The Role of Mast Cell Derived Mediators in Airway Hyperresponsiveness*

Edward S. Schulman, M.D., F.C.C.P†

Clinically, asthma is recognized by airway hyperreactivity and reversible airways obstruction. At the tissue level, pathologic derangements include constriction of airway smooth muscle, increased vascular permeability resulting in edema of airways, outpouring of mucus from goblet cells and mucus glands, parasympathetic nervous system activation, denudation of airway epithelial lining cells and influx of inflammatory cells including neutrophils and eosinophils.1 Underlying these tissue effects are potent chemical mediators secreted following the physical, inflammatory or immunologic activation of the human lung mast cell (HLMC).

Though mast cells are found in all human organs, they exist in highest concentrations at portals of entry into the body: the skin and the respiratory and gastrointestinal tracts. In the lung, small numbers are found free in the bronchial lumen5,3 and within the epithelial lining;4 thus, these mast cells may represent the true sentinel cells of hypersensitivity. Their perturbation may actually initiate airway reactions that lead to permeability changes and allow penetration of antigen into mast cell-rich submucosal areas: the basement membranes of airways, and areas adjacent to submucous glands, near blood vessels, and in muscle bundles. Large numbers of mast cells are also localized to inter-alveolar septa in lung parenchyma.5,6

The recent development of techniques to purify HLMC* has allowed detailed studies of their biology including ultrastructure,7,8 biochemistry of activation,9 the nature of mediators released,10-13 pharmacologic modulation of the release mechanism,6,14 and heterogeneity.

Biochemistry of Release

An understanding of the biochemistry of the release reaction provides a rationale for present and future drug therapies of human hypersensitivity reactions. The mechanism of human mast cell activation most studied and therefore best understood is that involving antigen-induced cross-linking of antigen-specific immunoglobulin E (IgE) on the surface of mast cells and basophils. This leads to further cross-linking of Fc epsilon receptors within the cell membrane and initiation of a series of biochemical events which eventually result in mediator release.15 These biochemical events include the conversion of phosphatidylethanolamine to phosphatidylcholine by two membrane methyltransferases at 30 seconds following activation, a rise in intracellular cyclic AMP at 1 minute, calcium influx at 2-3 minutes and histamine release which nears completion at 5-10 minutes.16 Clearly, many biochemical activation pathways remain uncharacterized and some may hold promise for future avenues of pharmacologic intervention.

MEDIATORS

The interplay of mast cell mediators is undoubtedly critical to pathophysiology. Mast cell mediators can be subdivided into those that are preformed or granule associated, and non-preformed or newly synthesized following activation (Table 1).

Preformed Mediators

Histamine: Histamine has numerous pro-inflammatory effects including enhancement of vascular permeability, chemotraction for eosinophils,17 and initiation of airway smooth muscle contraction. Plasma histamine levels have been found to increase two to five-fold following specific antigen but not meth-

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Abbreviations used: HLMC, human lung mast cell; SRS-A, slow reacting substance of anaphylaxis; LTC4, leukotriene C4; IgE, immunoglobulin E; PGD2, prostaglandin D2; HMW-NCF-A, high molecular weight neutrophil chemotactic factor of anaphylaxis; HMW-ECF-A, high molecular weight eosinophil chemotactic factor of anaphylaxis; IC50, concentration of drug which inhibits histamine release by 50%.

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Table 1—Human Lung Mast Cell Mediators

<table>
<thead>
<tr>
<th>Preformed</th>
<th>Non-Preformed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>Prostaglandin D2</td>
</tr>
<tr>
<td>Heparin</td>
<td>Leukotriene C4</td>
</tr>
<tr>
<td>ECF-A</td>
<td>Platelet-Activating Factor</td>
</tr>
<tr>
<td>NCF-A</td>
<td>Leukotriene B4</td>
</tr>
<tr>
<td>Elastase</td>
<td>Kininogenase</td>
</tr>
<tr>
<td>Tryptase</td>
<td>Cathepsin-G-like (Chymotryptic) Enzyme</td>
</tr>
<tr>
<td>Cathepsin-D</td>
<td>β-glucuronidase</td>
</tr>
<tr>
<td>β-hexosaminidase</td>
<td>Arylsulfatase</td>
</tr>
</tbody>
</table>

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578 Mast Cell Derived Mediators in Airway Hyperresponsiveness (Edward S. Schulman)
acholine inhalation challenge in humans. However, it has long been appreciated that inhibiting histamine end organ effects by histamine H1 receptor blocking agents has marginal value in the management of asthma. The reasons for this include: 1) high local tissue concentrations of histamine may be unaffected by clinical doses of H1 blocking agents, and 2) histamine is only one of many mediators producing end organ effects.

