

Review

The Dynamic and Complex Role of Mast Cells in Allergic Disease

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Abstract. Mast cells (MCs) are found widely distributed in tissues and contribute to regulation of inflammatory responses and ongoing modulation of the tissues. Although MCs are important in a variety of processes, including innate immunity, their role in allergic disease has received increasing attention in the past decade. MCs are located throughout the human body and, upon allergen exposure, they are stimulated via the immunoglobulin E (IgE) receptor (FcεRI) to release several pro-inflammatory mediators such as tumor necrosis factor (TNF), reactive oxygen species such as nitric oxide (NO), proteases, and lipid-derived mediators. However, we now recognize that MCs can be activated by a variety of mechanisms and that mediator release is a consequence of several intra- and extracellular signals. Some of these mechanisms, such as Fc receptor aggregation and proteinase-activated receptor (PAR)-mediated activation facilitate and augment local inflammatory responses. Other mechanisms, such as interferon γ (IFN- γ) induction of NO, may inhibit MC function and downregulate inflammatory responses. Increased understanding of these complex pathways has encouraged the development of therapies for allergic inflammation that target specific MC functions and mediators. Some novel strategies include oligonucleotides that induce or inhibit the production of specific mediators. Such approaches may yield useful therapies for allergic individuals in the near future.

Key words: mast cells; allergy; IgE; proteases; cytokines.

Introduction

Mast cells (MCs) are important effector cells in innate immunity and inflammatory responses. Their activation releases several pro-inflammatory mediators that have a variety of targets, including endothelium, epithelium, mesenchymal cells and leukocytes³⁰. Tissue-specific heterogeneity and near ubiquitous distribution make MCs ideal switches for the localized, often tissue-

-specific, inflammatory responses characteristic of allergic disease. Identifying mechanisms underlying MC activation and mediator release is therefore important in the development of rational treatments for allergic diseases.

An allergic reaction (or type I hypersensitivity) is characterized by the production of immunoglobulin E (IgE) in response to a normally innocuous allergen⁵⁶. An allergen crosslinks IgE bound to IgE receptors

Abbreviations used: MC – mast cell, NO – nitric oxide, PAR – proteinase-activated receptor, PAF – platelet activating factor, LC – Ig-free light chain, LTC₄ – leukotriene C₄, VIP – vasoactive intestinal peptide, SCG – sodium cromoglycate, NED – nedocromil sodium, iNOS – isoform of nitric oxide synthase, SCF – stem cell factor, OVA – ovalbumin.

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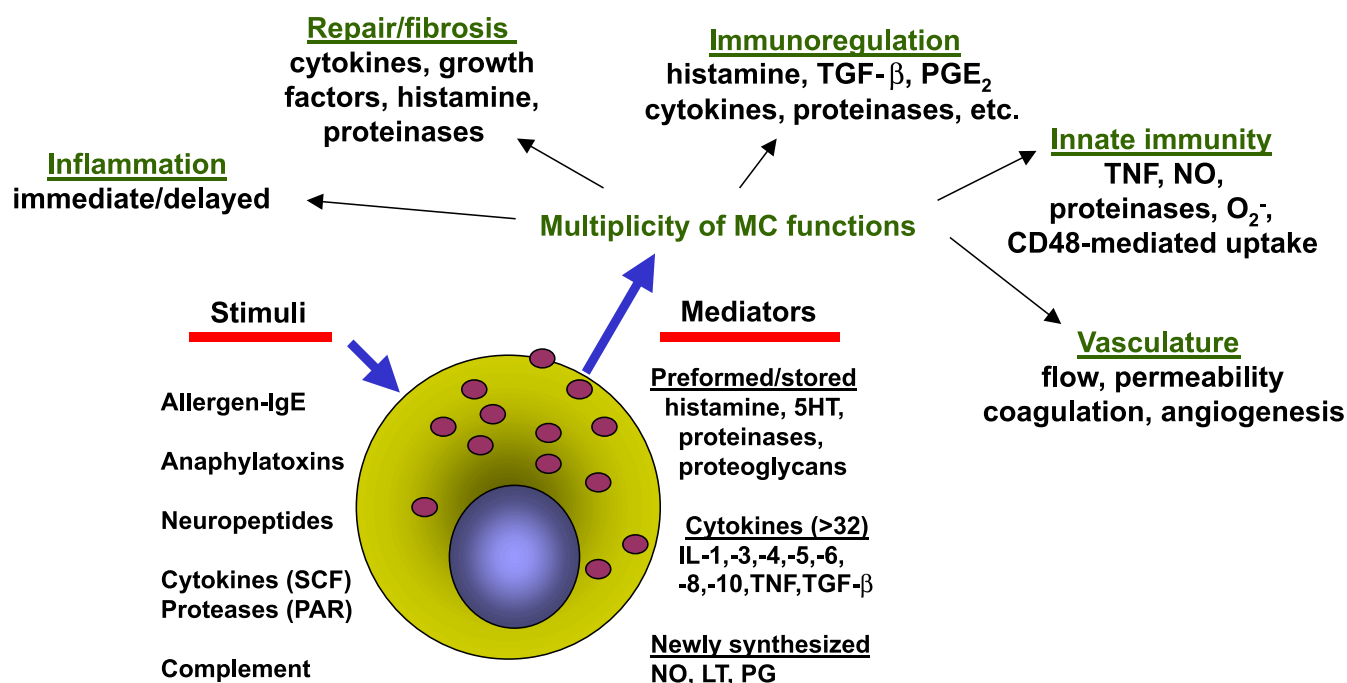


