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Mast Cell Mediators in the Blood of Patients with Asthma*

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The identification of human mast cell-dependent disease is usually made on historic grounds together with appropriate physical signs and symptoms. In asthma, these findings include reversibility of signs and symptoms, the characteristic triggers of airways dysfunction, and the cardinal signs of airway obstruction, including wheeze and cough. These findings are supported by objective measurement of pulmonary function.

Accumulating evidence suggests that in addition to signs, symptoms, and pulmonary functional abnormalities which are reversed after β-adrenergic agonist inhalation, it may be possible to identify in the blood markers suggestive of mast cell-mediated bronchoconstriction. This review will focus upon those mediators identified in human diseases with

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special focus upon asthma and with additional reference to mass cell-dependent physical urticaria.

**Antigen-Provocation Bronchospasm**

It has long been known that asthma may be provoked by exposure of sensitive individuals to appropriate antigens. This knowledge has been employed using standardized methodology to identify patients with allergen-induced asthma. Exploration of this model system has also greatly expanded our understanding of the role of mast cells in asthma and of the natural history of allergen-exposure asthma.

The exposure of a sensitive individual to an aerosol antigen is followed by rapid, and dose-related, diminution in pulmonary function as assessed by a variety of pulmonary function measures. The duration of antigen-induced asthma is variable, but generally normal function is restored, without treatment, in a matter of 30 to 90 minutes. In many patients, a second (and also dose-related) fall in pulmonary function ensues. This later response is often of greater magnitude and almost always lasts longer than the initial response. The second phase generally begins two to four hours after initial antigen exposure but may be delayed for 12 to 18 hours, and the response may last for many hours. Since the late-phase response is blunted by prior treatment with glucocorticoids, and both early and late responses are abrogated by cromolyn, it has become possible to attribute the entire response pattern to initial antigen-mediated mast cell activation with a secondary mast cell-dependent inflammatory response.

In addition to permitting such speculative insights, antigen aerosol challenges have permitted the search for and identification of a variety of compounds capable of mediating spasmogenic and/or chemotactic reactions. These studies have been complemented by similar analyses of blood of patients after physically (nonantigen) mediated mast cell activation in patients with physical urticaria. These model systems have been particularly informative not only because they are accomplished in humans, but also because patients can act as their own controls, and time-dependent changes in mediator concentration can be used to identify appropriate compounds of interest.

**Histamine**

Because of its unequivocal localization to mast cells (as well as basophils), histamine has been used as the index molecule to define mast cell activation. Unfortunately, the assay systems available to measure histamine have, until quite recently, proved inadequate to the task of measuring small amounts of this amine in complex biologic mixtures. Using an enzymatic assay in which the amine was methylated with radioactive methyl groups by crude histamine N-methyl transferase and then separated from unalabeled histamine by organic extraction, Kaplan and co-workers successfully identified histamine in the blood of patients with cold urticaria after cold challenge. This finding has been repeatedly confirmed, utilizing ever more specific assays with more active methylation enzymes of greater purity and thin-layer chromatographic separation of methylated histamine (from nonhistamine methyl acceptors). Thus, from baseline levels of 0.3 ng/ml, rises in plasma histamine to 10 to 50 ng/ml have been unequivocally demonstrated. Similar studies in asthma have been more difficult because much less histamine is released into the circulation after aerosol antigen challenge than in physical urticaria (in which the draining venous effluent may be sampled directly). However, using the most sensitive assays available, twofold to fivefold increases in plasma histamine have been demonstrated to accompany bronchospasm in antigen-sensitive asthmatic patients exposed to aerosol antigens. Increases in histamine are specific in that antigens to which patients do not possess IgE antibodies are without effect, and bronchospasm itself, as engendered by methacholine, does not alter plasma histamine levels. Unfortunately, the levels of plasma histamine are so low, the assay conditions required to demonstrate them sufficiently rigorous, and the ease with which basophil histamine may spuriously affect plasma levels make it such that the routine assay of plasma histamine cannot be utilized as a measure of asthma severity or in the differential diagnosis of the etiology of asthma.