High Molecular Weight Eosinophil Chemotactic Factor of Anaphylaxis (HMW-ECF-A): The presence of eosinophils in the vicinity of mast cell-mediated reactions is well documented. The release of HMW-ECF-A, histamine and possibly leukotriene B4 (LTB4) from mast cells may serve as the biochemical mediators of this response. Once present, eosinophils may play both pro- and anti-inflammatory roles. The eosinophil granule contains pro-inflammatory mediators including major basic protein (MBP), peroxidase and cationic protein. MBP has potent airway inflammatory properties and also induces noncytotoxic histamine release from human basophils and rat mast cells. Therefore, this protein may serve to propagate hypersensitivity reactions. Eosinophils are also capable of both releasing and inactivating leukotriene C4 (LTC4). One mechanism of inactivation involves production of superoxide following a respiratory burst which dismutates to hydrogen peroxide. Hydrogen peroxide in the presence of cellular peroxidase and chloride ion forms hypochlorous acid that attacks leukotrienes C4, D4, and E4, forming inactive metabolites. Furthermore, eosinophil histaminase is capable of inactivating mast cell histamine.

High Molecular Weight Neutrophil Chemotactic Factor of Anaphylaxis (HMW-NCF-A): HMW-NCF-A can be measured in the circulation of sensitive asthmatic patients following antigen, aspirin, and, according to some investigators, exercise challenge. Its presence correlates with the occurrence of airway obstruction in both immediate and late-phase responses following antigen challenge. Tissue neutrophilia has been seen during the late-phases of IgE-mediated responses both in animals and humans.

Enzymes

Trypsin: Trypsin is the predominant neutral protease of the mast cell granule. The role played by this mediator in IgE-mediated reactions is conjectural, but trypsin is capable of inactivating high molecular weight kininogen. Therefore, this activity, along with the anticoagulant activity of the mast cell proteoglycan heparin, may inhibit clot formation allowing rapid resolution of edema reactions in the airways and urticarial reactions in the skin.

Cathepsin-G-Like (Chymotryptic) Enzyme: This cathepsin G-like neutral protease has chymotryptic specificities. A similar, if not identical enzyme has been identified in human skin mast cells. These chymotryptic enzymes are more potent than angiotensin-converting enzyme in converting angiotensin I to angiotensin II.

Elastase: Elastase is released from purified HLMC in an IgE-mediated reaction. It appears identical to what was previously called lung Hageman factor activator (LHFA) and is now measured in a radioimmunoassay developed to detect human neutrophil elastase. The role of this mediator in hypersensitivity is unknown.

Kininogenase: Purified mast cells release a kininogenase that cleaves low molecular weight kininogen to form biologically active kinin. Kinins have potent effects on blood vessels to promote vasodilation and edema.

Acid Hydrolases: These granule-associated enzymes include β-hexosaminidase, β-glucuronidase and arylsulfatase. The role of these enzymes is speculative, but they may contribute to epithelial cell denudation.

Non-Preformed Mediators: Recently, exciting insights have been gained into the critical contribution of arachidonic acid metabolites in asthma and other allergic disorders. Following HLMC activation, arachidonic acid appears to be liberated from electron dense cytoplasmic lipid bodies and from membrane phospholipids. It is then metabolized to prostaglandin D1 (PGD1) and LTC4 as the major products of the cyclooxygenase and lipooxygenase pathways, respectively. It is still unclear whether HLMC generate small quantities of the chemotactic lipooxygenase products 5-HETE and LTB4, the latter being more potently chemotactic for both neutrophils and eosinophils than the former.

PGD1. The IgE-dependent release of PGD1 from human lung was first demonstrated in both parenchymal and airway fragments. This product was not released spontaneously or in response to a maximal smooth muscle contraction induced by methacholine (10-4 M), suggesting the HLMC as the cell of origin. Subsequent studies with purified HLMC confirmed this theory. Physiologically, when inhaled by asthmatic patients, PGD1 was shown to be the most potent of the cyclooxygenase bronchoconstrictors, being 3.5 fold more potent than PGE2 alpha in producing a 35 percent fall in specific airway conductance. PGD1 also acts as a vasodilator, produces increased capillary permeability, and causes random migration of neutrophils and eosinophils (chemokinesis).

