Advances in mechanisms of asthma, allergy, and immunology in 2008

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This review summarizes selected articles appearing in 2008 in the Journal. Articles chosen include those improving our understanding of mechanisms of allergic diseases by focusing on human basophil, mast cell, and eosinophil biology; IgE and its high-affinity receptor on various cells; novel properties of omalizumab; airways remodeling; and genetics. Articles from other journals have been included to supplement the topics presented. (J Allergy Clin Immunol 2009;123:569-74.)

Abbreviations used

ADAM:	A disintegrin and metalloprotease
AHR:	Airway hyperresponsiveness
FIP1L1-PDGFRA:	Fip1-like1–platelet-derived growth factor receptor α-chain
MMCP:	Mouse mast cell protease
PI-3K:	Phospatidylinostol-3-phosphate kinase
Siglec:	Sialic acid–binding immunoglobulin-like lectin
Syk:	Spleen-type tyrosine kinase
Treg:	Regulatory T
VEGF:	Vascular endothelial growth factor

The JACI is one of the premier journals for publication of human cell biology and genetics relevant to asthma and allergic diseases, and this past year was no exception. Studies published in 2008 have advanced our knowledge regarding pathways controlling degranulation of IgE receptor bearing cells and have further elucidated the ability of IgE and anti-IgE therapies to modulate cellular responses. The list of mechanisms by which eosinophils can be activated and kept alive was expanded, as was the list of mechanisms by which viruses, cytokines, and other agents may contribute to expression of remodeling genes in the airway. Murine studies explored the potential of immunomodulator therapies to influence airway remodeling. Additional studies furthered our understanding of genes related to atopic disorders including asthma, especially those involved in innate immune responses (Table 1).

MAST CELLS AND BASOPHILS

Spleen-type (Syk) tyrosine kinase is required for the activation of mast cells and basophils occurring in response to FcεRI cross-linkage. Mazuc et al[1] used an ingenious strategy to identify a novel Syk inhibitor. These investigators had previously reported that an antibody (termed G4G11) directed against an amino acid sequence that is conserved among the Src homology 2 (Sh2) domains of human, mouse, and rat Syk, introduced into RBL-2H3 cells (a rat mast cell line), blocks FcεRI-mediated activation.[2] The investigators screened an extensive panel of small molecules for their ability to displace G4G11 from its target epitope.[1] They identified 15 molecules that displaced G4G11 binding, and tested 1 of these (termed C-13) for the ability to block Syk-mediated activation of mast cells. These investigators determined that C-13 binding required Arg68, Glu121, and Glu155 of Syk. C-13 was cell-permeable and blocked FcεRI-mediated activation of RBL-2H3 cells by preventing Syk-dependent phosphorylation of Bruton tyrosine kinase (Btk) and several downstream signaling events. Orally administered C-13 blocked both passive cutaneous and systemic anaphylaxis in mice. This study is an exciting step in the development of Syk-targeted drugs for allergic diseases.

Curcumin (a pigment of curry powder) was previously reported to have anti-allergic properties in animal models of allergy.[3] Lee et al[4] showed that treatment of RBL-2H3 cells with curcumin inhibited their FcεRI-mediated degranulation, production of both TNF-α and IL-4, and activation of mitogen-activated protein kinase. Like C-13, curcumin inhibited Syk enzymatic activity without blocking its FcεRI-mediated phosphorylation. Although it is not known whether curcumin targets a region of Syk that is similar to that bound by C-13, this study once again highlights the therapeutic potential of interference with Syk in allergic disease.

Syk is transiently inactivated after FcεRI-mediated cell activation, resulting in a refractory period during which mast cells or basophils are resistant to a second activation after cross-linkage of FcεRI. MacGlashan and Undem[5] demonstrated that a Syk inhibitor (NVP-QAB205) completely blocked the release of mediators by mast cells challenged with anti-IgE but had no effect on Syk inactivation (as indicated by the lack of mediator release in

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TABLE 1. Key advances in mechanisms of asthma, allergy, and immunology in 2008

1. For mast cell and basophil biology, advances include involvement and antagonism (by novel molecules and curcumin) of Syk kinase in FcεRI signaling; inhibition of FcεRI signaling by Siglec-8; role of adenosine A3 receptors and activated T-cell surface molecules in mast cell activation; contribution of serglycin to mast cell granule storage function; evolution of mast cell proteases across species.
2. For IgE biology, advances include comparison of clinical assays for measuring specific IgE; characterization of multiple functional properties of IgE affecting affinity for ligand and degranulation responses in basophils.
3. For omalizumab, advances include effects on free IgE levels and skin test responses over time; effects on asthma and tissue eosinophilia in a patient developing Churg-Strauss syndrome while receiving omalizumab; benefits in autoimmune urticaria.
4. Eosinophils: activating activity of IL-33 via its receptor ST2; ability of extracellular cationic charges to enhance eosinophil degranulation; report of a patient with chronic eosinophilic leukemia manifesting resistance to imatinib caused by mutations in the FIP1L1-PDGFRA kinase domain.
5. Airway remodeling and AHR in asthma: causative contributions of rhinovirus, VEGF, sADAM33, and mast cells within airway smooth muscle; use of DCregs to reduce AHR in mice; inhibitory effect of corticosteroids on cysteinyl leukotriene receptor expression on leukocytes incubated with IL-4.
6. Genetics of allergic diseases: influence of polymorphisms in innate immune receptors including TLRs and filaggrin on asthma and atopic dermatitis by altering both adjuvant effects of microbial agents and local responses to viral and bacterial infections.