Fig 1. Mast cell (MC) activation and mediator release. MCs can be activated by allergens via IgE and the FcεRI. MCs can also be activated by anaphylatoxins, neuropeptides, cytokines and complement. Activated MCs release several mediators including serotonin (5HT), interleukins (IL)-1, -3, -4, -5, -6, -8, and -10, tumor necrosis factor (TNF), leukotrienes (LT) and prostaglandins (PG). These mediators initiate several effects, including inflammation, repair/fibrosis, immunoregulation, innate immune effects and alterations in the vasculature

(FcεRI), leading to MC activation and release of pro-inflammatory mediators (Fig. 1). There are circumstances in which IgE-mediated hypersensitivity is protective, especially in response to parasitic infection⁴⁹. However, IgE responses to innocuous antigens predominate in industrialized countries such as Canada, costing the health care system millions of dollars each year. Therefore, understanding the pathophysiological consequences of IgE-mediated activation of MCs is important. This review examines: 1) the mechanisms of allergen-induced MC activation, 2) the effect of MC mediator release on surrounding tissue cells, resulting in some of the symptoms associated with an allergic response, and 3) some innovative therapies currently in development that target signaling pathways in MCs and other cell types.

Mechanisms of MC Activation and Mediator Release

Although MCs can be stimulated by various pathways, one of the best characterized mechanisms of activation is allergen-IgE-mediated crosslinking of FcεRI.

Allergen-mediated FcεRI aggregation stimulates the release of several mediators that are either stored in MC granules or synthesized upon activation. These include mediators such as tumor necrosis factor (TNF), reactive oxygen species such as nitric oxide (NO), proteases, and lipid-derived mediators including platelet-activating factor (PAF) and arachidonic acid metabolites prostaglandin D₂ and leukotriene C₄ (LTC₄)³⁰. Recent studies suggest that MCs are also activated by an alternative pathway involving IgG and the FcγRI and FcγRIII receptors^{53, 54}. This alternate pathway facilitates antigen-induced anaphylaxis in mouse models and likely activates other cell types, such as macrophages⁶⁹. Antigen-specific IgG alone can facilitate the initial period of bronchoconstriction characteristic of the early asthmatic response in the mouse model¹⁸. A recent study of children with cow's milk allergy measured antibodies to milk proteins in the duodenum and showed a correlation between gastrointestinal cow's milk allergy and high levels of IgG and IgA class antibodies to milk and its fractions⁴². In a similar study, IgG and IgE levels to inhaled and food allergens were compared and the data showed that children with an increased IgG antibody level to a mixture of wheat-rice or orange had

an increased risk of developing IgE to cat, dog or mite allergens²⁴. These observations suggest that some types of allergic responses, such as those to food allergens, may be IgE independent and may involve IgG responses.