LTC4. The demonstration of the IgE-dependent release of slow reacting substance of anaphylaxis or SRS-A (subsequently found to consist of the conjugated trienes LTC4, LTD4, and LTE4) from human lung fragments goes back many years, but the cellular source was unclear. Recent studies showed that
during IgE-dependent human lung reactions, the HLMC was the predominant source of the LTC\textsubscript{4} released.\textsuperscript{11} Also, it was not necessary for an accessory cell type such as the macrophage to be present. In human lung suspensions, it does not appear that LTC\textsubscript{4} is further metabolized to LTD\textsubscript{4} or LTE\textsubscript{4}.\textsuperscript{18}

The pathophysiologic consequences of mast cell LTC\textsubscript{4} release are protean. In normal human subjects, inhalation of aerosolized LTC\textsubscript{4} was found to induce contraction of both large and small airways, and was far more potent than histamine.\textsuperscript{35,38} LTC\textsubscript{4} was also found to be a potent inducer of human airway explant mucous secretion,\textsuperscript{46,50} and capillary permeability.\textsuperscript{51,52}

Until the past three years, quantification of SRS-A release required a cumbersome and highly unpredictable guinea pig ileum bioassay. The recent availability of highly specific LTC\textsubscript{4} radioimmunoassays has had immediate application in purified HLMC experiments to explore mechanisms underlying its release.\textsuperscript{53} For example, the mechanisms of aspirin-induced asthma have remained poorly understood. Some insight has been gained from recent studies in HLMC that showed that the cyclooxygenase inhibitor, indomethacin (10\textsuperscript{-8} M) failed to inhibit histamine release while causing significant enhancement of LTC\textsubscript{4} release (unpublished).

**Platelet Activating Factor:** This phospholipid mediator has recently been chemically characterized as acetyl glyceryl ether phosphorylcholine (AGEPC).\textsuperscript{55} On a per-cell basis, the mast cell synthesizes 10 to 100 times greater AGEPC than human neutrophils. The effects of AGEPC include platelet alpha granule content release,\textsuperscript{54} aggregation,\textsuperscript{55} increased neutrophil chemokinesis,\textsuperscript{56} vasoconstriction, bronchoconstriction,\textsuperscript{57} and when administered to baboons, an anaphylactic response.\textsuperscript{58}

**Pharmacologic Inhibition of Release**

The common classes of antiasthmatic drugs used in clinical practice have been the most obvious to test for their specificity of effects in HLMC (Table 2). To date, the beta-agonist pharmacologic agents, as typified by fenoterol, are by far the most potent inhibitors of human mast cell mediator release.\textsuperscript{8} In the chopped human lung model, the concentration of fenoterol which inhibits histamine release by 50 percent IC\textsubscript{50} is <10\textsuperscript{-10} M, though this drug is less potent on the purified mast cell (IC\textsubscript{50} = 10\textsuperscript{-8} M). The theophylline class of drugs typified by isobutylmethylxanthine (IBMX), are also effective inhibitors of HLMC release. Though these drugs inhibit mast cell phosphodiesterase, they also may function to inhibit the nucleoside mediator, adenosine\textsuperscript{40} from attaching to its adenyl cyclase-linked membrane receptor. Low concentrations of adenosine (10 \mu M), enhances mast cell mediator release,\textsuperscript{41,42} high doses (1 mM) inhibits release.\textsuperscript{44} Prostaglandin E\textsubscript{2} (PGE\textsubscript{2}), in addition to being a smooth muscle dilator, is an effective inhibitor of HLMC mediator release (IC\textsubscript{50} = 10\textsuperscript{-8} M). Interestingly, sterol binder drugs such as amphotericin B inhibit human mast cell release in submicromolar concentrations.\textsuperscript{41}

Though widely touted as a "mast cell stabilizer," cromolyn sodium poorly inhibits purified HLMC histamine release in response to anti-IgE or calcium ionophore. We have also been unable to identify HLMC subsets (see below) in which this drug is active (unpublished data). Likewise, corticosteroids such as dexamethasone, though useful in hypersensitivity disorders, do not work directly on the mast cell but may exert effects on accessory cell types.\textsuperscript{82}

