

# Mediators of inflammation and the inflammatory process

Martha White, MD Washington, D.C.

A complex interplay of inflammatory cells and chemical mediators is responsible for allergic inflammation. It is now understood that the allergic reaction consists of an early-phase response involving mast cell degranulation with the release of histamine and a late-phase response characterized by the migration of inflammatory cells. This review provides a summary of the early- and late-phase events associated with allergic inflammation and an overview of the principal chemical mediators involved in the inflammatory process. (*J Allergy Clin Immunol* 1999;103:S378-81)

The discovery of the role of histamine in immediate hypersensitivity reactions and the identification of subclasses of histamine receptors ( $H_1$  and  $H_2$ )<sup>1</sup> led to the development of selective  $H_1$ -receptor antagonists for managing the histamine-related symptoms of allergic rhinitis. As research progressed, it became clear that the pathophysiology of allergic rhinitis was the result not only of histamine being released during the allergic reaction but also of several other chemical mediators. Included among these mediators are arachidonic acid derivatives (leukotrienes and prostaglandins), vasoactive peptides (kinins), phospholipid mediators (platelet activating factor), and cytokines (interleukins and other bioreponse modifiers).

## OVERVIEW OF ALLERGIC INFLAMMATION

Allergic inflammation involves a complex interaction of many different inflammatory cells that release a spectrum of chemical mediators ultimately affecting various target tissues. Although the clinical manifestations of the allergic response vary depending on the tissue and antigen involved, it is now understood that the allergic reaction consists of an early-phase response primarily involving mast cell degranulation accompanied by the release of histamine and other mediators including cytokines and a late-phase response that is characterized by the migration of inflammatory cells from the circulation. Eosinophil infiltration is a hallmark feature of allergic rhinitis and distinguishes allergic inflammation from other inflammatory conditions of the upper respiratory tract.

The sensitization phase of the allergic response begins when antigen-presenting cells (Langerhans' cells) display allergen fragments to T lymphocytes. In a process that involves the secretion of IL-4 by T lymphocytes, B

### Abbreviations used

LT: Leukotriene

PAF: Platelet activating factor

lymphocytes are activated and mature into plasma cells that produce allergen-specific IgE antibodies. The IgE antibodies then bind to receptors on tissue mast cells and circulating basophils. On subsequent allergen exposure allergen molecules bind to IgE antibodies, which results in cross-linking of IgE molecules and activates enzyme cascades in the cell membrane involving tyrosine kinase, protein kinase C, phospholipase C, phospholipase A<sub>2</sub>, and an influx of calcium ions that results in the release of preformed (histamine) and newly synthesized chemical mediators (leukotrienes, prostaglandins) in the early-phase response.

Mast cells also secrete interleukins and other cytokines that regulate the duration and intensity of the immune response by promoting the expression of adhesion molecules and by recruiting inflammatory cells, particularly eosinophils, to the site of the allergic reaction. These inflammatory cells release additional chemical mediators in the late-phase response, leading to chronic inflammation, priming of nasal tissues, and tissue damage.

## MEDIATORS OF ALLERGIC INFLAMMATION

### Histamine

Histamine ( $\beta$ -Imidazolylethylamine) is a vasodilator, a constrictor of smooth muscle, and a potent stimulant of vascular permeability, respiratory mucus, and gastric acid secretion. It exerts its effects on a variety of cell types including smooth muscle cells, neurons, glandular cells (endocrine and exocrine), blood cells, and cells of the immune system.<sup>2</sup> In addition to its role in immediate hypersensitivity reactions, histamine can exert  $H_2$ -receptor-mediated anti-inflammatory activity including inhibition of human neutrophil lysosomal enzyme release, inhibition of IgE-mediated histamine release from peripheral leukocytes, and activation of suppressor T-lymphocytes.<sup>3</sup>

Histamine is synthesized in the Golgi apparatus of mast cells and basophils by decarboxylation of the amino acid histidine and is then stored in secretory granules in complexes with heparin, protein, or both.<sup>4</sup> In sensitized individuals histamine release occurs with the binding of specific allergens to IgE antibodies on mast cells and basophils, initiating a  $Ca^{++}$ -dependent degranulation reaction.<sup>5</sup>

From the Institute of Allergy and Asthma.