However, responses to inhaled allergens do not appear to correlate with IgG levels. Children exposed to a damp school environment and tested for IgG antibodies specific to 24 molds showed no significant differences in mold-specific IgG concentrations between exposed and non-exposed school children, and although mold-specific IgE levels correlated with allergic disease, the association between asthma, wheezing or cough symptoms and high mold-specific IgG levels was not significant⁷². Therefore, the route of allergen sensitization/challenge may determine whether the allergic response is primarily IgG or IgE driven and can ultimately initiate MC activation by either the FcγR or FcεR.

A novel report recently published in "Nature Medicine" has shown that Ig-free light chain (LC) can transfer antigen-specific immediate hypersensitivity responses in mice, a response that is absent in MC-deficient mice⁵⁸. Evidence in this report also suggests that MCs express a receptor for LC and that crosslinking MC surface proteins with LC results in MC activation. This observation may explain some of the early events observed in the initiation of contact allergy responses in which Ig and MC are not required for T cell priming, but both MCs and antigen-specific Ig are necessary for the effector phase^{8, 74}. This report is also the first to show a function for secreted LC which may play a role in the pathogenesis of autoimmune diseases associated with increased plasma levels of LC, such as multiple sclerosis or rheumatoid arthritis^{20, 26, 51, 65}.

MC can also be activated by peptides and complement-derived anaphylatoxins which signal through FcR-independent pathways. The bee venom peptide, mellitin, and adrenocorticotrophic hormone bind to MCs and induce second messengers, such as phospholipase A₂, which resemble responses induced by 48/80^{38, 59}. Neuropeptides, such as substance P, induce MC activation in an FcεRI-independent mechanism through activation of G proteins⁵⁰, and complement peptides, such as C5a, bind to specific receptors on MCs and stimulate degranulation and potentiate anaphylactic reactions²³.

Histamine's Many Targets

MC activation is rapid and releases large stores of histamine, proteoglycans, and MC-specific proteases. Smooth muscle cells express histamine receptors and

early studies on asthmatic patients showed that histamine could induce smooth muscle contraction and bronchoconstriction¹⁹. Over the past 50 years, the effects of histamine have been expanded to include almost any cell type in almost any part of the body, and evidence of histamine receptor expression on a variety of immune and non-immune cells suggests a much wider and more critical role for histamine in allergic disease than is currently understood. The interaction of histamine with its G-protein-coupled receptors (H1–H4) in various cell types activates an IP₃, cAMP and Ca²⁺-dependent pathway, eventually leading to sneezing, itching and discharge in rhinitis and itchy skin wheals/flare in urticaria. Pharmacologic studies show that purified human conjunctival MCs express histamine receptors and that MC mediator release can be inhibited by antihistamine drugs such as olopatadine, cetirizine and terfenadine^{25, 70, 79}. Olopatadine and terfenadine, for example, are used to treat ocular conjunctivitis and are given either orally or as eye drops^{2, 4}. Antihistamines are thought to function mainly by blocking histamine receptors and preventing histamine-induced signaling in these cells.

The Role of MC-Derived Cytokines in Allergic Disease

Upon activation, MCs also synthesize and secrete a wide range of cytokines, such as interleukin 3 (IL-3), IL-4, IL-5, IL-6, IL-8, IL-9, IL-13, IL-16, TNF and granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemokines, such as monocyte chemoattractant protein-1, monocyte inhibitory protein-1 α/β and regulated upon activation normal T cell expressed and secreted protein (RANTES)^{30, 47}. These cytokines activate and recruit other cells and may eventually lead to tissue damage. IL-4 and IL-13 induce IgE synthesis in B cells and amplify a local allergic reaction⁵⁷. IL-5 and RANTES recruit neutrophils and eosinophils to the lung^{34, 35, 39, 55}, resulting in local increases of PAF, LTC₄, major basic protein, eosinophil cationic protein and eosinophil peroxidase, which contribute to airway hyperresponsiveness and tissue damage^{6, 34, 63}. Activated MCs also release IL-16 and lymphotactin which recruit lymphocytes to the lung^{61, 62}. The production of cytokines is a highly controlled process likely regulated by a number of feedback mechanisms. Through the release of cytokines, chemokines and growth factors, MCs can also contribute to the chronic inflammatory infiltrate and structural changes that are associated with