response to a second stimulation with anti-IgE).[6] A similar result was obtained with basophils. An inhibitor of phospatidylinostol-3-phosphate kinase (PI-3K) also completely blocked mediator release by anti-IgE–activated basophils without altering inactivation of Syk. Thus, neither Syk activation nor PI-3K activity is necessary for the transient inactivation of Syk after mast cell or basophil activation. These findings suggest that pharmacologic antagonists that target either Syk or PI-3K should block anaphylactic mediator release without interfering with the potentially desired effect of Syk inactivation during desensitization to antigen.

Sialic acid–binding immunoglobulin-like lectins (Siglecs) are a family of receptors that bind to extracellular glycan structures. Many Siglecs are predicted to have inhibitory functions based on the presence of immunoreceptor tyrosine inhibitory motifs. One member of this family, Siglec-8, is selectively expressed on human eosinophils, basophils, and mast cells. Yokoi et al[6] demonstrated that Siglec-8 engagement inhibited exocytosis by human mast cells *in vitro*, as well as their production of prostaglandin D_2, but not release of IL-8. Siglec-8 engagement also inhibited the contraction of isolated human bronchi in response to stimulation with anti-IgE. The authors generated a series of mutated Siglec-8 constructs, and demonstrated in RBL-2H3 cells that the immunoreceptor tyrosine inhibitory motif domain was crucial for the inhibitory function of Siglec-8. Thus, antibodies or molecules that mimic Siglec-8 ligands could be developed as potential therapeutic agents that prevent mediator generation by mast cells and its physiologic consequences.

Adenosine, a product of neurons and vascular cells released in response to stress, hypoxia, and inflammation, is a known agonist of mast cell activation *in vitro*, and induces bronchoconstriction by a mechanism that depends on mast cell–derived mediators. Hua et al[7] studied the potential role of adenosine in mediating airway hyperresponsiveness (AHR) to methacholine. Inhalation of the stable adenosine analogue adenosine-5′ N-ethylcarboxamide induced AHR in mice. This feature was absent in mast cell–deficient Wsh/Wsh mice, as well as in mice lacking the A3-type adenosine receptor. The direct role of A3 receptors on mast cells was confirmed by adoptive transfer experiments, in which engraftment of the Wsh/Wsh mice with mast cells derived from wild-type mice, but not with mast cells derived from A3 receptor–deficient mice, restored AHR after inhalation of adenosine-5′ N-ethylcarboxamide. The adenosine pathway may thus be an important IgE-independent mechanism by which mast cells contribute to AHR.

Salamon et al[8] used a microarray to identify the profile of mRNA transcripts inducibly expressed by the human LAD2 mast cell line when these cells were incubated in the presence of membranes derived from activated T cells. This condition induced the expression of 200 transcripts that were not expressed in response to FcεRI cross-linkage. These included several cytokines and chemokines, such as oncostatin M, a cytokine belonging to the IL-6 family with profibrotic properties. LAD2 cells and cord blood–derived mast cells secreted oncostatin M protein when incubated with membranes from activated but not from resting T cells. The production of oncostatin M was sensitive to dexamethasone and could also be modestly suppressed by curcumin. The quantities of oncostatin M secreted by mast cells were sufficient to induce the proliferation of fibroblasts *in vitro*. Thus, oncostatin M, produced by mast cells in response to contact with activated T cells, may promote remodeling of the airway and other tissues.

Mast cells and basophils store preformed histamine (and mouse mast cells also store serotonin) in secretory granules. Storage of these mediators involves proteoglycans that contain a core peptide termed *serglycin*. Ringvall et al[9] studied the storage of histamine and serotonin in mast cells from mice lacking serglycin. The granules of the mast cells from the serglycin knockout strain were poorly developed and contained less histamine and serotonin than did the mast cells from the wild-type strain, particularly *in vivo*. Although *in vivo*-derived and *in vitro*-derived mast cells from both strains released histamine and serotonin when activated by using calcium ionophore, the quantities released from the cells of the serglycin knockout mice were substantially lower. Interestingly, although release depended entirely on preformed stores, some of the serotonin released was synthesized *de novo* in response to cell activation. The ability of mast cells to generate serotonin, but not histamine, *de novo* in response to activation suggests a mechanism for the release of this important vasoactive substance that occurs independently of classic degranulation.

Mouse mast cells express 2 functionally divergent tryptases, termed *mouse mast cell protease* (MMCP)–6, a homolog of human mast cell tryptase β, and MMCP-7. Human mast cell granules lack a true ortholog of MMCP-7, instead expressing small amounts of tryptase δ, an enzyme that does not exist in mice and that has weak protease activity. Trivedi et al[10] determined how tryptase δ changed during primate evolution, and why the human enzyme possesses such weak activity. All primates studied (including lemurs, macaque, and great apes) possess genes encoding tryptase δ, which evolved by transformation from an ancestral MMCP-7–like gene. The human tryptase δ gene is truncated, affecting its substrate-binding pocket, resulting in a loss of function.