**Chemotactic Factors**

The long association of eosinophils with allergic disease and asthma, as well as the seeming inflammatory nature of late-phase asthma, made it logical to search for chemotactic factors in this disorder. Several groups have identified in the blood of patients with bronchospasm induced by antigen or exercise but not cold air or methacholine a high molecular weight, neutral to slightly acidic, protease-sensitive, factor which possesses chemotactic activity for human neutrophils. This factor is released into the circulation within minutes of antigen inhalation in sensitive individuals, and its presence may be appreciated for hours. In those patients experiencing a late-phase response, a second rise in its concentration occurs. A very similar material has been identified in patients with physical urticaria after challenge. In asthma, its release may be diminished by cromolyn sodium and glucocorticoids. Termed NCA or HMW-NCF, the presence of this factor is determined by a biologic assay of its ability to induce directed migration of human neutrophils in vitro. Until the cell of origin of this factor is identified and a nonbiologic assay for its quantitation developed, it will be difficult to use this activity as a marker in the management or assessment of asthma.

In physical urticaria and in asthma, several families of eosinophil chemotactic activities have been noted. One of these materials, identified in physical urticaria, shares a specificity for mononuclear leukocytes. The chemical characterization and nonbiologic assay of these materials has yet to be accomplished.

**Platelet-activating Factor**

The platelet α granule constituent platelet factor 4 (PF4) has been identified in the blood of antigen-challenged asthmatics patients and patients with physical urticaria. The PF4 release occurs rapidly after challenge, and levels return to baseline within 15 to 40 minutes. Recent studies in physical urticaria suggest that PF4 release may be a consequence of the generation of platelet-activating factor (PAF). A molecule known to be synthesized by mast cells (among others), assay of this mediator requires organic extraction of blood which has been acidified (to inhibit an enzyme known to degrade PAF). Further analysis requires thin-layer chro-
Table 1—Mast Cell Mediators Identified in Human Disease

<table>
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<tr>
<th>Mediator</th>
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<tbody>
<tr>
<td>Histamine</td>
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<tr>
<td>Neutrophil chemotactic factor</td>
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<tr>
<td>Eosinophil chemotactic factors</td>
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<tr>
<td>Monocyte chemotactic factor</td>
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<tr>
<td>Platelet-activating factor</td>
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<tr>
<td>Adenosine</td>
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matography along with authentic PAF standards, followed by biologic assay of the ability of the mediator to cause shape change and mediator release from human or rabbit platelets.

**ADENOSINE**

The nucleoside adenosine is known to augment mast cell mediator release by its action on a putative theophylline-inhibitable cell surface receptor linked to adenylate cyclase. Inhalation of adenosine by asthmatic patients, but not by normal individuals, is associated with bronchospasm. Recent studies indicate that activated mast cells generate and release large quantities of adenosine, which, in humans, may be identified in the blood. Due to its source in many cells, its rapid metabolism, and the requirement of extraction and high-pressure liquid chromatography for analysis, this molecule has yet to be extensively analyzed in the blood of asthma.

Other mast cell constituents, such as tryptase and heparin, or the newly generated mast cell metabolites of arachidonic acid, PGD₂, and sulfidopeptide leukotrienes, are likely present in asthma, but their identification in blood in this disease has not yet been reported.

**SUMMARY**

 Mast cell activation occurs in allergic asthma and may play a role in a variety of nonallergic asthmatic states. The defined mast cell constituent histamine has been identified in blood of antigen-sensitive challenged asthma patients, while other mediators, whose cell of origin is not fully defined, accompany this amine in blood (Table 1). Due to technical difficulty in accurate assessment, the rapid metabolism of various constituents, and the need for biologic rather than chemical assay of some mediators, it is not yet possible to assess blood constituents for the unequivocal attribution of asthma to activation of a particular cell type. Likewise, the usefulness of blood studies in the prediction of the course of asthma or as serial measurements to define the severity of asthma remains limited. However, it is only with analysis of the appropriate biologic fluids, blood and/or bronchoalveolar lavage materials, that it will be possible to define which potential mediators are, in fact, present and active in asthma. Until such analysis is completed, it is not possible to assign a function in this disease to the numerous potent inflammatory mediators known to be active in in vitro or in vivo models of asthma.

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