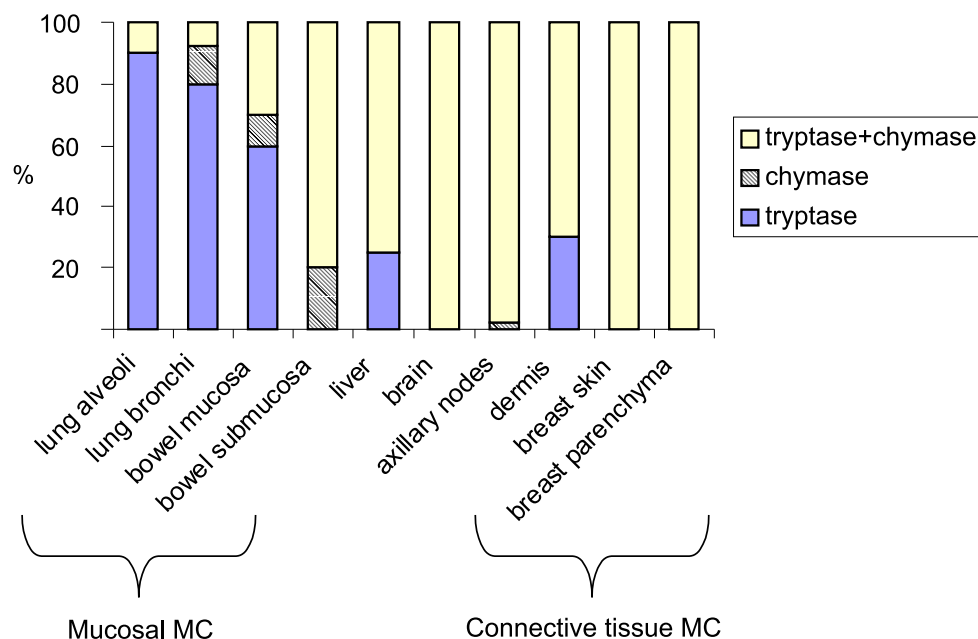


Fig. 2. Distribution of mast cells (MCs) with different proteinase content in human tissues. The two most abundant MC serine proteinases, tryptase and chymase, are found in separate cells or in the same cells, and the proportions of these different cell populations vary among tissues. Few lung alveolar (mucosal) MCs express chymase, whereas most breast parenchymal (connective tissue) MCs express both tryptase and chymase

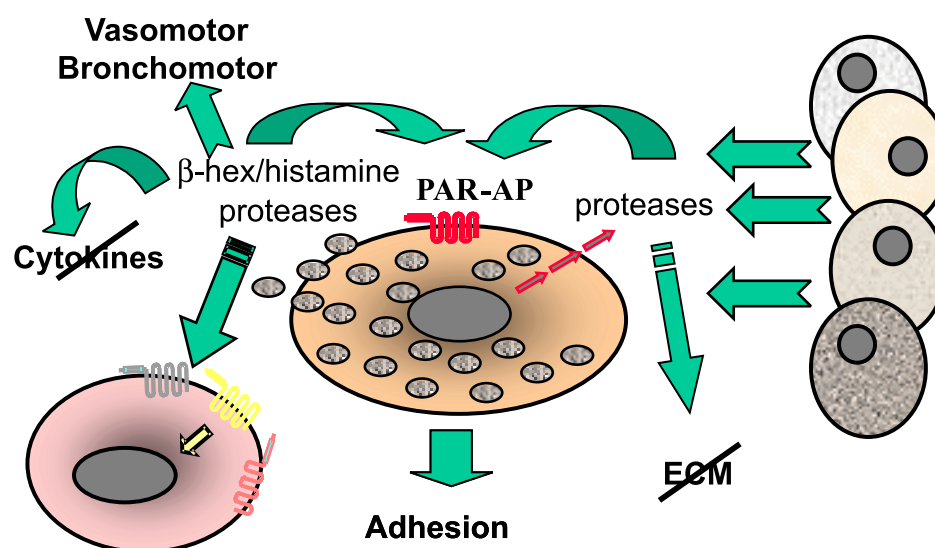


Fig. 3. Effect of proteinase-activated receptors 2 (PAR-2) activation on mast cell (MC)-mediated inflammation. The release of MC proteinases can activate PAR on nearby cells, and on the MC surface in an autocrine fashion. The autocrine activation of MC PAR can induce release of β -hexosaminidase (β -hex), histamine and proteinases, further amplifying the response. Through their proteolytic activity MC proteinases can degrade cytokines and extracellular matrix (ECM). MC proteinases are also chemotactic for inflammatory cells and can alter vasomotor and bronchomotor activity

some long-term allergic inflammatory diseases such as asthma.