Reprint requests: Martha White, MD, Institute for Asthma and Allergy at Washington Hospital Center, 106 Irving St., NW., Suite 108, Washington, DC 20010.

Copyright © 1999 by Mosby, Inc.

0091-6749/99/\$8.00 + 0 1/0/96500

After continuity is established between the secretory granule and the plasma membrane of the mast cell, histamine dissociates from the partially solubilized granular matrix by exchanging with sodium ions in the extracellular environment and is extruded into tissue spaces.<sup>6</sup> Recent studies provide evidence for an alternate secretory process in humans, referred to as piecemeal degranulation, in which histamine is released from mast cells with retention of empty granule containers within the cytoplasm.<sup>7</sup> The release of histamine from mast cells and basophils can occur by immunologic (IgE-mediated) and nonimmunologic (exercise-induced asthma) mechanisms, thereby contributing to the symptoms of both allergy and anaphylaxis.<sup>8</sup> Research also suggests that activated T lymphocytes may participate in the process of mast cell degranulation by direct cell-to-cell contact.<sup>9</sup>

### Leukotrienes

Leukotriene (LT) C<sub>4</sub> and its products, LTD<sub>4</sub> and LTE<sub>4</sub>, make up the biologic mixture previously known as the slow-reacting substance of anaphylaxis. Leukotrienes are generated by most cell types that participate in inflammatory reactions including mast cells, basophils, eosinophils, neutrophils, and monocytes.<sup>10</sup> As chemical mediators of inflammation, they have biologic activity similar to that of histamine. Studies of the effects of H<sub>1</sub>-receptor antagonists on leukotriene release suggest that the mechanism may involve blocking the activity of receptor-coupled G proteins.<sup>11</sup>

Leukotrienes are derived from arachidonic acid, which is made available from cell membrane phospholipids by the action of phospholipase A<sub>2</sub><sup>12</sup> or by the sequential action of phospholipase C and diacylglycerol lipase.<sup>13</sup> After being released from cell membranes by the action of phospholipase A<sub>2</sub>, arachidonic acid is converted by 5-lipoxygenase to 5S-hydroperoxy-6,8-*trans*-11,14-*cis*-eicosatetraenoic acid.<sup>14</sup> The same 5-lipoxygenase pathway catalyzes the conversion of 5S-hydroperoxy-6,8-*trans*-11,14-*cis*-eicosatetraenoic acid to LT<sub>4</sub> (LTA<sub>4</sub>).<sup>15</sup> LTA<sub>4</sub> can then be converted to LTB<sub>4</sub><sup>16,17</sup> or conjugated with reduced glutathione to form LTC<sub>4</sub>.<sup>18</sup> In the later phases of the allergic reaction, after transport into the extracellular environment, LTC<sub>4</sub> can undergo further metabolism to LTD<sub>4</sub> and LTE<sub>4</sub>.<sup>10</sup>

### Prostaglandins

Another group of arachidonic acid-derived molecules that mediate allergic reactions are prostaglandins. The most abundant cyclooxygenase product generated by the immunologic activation of human lung mast cells is PGD<sub>2</sub>.<sup>19</sup> PGD<sub>2</sub> is generated by human mast cells after they are activated through the IgE receptor or by calcium ionophore.<sup>20</sup> The biologic effects of prostaglandins generated during mast cell-dependent reactions in tissues include modulation of smooth muscle contractility, vascular permeability, sensations of pruritus and pain, and platelet aggregation and degranulation.<sup>21</sup>

