevated levels of serum tryptase accompanied by clinical symptoms consistent with MC activation (MCAS-T), including 19 with histories of anaphylaxis. None of these patients met clinical or molecular criteria for a clonal MC disorder. Bone marrow biopsies from patients with MCAS-T showed unique morphologic and histologic features

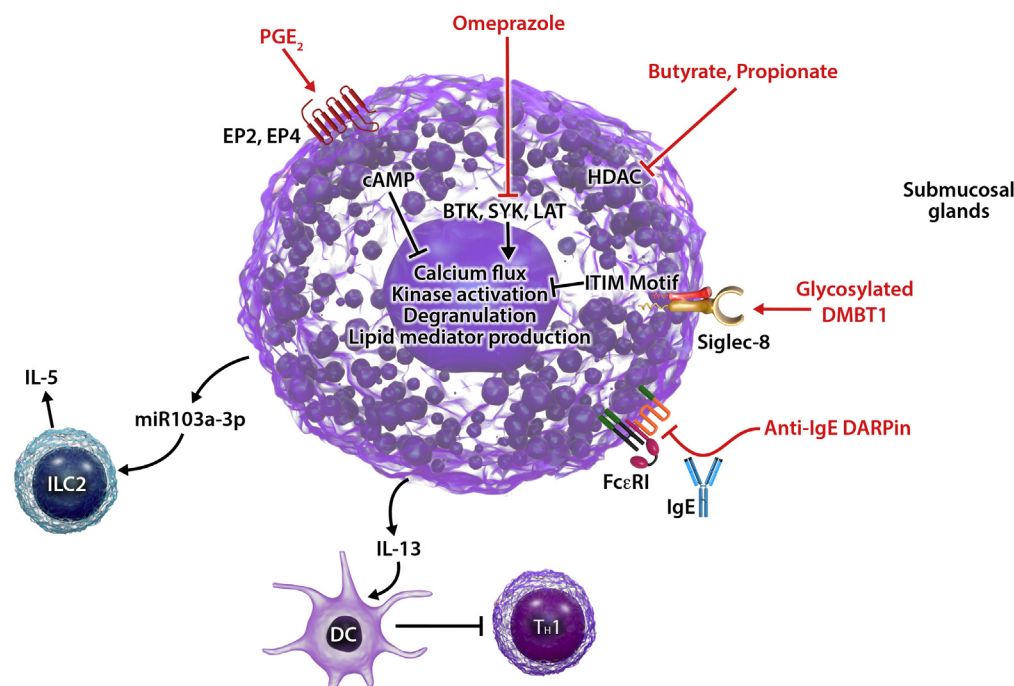


FIG 1. Recently identified mechanisms and pathways controlling the function of MCs, and by which MCs in turn influence the immune response. Several of these (EP receptor agonists, omeprazole, anti-IgE DARPins, fatty acids, Siglec 8 ligands) have substantial potential as therapeutic targets. *BTK*, Bruton's tyrosine kinase; *cAMP*, cyclic adenosine monophosphate; *DMBT1*, deleted in malignant brain tumors 1; *EP*, E prostanoic; *ITIM*, immunotyrosine inhibitory motif; *LAT*, linker for activation of T cells; *SYK*, spleen associated tyrosine kinase.

when compared with controls, including enlarged, hypogranular MCs frequently detected in paratracheal and perivascular locations, and associated with bone marrow eosinophilia. All 10 patients for whom DNA samples were available had duplications in the *TPSAB1* allele. Thus, patients with HAT and clinical symptoms consistent with MC activation may display aberrant bone marrow MC distribution and morphology that is distinct from the clustered, elongated MCs reported in SM and clonal MC disorders driven by the KIT D816V mutation.³⁵ Because many patients with HAT lack clinical symptoms,³² and no asymptomatic patients were examined in this study, the causative role of the *TPSAB1* duplication per se to the bone marrow findings and MC activation-related symptoms remains to be proven.

Severe hypotensive reactions to hymenoptera stings are common in patients with SM and clonal MC disorders.³⁵ Baseline serum tryptase levels are often used to select hymenoptera-sensitized patients for genotyping for KIT D816V. Selb et al³⁶ prospectively evaluated 374 patients presenting for evaluation of hymenoptera allergy to a single center. Twenty-three of these patients had elevated baseline serum tryptase levels, of whom 15 proved to have *TPSAB1* duplications diagnostic of HAT, 6 had a KIT D816V mutation, and 2 had neither. Remarkably, of the remaining 351 who had normal serum tryptase levels, 28 (8%) had a KIT D816V mutation. Patients with KIT D816V mutations, both with normal and elevated tryptases, were strongly overrepresented among patients whose reactions were severe, whereas *TPSAB1* duplications were not. This study highlights the utility of screening patients presenting for evaluation of systemic reactions to hymenoptera stings for clonal MC disorders, even when baseline levels of tryptase

are normal. The use of such screening may be important for risk stratification (Table II).