A group of MC mediators receiving increasing attention in the current proteomics boom are the MC proteinases. Neatly packaged in the granule proteoglycan matrix, these proteinases are both abundant and MC-spe-

cific. Although there are over 50 characterized MC-derived proteinases⁵, very little is known about their specific role in allergic disease (Fig. 2). However, increasing evidence suggests that proteolytic cleavage may be an important event in allergic reactions. For example, two common features of gut allergic reactions are

duodenal contraction and intestinal permeability, both of which may be regulated by MC proteases. MC-derived tryptase hydrolyses neuropeptide vasoactive intestinal peptide (VIP) and MC-derived chymase cleaves both VIP and substance P, possibly modulating neurogenic inflammatory responses and control of peristalsis¹⁵. Rat MC protease 2 increases intestinal epithelial paracellular permeability in rat intestine, possibly via disruption of tight junctions, and may facilitate egress of MCs into the gut lumen⁶⁴. The recruitment of inflammatory cells is also an important feature of allergic reactivity for which MC proteases are responsible. Intraperitoneal injection of human MC tryptase or chymase in mice generates a marked neutrophilia and eosinophilia which is likely mediated by induction of cytokines such as IL-8 from epithelial and endothelial cells³⁷.

MC-derived proteases are also responsible for the tissue remodeling associated with the long-term allergen exposure that occurs in asthma. In the lung, tryptase and chymase contribute to tissue remodeling through selective proteolysis of matrix proteins and through activation of proteinase activated receptor (PAR) and matrix metalloproteinases^{1, 44} (Fig. 3). Several cell types express PAR, including MCs. In fact, PAR-activating peptides induce MCs to release several mediators including IL-6, TNF and proteases, suggesting that MCs express PAR^{32, 67}. Although the *in vivo* significance of these findings has yet to be determined, blocking MC tryptase and chymase may prove a useful therapeutic tool for asthma.

Inhibition of MC Mediator Release

Drugs that inhibit MC function include theophylline, prostaglandin analogues³⁶, corticosteroids, β -agonists, and cromolyn compounds such as nedocromil sodium (NED) and sodium cromoglycate (SCG). The precise mechanisms by which some of these drugs inhibit MC activation are poorly understood, although they appear to target intracellular signaling pathways that lead to release of both stored and newly synthesized mediators. Theophylline is a phosphodiesterase inhibitor⁷⁵ and increases intracellular cAMP concentration⁷¹. β_2 -agonists such as salbutamol and salmeterol inhibit the release of preformed and newly synthesized MC mediators⁹. NED and SCG downregulate TNF release by up to 40%^{10, 11, 21}, possibly via inhibition of Cl^- channel activity⁶⁰. Although these drugs may be effective, they are not MC-specific and can have effects on other cell types.

Cytokines such as IL-10⁴⁶, transforming growth factor β (TGF- β)¹² and interferon γ (IFN- γ)¹⁷ can also downregulate MCs mediator release. In immediate type hypersensitivity diseases, where MCs play a significant effector role, IFN- γ production is often abnormal. Studies comparing cytokine production of peripheral blood T CD4⁺ lymphocytes in normal and allergic asthmatic patients show significant differences in the numbers of IFN- γ -producing T cells. In patients with allergic asthma, the percentage of IFN- γ -producing T lymphocytes in the peripheral blood is considerably