Prostaglandins are generated by cyclooxygenase, an enzyme associated with the endoplasmic reticulum of

mast cells. Similar to 5-lipoxygenase, cyclooxygenase catalyzes the formation of relatively unstable intermediates PG<sub>2</sub> and PGH<sub>2</sub>.<sup>22</sup> These intermediates may then be converted nonenzymatically or enzymatically by specific isomerase/peroxidase or synthase enzymes to yield the primary prostaglandins PGD<sub>2</sub>, PGE<sub>2</sub>, and PGF $\alpha$ .<sup>21</sup>

### Kinins

Kinins are potent peptide hormones formed *de novo* in body fluids and tissues during inflammation. They are derived from  $\alpha$ 2 globulins (high and low molecular weight kininogens) through proteolytic cleavage by a variety of enzymes, the most important of which are plasma and tissue kallikreins.<sup>23</sup> Three distinct kinins have been identified in humans: bradykinin, kallidin (lys-bradykinin), and met-lys-bradykinin.<sup>24</sup> Although some quantitative differences exist, all of the kinins possess the same basic pharmacologic properties including the induction of smooth muscle contractility, vasodilation, and increased capillary blood flow.<sup>25</sup>

The generation of kinins in the inflammatory response occurs through a plasma pathway, a tissue pathway, and a plasma/tissue-independent pathway. Kinin production by the plasma pathway is initiated by the interaction of activated factor XII (Hageman factor) with prekallikrein<sup>26</sup> and high molecular weight kininogen.<sup>27</sup> Activated Hageman factor (factor XIIa) initiates the conversion of prekallikrein to kallikrein, which furthers the conversion of factor XII to XIIa. The action of kallikrein to further conversion of factor XII to XIIa is augmented by high molecular weight kininogen. The active kallikrein released by this sequence of events cleaves high molecular weight kininogen to release bradykinin.

The tissue pathway for bradykinin synthesis involves tissue kallikreins that are physiochemically and immunologically distinct from plasma kallikrein.<sup>28</sup> Tissue kallikreins release kinins from both high molecular weight kininogens and low molecular weight kininogens, with low molecular weight kininogen making up approximately 70% of the total human kininogen pool.<sup>23</sup> The ability of tissue kallikreins to react equally well with high molecular weight kininogen and low molecular weight kininogen provides a larger pool of available substrate than is available to plasma kallikrein. Once activated, tissue kallikreins are less susceptible to inhibition by protease inhibitors than plasma kallikrein, and they are the only known enzymes to generate the bradykinin precursor kallidin. Kallidin can then be converted to bradykinin by the enzymatic removal of the N-terminal lysine. The independent pathway involves the action of kininogenases other than kallikreins in the cleavage of high and low molecular weight kininogen to form bradykinin.

Although the relevance of kinins as mediators of allergic airway disease is not clearly established, observations that human mast cells<sup>29</sup> and basophils<sup>30</sup> possess IgE-mediated kininogenase activity, that bradykinin receptors are present in nasal mucosa,<sup>31</sup> and that elevated levels of kinins are present in nasal lavages of patients with allergic rhinitis<sup>32</sup> suggest a role for these peptides in allergic inflammation.

## Platelet activating factor

Platelet activating factor (PAF) is an ether-linked phospholipid, designated as such because of its discovery as a basophil-derived mediator of rabbit platelet activation.<sup>33</sup> PAF may be produced by several of the cells that participate in the inflammatory response including mast cells, macrophages, neutrophils, and eosinophils.<sup>34</sup> The synthesis of PAF occurs as a 2-step pathway in which phospholipase A2 hydrolyzes a 1-O-alkyl-2-acyl-glycerol-3-phosphorylcholine to produce 1-O-alkyl-2-acyl-glycerol-3-phosphocholine (lyso-PAF), which is then converted by an acetyltransferase enzyme to PAF.