MC FUNCTION IN TYPE 2 IMMUNITY

Although classical MC-derived mediators (histamine, eicosanoids) play validated roles in type 1 hypersensitivity, less is understood regarding the role of MCs in the maintenance of type 2 immunity. Extracellular vesicles (microparticles, exosomes) are subcellular particles that contain cytoplasmic and surface constituents of their cells of origin.³⁷ They are often released in response to cell activation, and can carry proteins and nucleic acids through the serum to distant sites, reflecting a potential means of cell-cell communication across substantial distances. MC-derived microparticles are present in nasal lavage fluid from patients with CRSwNP, especially in patients with AERD,³⁸ a severe disease variant. Toyoshima et al³⁹ examined the potential for MC-derived extracellular vesicles to influence the function of group 2 innate lymphoid cells (ILC2s). Vesicles released by FcεRI-activated MCs markedly enhanced the production of IL-5 by ILC2s stimulated with recombinant IL-33 *ex vivo*.³⁹ The authors used a combination of approaches to implicate miR103a-3p, a microRNA that was enriched in MC-derived vesicles, in potentiating the production of IL-5 (Fig 1). Notably, IL-13 was not potentiated. miR103a-3p promoted demethylation of an arginine residue of GATA3 by downregulating protein arginine methyltransferase 5 mRNA. Reduction of protein arginine methyltransferase 5 expression in ILC2s by using a small-interfering RNA technique resulted in upregulation of IL-5 production by IL-33-stimulated ILC2s. Furthermore, the level of

TABLE II. Relationship of serum tryptase levels, *TPSAB1* copy number, and KIT D816V mutation status to risk of severe hymenoptera anaphylaxis

Elevated serum tryptase	Increased <i>TPSAB1</i> copy number	Positive KIT D816V mutation test result	Elevated risk for severe anaphylaxis
Yes	Yes	No	No
Yes	No	Yes	Yes
No	No	Yes	Yes

More than 8% of subjects with normal serum tryptase levels were positive for KIT D816V.³⁶

miR103a-3p expression was significantly higher in extracellular vesicles from sera of patients with atopic dermatitis than in extracellular vesicles from nonatopic healthy control subjects. These findings suggest that MCs may use microvesicles as vehicles to regulate the functions of other cell types through miRs to drive type 2 inflammation.

Like T_H2 cells and ILC2s, MCs can generate substantial quantities of type 2 cytokines, including IL-13, when activated.⁴⁰ The physiological function of MC-derived IL-13 is unknown. Using a model of atopic dermatitis initiated by skin barrier disruption in mice, Leyva-Castillo et al⁴¹ demonstrated an essential role for MC-derived IL-13 in suppressing T_H1 responses to cutaneously applied antigens. Skin tape stripping to induce epidermal disruption resulted in IL-33-mediated cutaneous MC activation and their induced expression of IL-13. Using mice with *Il13* expression selectively deleted in MCs, the authors demonstrated that MC-derived IL-13 acted on dendritic cells (DCs) from the draining lymph nodes of ovalbumin-sensitized skin to selectively suppress their DC-dependent polarization of naive ovalbumin-TCR transgenic CD4(+) T cells into IFN- γ -secreting cells (Fig 1). MC-derived IL-13 also inhibited the T_H1-cell response in a model of contact hypersensitivity induced by cutaneous sensitization to dinitrofluorobenzene. IL-13 suppressed IL-12 production by mouse skin-derived DCs *in vitro* and *in vivo*. The authors also demonstrated that scratching upregulated IL-13 expression in human skin, and that IL-13 suppressed the capacity of LPS-stimulated human skin DCs to express IL-12 and promote IFN- γ secretion by human CD4(+) T cells. These findings suggest that MCs produce IL-13 through an innate IL-33-driven mechanism in response to barrier disruption, and that MC-derived IL-13 plays a specific role in suppressing T_H1-type immunity to antigens that gain access to the adaptive immune system through the skin.⁴¹