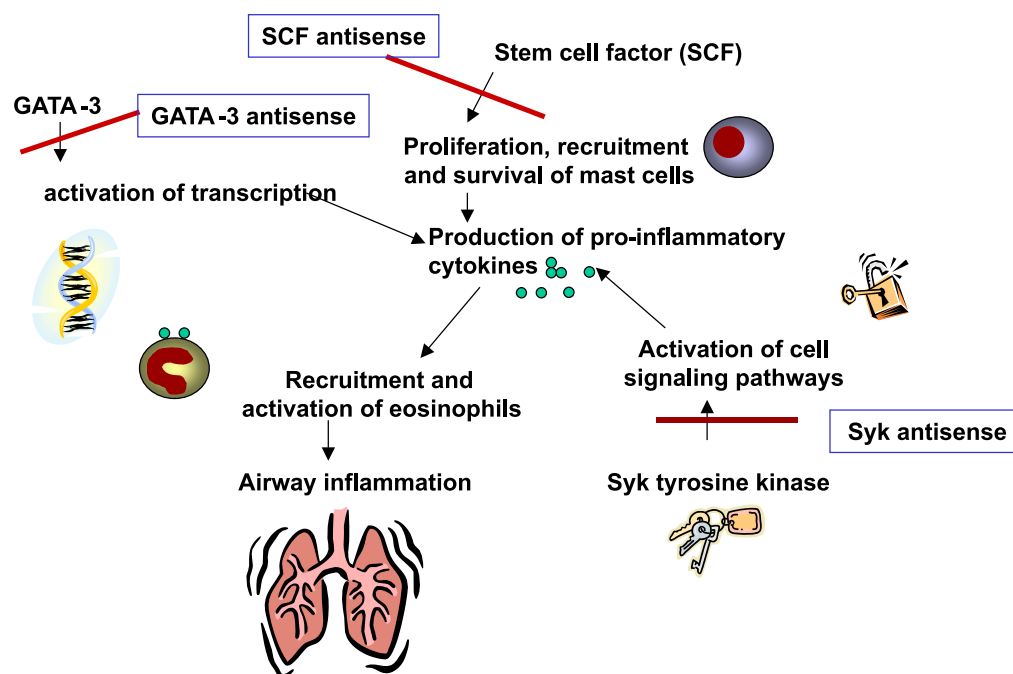


Fig. 4. Novel approaches to allergic therapies. Some current therapies have employed antisense oligonucleotides to block the production of pro-inflammatory mediators which are responsible for eosinophil recruitment and allergic inflammation in the lung

lower than in normal subjects (5.7% versus 23.5% in normal subjects, $p < 0.001$) but the level of IL-4-producing T lymphocytes is the same⁷⁷. Studies of atopic asthmatics have revealed reduced expression of IFN- γ mRNA in bronchoalveolar lavage, 24 h post endobronchial allergen challenge¹³. In adoptive transfer experiments, IFN- γ has been shown to have a suppressive effect on airway eosinophilia¹⁶. Peripheral blood mononuclear cells isolated from children with food allergy, however, show normal levels of IFN- γ when stimulated with phytohemagglutinin¹⁴, suggesting that decreased IFN- γ production may be antigen-specific.

Many IFN- γ -mediated effects are induced indirectly and are the result of pathways that are initiated as a result of IFN- γ regulation of gene transcription. One of those indirect messengers is NO. NO is a radical synthesized from L-arginine and molecular oxygen by many cell types, including MCs. IFN- γ initiates production of the inducible isoform of NO synthase (iNOS) in MCs as well as some of the second messengers involved with NO production^{29, 31}. NO exerts a range of physiological, toxicological and immunoregulatory effects and may be important in immunity by influencing the balance between functional subsets of T helper (Th) cells. NO may also be involved in IgE-mediated allergic reactions by inhibiting mast cell activation and histamine release^{22, 29, 43}, since IFN- γ -induced release of NO from MCs inhibits histamine secretion and adhesion to fibronectin⁷⁶. Yet, exogenously applied NO upregulates expression of CD8 α , an important co-stimulatory molecule that can activate the release of newly synthesized mediators, such as TNF and NO, on MCs⁵².

Although the significance of increased CD8 α expression on MCs has yet to be explored, it is possible that NO modulation of MC mediator release may be closely linked to the severity of allergic inflammation in diseases such as asthma. Individuals with asthma show increased expression of iNOS and NO production in their airways³³. iNOS knock-out mice sensitized and challenged with aerosolized antigen show decreased pulmonary allergic inflammation compared with wild-type animals⁷⁸, suggesting that NO production is required for manifestation of allergic disease.

New and Current Therapies and Treatments

Increased understanding of the molecular pathways involved in allergy and MC activation has opened up new opportunities for interventions. As with many other therapies, allergy treatments have been moving

toward molecular interventions for the past decade and have lead to such approaches as anti-IgE antibodies, vaccination with plasmid DNA, the use of immunostimulatory DNA sequences, cytokines and bacterial agents, immunotherapy with mutated proteins and peptides, and complementary medicine such as Chinese herbs. In the past year, several studies that utilize DNA targeting strategies have shown that blocking allergic inflammation is possible through the targeted and selective inhibition or expression of specific mediators (for a recent review see⁴⁰). These therapies can be divided into two categories: antisense blockade of mediator expression or targeted expression of specific gene products.