The biologic activities of PAF include platelet activation, activation of neutrophils, and smooth muscle contraction.<sup>21</sup> Because PAF is rapidly inactivated *in vivo*, it is likely that it triggers a chain of inflammatory events. PAF stimulates the accumulation of eosinophils to endothelial surfaces, which may be the first step in the recruitment of eosinophils into tissues. Eosinophils also are a source of PAF and can attract additional eosinophils, which can potentiate the inflammatory reaction. PAF stimulates eosinophils to release basic proteins, leading to epithelial cell damage, and causes an increased expression of low-affinity IgE receptors on eosinophils and monocytes.<sup>34</sup>

## Cytokines and chemokines

An increasing body of evidence suggests that cytokines play a critical role in late-phase inflammatory events associated with allergic rhinitis and asthma, and that the mast cell is a source of many multifunctional cytokines.<sup>35</sup> Cytokines such as IL-1, -2, -4, -5, -6, TNF- $\alpha$ , and GM-CSF influence the course of allergic inflammation by modulating the activity of inflammatory cells including macrophages, T cells, B cells, and eosinophils. IL-1 enhances the growth of T-helper cells and the growth and proliferation of B cells, whereas IL-2 causes proliferation of T lymphocytes and activation of B lymphocytes. IL-4 causes differentiation of B lymphocytes into IgE-secreting plasma cells and, along with TNF- $\alpha$ , upregulates both high- and low-affinity IgE receptor expression on antigen presenting cells. IL-5 causes activation of B lymphocytes and the differentiation and increased longevity of eosinophils, and IL-6 causes increased synthesis and secretion of immunoglobulins by B lymphocytes. In clinical studies in patients with seasonal allergic rhinitis, nasal biopsy specimens showed immunoreactivity for IL-4 and IL-6 localized predominantly to mast cells, whereas IL-5 was found in both mast cells and eosinophils.<sup>36</sup> Similar findings of increased levels of cytokines (IL-4 and IL-10) in nasal fluids were reported in a study of children with seasonal allergic rhinitis.<sup>37</sup>

Chemokines such as IL-8, macrophage inflammatory protein-1 $\alpha$ , macrophage chemoattractant protein-1, -2, and -3, and RANTES are cytokines that function primarily as chemoattractant molecules for macrophages and circulating leukocytes<sup>38</sup> but also induce other inflammatory effects including histamine release from basophils

and mast cells and activation of eosinophils.<sup>39,40</sup> Increased levels of chemokines IL-8, macrophage inflammatory protein-1 $\alpha$ , and RANTES, which correlated with total symptom scores, together with cytokines TNF- $\alpha$  and GM-CSF, have been detected in nasal secretions after allergen challenge in patients with allergic rhinitis.<sup>41</sup>

The inflammatory changes that occur during the allergic response produce priming of the nasal tissues. The resultant nasal hyper-responsiveness leads to escalation of both the symptomatic and inflammatory response to additional allergic or irritant stimulation to the nasal mucosa.

## CONCLUSIONS

A complex interplay of inflammatory cells and chemical mediators is responsible for allergic inflammation. The understanding of how the mast cell-mediated events of the early-phase response lead to the late-phase response and chronic inflammation indicates that effective management of allergic diseases must address the inflammatory component. However, the numbers of mediators, their multiple effects and sites of action, and the interplay among the mediators and effector cells in the inflammatory process suggest that no single mediator can be targeted. The most effective therapies will combine the receptor antagonist approaches of the antihistamines with the anti-inflammatory properties of corticosteroids. Recently, specific leukotriene antagonists have been developed for use in chronic bronchial asthma,<sup>42</sup> and further studies into the role of cytokines and chemokines in the inflammatory process may lead to new treatment approaches for respiratory disease.