MC-DIRECTED THERAPEUTICS

Omalizumab is a humanized mAb that binds to the Fc portion of free IgE, preventing its interaction with Fc ϵ RI on MCs, basophils, and other cell types. Although effective for preventing asthma exacerbations in atopic individuals, it is not uniformly efficacious, and does not efficiently liberate IgE that is bound to the cell surface of effector cells. Delgado et al⁴² studied the effect of a disruptive anti-IgE–designed ankyrin repeat protein (DARPin) referred to as E002 in a model of bronchoconstriction in allergen-sensitized precision-cut human lung slices. In contrast to omalizumab, anti-IgE–designed DARPins are highly effective at disassociating bound IgE from Fc ϵ RI⁴³ (Fig 1). Lung slices sensitized with sera from dust mite–allergic donors displayed bronchoconstriction and histamine release (indicative of local MC activation) when challenged *ex vivo* with dust mite antigens. Omalizumab was highly effective in blocking this response when

administered before allergen sensitization, but had no effect when administered after sensitization and before challenge. In contrast, E002 markedly reduced both bronchoconstriction and release of histamine in a dose-dependent manner when administered after sensitization and before challenge, achieving inhibition at doses as low as 0.5 μ mol/L and complete abrogation at 50 μ mol/L. These findings suggest a potential therapeutic application for E002 or other IgE-directed DARPins in the treatment of asthma and other disorders involving IgE-dependent sensitization and MC activation.

Like many type 2 inflammatory diseases, eosinophilic esophagitis is associated with marked expansion of MCs in the involved epithelial surface.⁴⁴ Curiously, a substantial subset of patients with eosinophilic esophagitis responds clinically to proton pump inhibitors by unclear mechanisms.⁴⁵ Using a combination of *ex vivo* and *in vivo* approaches, Kanagaratham et al⁴⁶ demonstrated that omeprazole, a commonly prescribed proton pump inhibitor, potently inhibits the activation of mouse and human MCs *ex vivo* in response to IgE receptor cross-linking. Treatment of MCs with omeprazole interfered with Fc ϵ RI-triggered Syk and extracellular regulated signal kinase phosphorylation and dramatically blocked histamine release, PGD₂ generation, and especially IL-13 and IL-5 production. Omeprazole also inhibited the expansion of MCs in an IL-3–dependent culture system. The systemic administration of esomeprazole to mice attenuated the severity of passive systemic anaphylaxis, including the levels of released MC products, and also blocked intestinal inflammation and expansion of MCs in a model of food allergy induced by ovalbumin in sensitized mice. These results suggest that MCs are prominent candidate targets of proton pump inhibitors,⁴⁷ and suggest the potential application of these drugs for other disorders associated with MC expansion and activation.

Short-chain fatty acids (SCFAs) are fermentation products generated by intestinal bacteria. Their effects on immune cells are thought to underlie some of the beneficial effects of high-fiber diets and early-life gut microbial diversification on the incidence of allergic diseases.⁴⁸ Folkerts et al⁴⁹ demonstrated that the SCFA butyrate blocked the allergen-induced release of MC products and bronchoconstriction in guinea pig airways. Both butyrate and another SCFA, propionate, inhibited the activations of human and mouse MCs *ex vivo*. Studies using gene-deleted mice revealed that these effects of SCFAs occurred independently of the SCFA receptors GPR41, GPR43, and peroxisome proliferator-associated receptors, but were instead associated with the inhibition of histone deacetylases and altered global histone acetylation in MCs. Notably, butyrate treatment decreased the levels of histone acetylation at the promoter regions of the critical signaling molecules Bruton's tyrosine kinase, spleen associated tyrosine kinase, and linker for activation of T cells, resulting in downregulation of the expression of the corresponding genes (Fig 1). These data suggest that SCFAs may modify the

epigenome of MCs as one of their immunologic targets in their modification of allergic disease pathogenesis.

CONCLUSIONS

This is an exciting era in MC biology. Recent technological advances have enabled investigators to circumvent some of the challenges associated with functional studies of resident MCs in human and mouse tissues. The resultant studies presented herein provide new knowledge into the origins of MCs and their physiological and pathological functions. These advances will likely guide therapeutic advances and strategies to target MCs, as has already been achieved in clonal MC disease. Future treatment strategies emerging from these and other studies may target MC hyperplasia in type 2 diseases, and offer novel means of preventing pathological MC activation that underlies anaphylaxis, asthma, CRSwNP, AERD, and other diseases in which MCs and their products play a prominent role.

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