The early discovery that both histamine and SRS-A were released following antigen challenge of human lung was followed by numerous pharmacologic studies to examine their release inhibition. In the early 1970s the studies suggested that pharmacologic release inhibition of histamine and SRS-A in monkey and human lung was closely associated and could not be dissociated by diethylcarbamazine.\textsuperscript{83,84} The notion of requisite coexpression of lipoxygenase product release with histamine release in human systems was strengthened by observations in the human basophil in the late 1970s. These studies suggested that a product of the lipoxygenase pathway was necessary for histamine exocytosis.\textsuperscript{86} These conclusions may be in error. More recently, studies in the HLMC show dose-dependent dissociation of LTC\textsubscript{4} and histamine release.\textsuperscript{66} There is currently an enormous worldwide effort underway to find effective inhibitors of the 5-lipoxygenase pathway and inhibitors of leukotriene receptors. These agents may represent the next class of agents to be clinically tested in asthma.

### Table 2—Pharmacologic Inhibitors of HLMC Histamine Release*

<table>
<thead>
<tr>
<th>IC\textsubscript{50} (Molar)</th>
<th>NA*</th>
<th>10\textsuperscript{-5}→10\textsuperscript{-7}M</th>
<th>10\textsuperscript{-7}→10\textsuperscript{-9}M</th>
<th>10\textsuperscript{-9}M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids</td>
<td>IBMX</td>
<td>PGE\textsubscript{2}</td>
<td>Amphotericin B</td>
<td>Fenoterol</td>
</tr>
<tr>
<td>Cromolyn sodium</td>
<td>Adenosine</td>
<td>Nondihydrogualic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimaprit (histamine H\textsubscript{4} agonist)</td>
<td>Dibutyryl cyclic AMP</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from refs 6, 14, 60, 61 and unpublished data.

†NA = IC\textsubscript{50} not achievable.
Table 3—Rodent Mast Cell Heterogeneity

<table>
<thead>
<tr>
<th></th>
<th>Mucosal (Bone Marrow-Derived) Mast Cell</th>
<th>Connective Tissue Mast Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>10 microns</td>
<td>20 microns</td>
</tr>
<tr>
<td>Density in gradient</td>
<td>less dense</td>
<td>more dense</td>
</tr>
<tr>
<td>Formalin Sensitivity</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Granules</td>
<td>few</td>
<td>many</td>
</tr>
<tr>
<td>Thymus-dependency</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Arachidonic acid product</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTB4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LTC4</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>PGD2</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Proteoglycan</td>
<td>Chondroitin Sulfate</td>
<td>Heparin Sulfate</td>
</tr>
<tr>
<td>Stimuli:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-IgE</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>48/80</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Pharmacologic Inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theophylline</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Cromolyn Sodium</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Doxantrazole</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Adapted from refs 67-72.

**Mast Cell Subclasses**

Though the discussion to this point has focused on “purified” HLMC, it is as yet unknown in human subjects if there are two or more distinct subpopulations. In 1966, Enerback67-68 first described in detail the two mast cell populations in the rodent. In the last few years, these observations were extended to important functional differences.70-72 The differences between rodent mucosal (bone marrow-derived) and connective tissue (peritoneal) mast cells are highlighted in Table 3. The important differences include those related to diameter, density, detection following formalin fixation, effective release triggers, pharmacologic responsiveness and arachidonic acid products generated. Studies of human mast cell heterogeneity are only beginning. Previous studies have demonstrated that HLMC separated on the basis of both diameter and density are functionally different.73-74 This difference extends not only to the content of mediators, but also to functional responsiveness to various release triggers. Most recently, we have demonstrated histologically both “connective tissue” and “mucosal” type mast cells in dispersed human lung parenchyma. Future studies will elucidate differences in the biology of these HLMC subpopulations.

In conclusion, mediators released by activation of the HLMC play a central role in respiratory tract hypersensitivity disorders. Mediators released from the HLMC are involved in neural stimulation, mucus gland and goblet cell hypersecretion, epithelial cell desquamation, vascular reactivity, chemoattractant functions and smooth muscle constriction. Perhaps the most important of these mediators are those products generated through arachidonic acid metabolic pathways. However, given the multitude of mediators released and their duplication in producing pathophysiology, new therapeutic agents aimed at inhibiting the synthesis or end organ effects of only one mediator may fail. Agents capable of globally inhibiting the release mechanism or stabilizing mast cells may be more likely to succeed therapeutically.

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