## REFERENCES

1. Ash ASF, Schild HO. Receptors mediating some actions of histamine. *Br J Pharmacol* 1966;27:427-37.
2. Pearce FL. Biological effects of histamine: an overview. *Agents Actions* 1991;33:4-7.
3. Metcalfe DD, Kaliner M, Donlon MA. The mast cell. *CRC Crit Rev Immunol* 1981;3:23-74.
4. Uvnas B, Aborg CH, Bergendoff A. Storage of mast cells. Evidence for an ionic binding of histamine to protein carboxyls in the granule heparin complex. *Acta Physiol Scand* 1970;336(Suppl):3-26.
5. Carswell H, Daum PR, Young JM. In: Ganellin CR, Schwartz JC, editors. *Frontiers in histamine research. Histamine H<sub>1</sub>-agonist stimulated breakdown on inositol phospholipids*. Oxford: Pergamon; 1985. p. 27-38.
6. Uvnas B. Release process in mast cells and their activation by injury. *Ann NY Acad Sci* 1964;116:880-90.
7. Dvorak AM. New aspects of mast cell biology. *Int Arch Allergy Immunol* 1997;114:1-9.
8. Pearce FL. The mast cell. *Immunol Allergy Clin North Am* 1990;10:251-62.
9. Bhattacharyya SP, Drucker I, Reshef T, Kirshenbaum AS, Metcalfe DD, Mekori YA. Activated T lymphocytes induce degranulation and cytokine production by human mast cells following cell-to-cell contact. *J Leukoc Biol* 1998;63:337-41.
10. Naclerio RM, Baroody FM, Togias AG. The role of leukotrienes in allergic rhinitis: a review. *Am Rev Respir Dis* 1991;143:S91-5.
11. Rihoux JP, Masliah J, Bereziat G, Konig W. G proteins as biological targets for anti-allergic drugs? *Int Arch Allergy Immunol* 1997;113:339-41.
12. Ho PC, Orange RP. Indirect evidence of phospholipase A activation in purified rat mast cells during reverse anaphylactic challenge. *FASEB Proc* 1978;37:1667.
13. Kennerly DA, Sullivan TJ, Sylvester P, Parker CW. Diacylglycerol