There are several new therapies in clinical trial, including various DNA-based therapeutics, that are effective in inhibiting allergic disease (Fig. 4). One mechanism has been to inhibit the production of Th2 cytokines that can activate and recruit MCs, eosinophils and basophils to inflammatory sites. The transcription factor GATA-3 is exclusively produced by Th2 cells and blocking GATA-3 synthesis through antisense oligonucleotides delivered to the airway in the ovalbumin (OVA) murine model of asthma reduced eosinophilia and detectable IL-4 in the bronchoalveolar lavage²⁸. Another recent study reported that intranasal administration of antisense to stem-cell factor (SCF) blocked intracellular SCF production in interstitial lung cells. SCF antisense administration to an allergen-challenged mouse model also reduced IL-4 production and eosinophil infiltration²⁷. We have found that administration of aerosolized antisense to the tyrosine kinase signaling molecule, Syk, suppresses airway inflammation, likely via inhibition of alveolar macrophage mediator release^{66, 68}. These studies suggest that the targets for these therapies are most likely a variety of cell types, including macrophages, MCs and epithelial cells.

As discussed earlier, IFN- γ is a potent inhibitor of some MC functions and IFN- γ production in allergic asthma appears to be downregulated. Another DNA-based therapeutic approach has artificially upregulated IFN- γ production in the airway through gene transfer of IFN- γ -inducing cytokines such as IL-18 and IL-12. Preliminary experiments using an IL-18 fusion plasmid showed that intramuscular injection of this plasmid prior to initial sensitization with OVA, increased OVA-stimulated splenocyte IFN- γ production and decreased serum levels of OVA-specific IgE⁴⁸. Several studies have shown that CpG oligodeoxynucleotides are effective in preventing and downregulating atopic responses in murine models of asthma even in the absence of IFN- γ and IL-12⁴¹, suggesting that other Th1 cytokines

such as IL-10, which can also inhibit MC mediator release, may be involved⁴⁶.

Potential targets of these molecular therapies may arise from genomic screening of allergic disease. There is an overwhelming collection of evidence to support a genetic component for allergic disease (for a recent review see ⁷³) and genomic wide screening and candidate gene analysis has shown that genes for cytokines, chemokines, their receptors, transcription factors and MHC molecules may all be potential candidate genes for allergy-predisposing genes. All of these genes may be potential targets of DNA-based therapies. For example, polymorphisms in cytokine genes, such as GM-CSF, IL-3, and IL-5, may predispose some individuals to allergic inflammation. In a recent study, a British group found that the frequency of a single nucleotide polymorphism in the 3'UTR region of the STAT6 gene was significantly increased in nut allergy patients compared with blood donor controls³. STAT6 is an important transcription factor induced in MCs following FcεR crosslinking and is involved in IgE-stimulated release of TNF and IL-6³. In another study of a rural population it was found that polymorphisms in the FcεRI-β gene have significant effects on IgE responsiveness to common inhaled allergens, especially pollen and dust mite⁶⁴. These observations suggest that allergic individuals signal abnormally through the IgE receptor.

Conclusions

Mature MCs are found throughout the body, predominantly located near blood vessels and nerves and beneath epithelia^{7, 30}. MC activation and mediator release can induce and regulate allergic inflammation associated with allergic responses. It is believed that, when activated by allergen systemically, MCs and basophil mediators contribute to vasodilation and exudation of plasma in the vascular beds throughout the body, resulting in a fall in blood pressure, constriction of the upper and lower airways, hypersensitivity of the gut, over-production of mucus in the gut and lung, and urticarial lesions (hives) on the skin⁴⁵.

Although MCs are central to allergic inflammation, they are certainly not the only cell involved in this complex process. Lymphocytes, eosinophils, basophils, epithelial cells and endothelial cells are all players in the complexity of an allergic reaction. However, MCs and their mediators appear to play a central role in initiating and perpetuating the inflammatory signal. Understanding the molecular mechanisms of MC activa-

tion may offer insights into possible treatments for allergic disease.

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