- metabolism in mast cells. A potential role in membrane fusion and arachidonic acid release. *J Exp Med* 1979;150:1039-44.
14. Soberman RJ, Harper TW, Betteridge D, Lewis RA, Austen KF. Characterization and separation of the arachidonic acid 5-lipoxygenase and linoleic acid  $\Omega$ -6 lipoxygenase (arachidonic acid 15-lipoxygenase) of human polymorphonuclear leukocytes. *J Biol Chem* 1985;260:4508-15.
15. Rouzer CA, Matsumoto T, Samuelsson B. Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A<sub>4</sub> synthase activities. *Proc Natl Acad Sci USA* 1986;83:857-61.
16. Borgeat P, Samuelsson B. Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. *J Biol Chem* 1979;254:2643-6.
17. Corey EJ, Marfat A, Goto G, Brion F, Leukotriene B. Total synthesis and assignment of stereochemistry. *J Am Chem Soc* 1980b;102:7984-5.
18. Corey EJ, Clark DA, Goto G, Marfat A, Mioskowski C, Samuelsson B, et al. Stereospecific total synthesis of a "slow reacting substance" of anaphylaxis, leukotriene C-1. *J Am Chem Soc* 1980a;102:1436-9.
19. Lewis RA, Holgate ST, Roberts LJ II, Oates JA, Austen KF. Preferential generation of prostaglandin D<sub>2</sub> by rat and human mast cells. In: Becker EL, Simon AS, Austen KF, editors. *Biochemistry of the acute allergic reactions*. New York: Liss; 1981. p. 239-54.
20. Schwartz L, Huff T. Biology of mast cells and basophils. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, editors. *Allergy: principles and practice*. 4th ed. St Louis(Mo): Mosby; 1993. p. 150.
21. Schwartz LB, Austen KF. Structure and function of the chemical mediators of mast cells. *Prog Allergy* 1984;34:271-321.
22. Hamberg M, Svensson J, Wakabayashi T, Samuelsson B. Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proc Natl Acad Sci USA* 1974;71:345-9.
23. Proud D, Kaplan AP. Kinin formation: mechanisms and role in inflammatory disorders. *Ann Rev Immunol* 1988;6:49-83.
24. Bhoola KD, Collier HOJ, Schachter M, Shorley PG. Actions of some peptides on bronchial muscle. *Br J Pharmacol* 1962;19:190-7.
25. Keele CA. Clinical and pathological aspects of kinins in man. *Proc R Soc Biol* 1969;173:361-9.
26. Weiss AS, Gallin JI, Kaplan AP, Fletcher factor deficiency. A diminished rate of Hageman factor activation caused by absence of prekallikrein with abnormalities of coagulation, fibrinolysis, chemotactic activity, and kinin generation. *J Clin Invest* 1974;53:622-33.
27. Colman RW, Bagdasarian A, Talamo RC, Scott CF, Seavey M, Guimaraes JA, et al. Williams Trait. Human kininogen deficiency with diminished levels of plasminogen proactivator and prekallikrein associated with abnormalities of the Hageman factor-dependent pathways. *J Clin Invest* 1975;56:1650-62.
28. Nustad K, Orstavik TB, Gautvik KM, Pierce JV. Glandular kallikreins. *Gen Pharmacol* 1978;9:1-9.
29. Proud D, Macglashan DW Jr, Newball HH, Schulman ES, Lichtenstein LM. Immunoglobulin E-mediated release of a kininogenase from purified human lung mast cells. *Am Rev Respir Dis* 1985;132:405-8.
30. Newball HH, Berninger RW, Talamo RC, Lichtenstein LM. Anaphylactic release of a basophil kallikrein-like activity: purification and characterization. *J Clin Invest* 1979;64:457-65.
31. Baraniuk JN, Lundgren JD, Mizoguchi M, Peden D, Gawin A, Merida M, et al. Bradykinin and respiratory mucous membranes. Analysis of bradykinin binding site distribution and secretory responses in vitro and in vivo. *Am Rev Respir Dis* 1990;141:706-14.
32. Shin MH, Baroody F, Proud D, Kagey-Sobotka A, Lichtenstein LM, Naclerio RM. The effect of azelastine on the early allergic response. *Clin Exp Allergy* 1992;22:289-95.
33. Barbaro JF, Zvaifler NJ. Antigen induced histamine release from platelets of rabbits producing homologous PCA antibody. *Proc Soc Exp Biol Med* 1966;122:1245-7.
34. Barnes PJ. Pathophysiology of allergic inflammation. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, editors. *Allergy: principles and practice*. 4th ed. St Louis(Mo): Mosby; 1993. p. 255-6.
35. Bradding P, Holgate ST. The mast cell as a source of cytokines in asthma. *Ann NY Acad Sci* 1996;796:272-81.
36. Bradding P, Feather IH, Wilson S, Holgate ST, Howarth PH. Cytokine immunoreactivity in seasonal rhinitis: regulation by a topical corticosteroid. *Am J Respir Crit Care Med* 1995;151:1900-6.
37. Benson M, Strannegard IL, Wennergren G, Strannegard O. Cytokines in nasal fluids from school children with seasonal allergic rhinitis. *Pediatr Allergy Immunol* 1997;8:143-9.
38. Luster AD. Chemokines: chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436-45.
39. Alam R, Lett-Brown MA, Forsythe PA, Anderson-Walters DJ, Kenamore C, Kormos C, et al. Monocyte chemotactic and activating factor (MCAF) is a potent histamine-releasing factor for basophils. *J Clin Invest* 1992;89:723-8.
40. Dahinden CA, Geiser T, Brunner T, von Tscharner V, Caput D, Ferrara P, et al. Monocyte chemotactic protein 3 is a most effective basophil- and eosinophil-activating chemokine. *J Exp Med* 1994;79:751-6.
41. Sim TC, Reece LM, Hilsmeier KA, Grant JA, Alam R. Secretion of chemokines and other cytokines in allergen-induced nasal responses: inhibition by topical steroid treatment. *Am J Respir Crit Care Med* 1995;152:927-33.
42. Fish JE, Kemp JP, Lockey RF, Glass M, Hanby LA, Bonucelli CM. Zafirlukast for symptomatic mild-to-moderate asthma: a 13-week multicenter study. *Clin Ther* 1997;19:675-90.