

16 The Molecular Pathogenesis of Bullous Pemphigoid

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The detection of immunopathologic abnormalities¹ prompted early studies that suggest that bullous pemphigoid is mediated by antibody-dependent complement activation following initiation by an epidermal basement membrane zone antigen.² These concepts will not be further considered in this chapter but are reviewed elsewhere (chapter 12) in this volume.

Study of the molecular pathogenesis of bullous pemphigoid has been aided by the ease of recognition of each clinical stage in lesional development and by easy access to a complex biologic fluid formed during the inflammatory process.³ Specimens of lesions at each stage of development from patients were examined for histologic changes by the 1- μ m-thick section technique⁴ and for immunopathologic abnormalities by direct immunofluorescence. In a single patient, lesions at each stage of development have been examined with the electron microscope for ultrastructural alterations.⁵ These studies permitted appreciation of the sequence of pathologic events that must be considered in understanding the molecular pathogenesis of bullous pemphigoid.

This chapter considers the pathologic events that characterize the tissue damage observed in bullous pemphigoid. In addition, the potential molecular contribution of cells that participate in the mediation of the inflammatory events characteristic of this unique bullous disease are examined.

Pathologic Correlates of the Morphologic Development of Clinical Lesions³

The Early Events

The earliest appreciable alterations occurring during the development of bullous pemphigoid lesions were detected in clinically normal skin adjacent to erythematous macules and included deposition of immunoreactants, subtle mast cell changes, a scant perivascular infiltration of lymphocytes, and dermal venular abnormalities. The third complement component was detected at the

dermal-epidermal junction in all patients, and immunoglobulin was detected in the two patients with circulating anti-basement membrane zone antibody. In three patients, mast cells were identified high in the papillary dermis, some adjacent to the dermal-epidermal junction. Endothelial cell hypertrophy was observed in dermal venules.

The Assembly of the Cellular Infiltrate

The progression of clinical lesions to erythematous macules and then erythematous plaques was characterized by the orderly assembly of an inflammatory infiltrate without alteration in the appearance or location of immunoreactants. In specimens of erythematous macules from all five patients, mast cells exhibiting apparent hypogranulation were observed throughout the papillary dermis, in the upper reticular dermis, and disposed about superficial dermal venules. Marked endothelial cell hypertrophy, focal endothelial cell damage, and focal obliteration of lumens were noted in venules. A perivenular infiltrate was evident, composed primarily of monocyte-macrophages and lymphocytes. Few eosinophils, basophils, or neutrophils were observed.

Further evolution of lesions into the erythematous plaque stage was characterized by more extensive mast cell and venular alterations and a change in composition of the dermal perivenular infiltrate. In each patient, apparently hypogranulated mast cells were noted high in the papillary dermis, and marked damage to endothelial cells, evidenced by vacuolar changes, and obliteration of venular lumens were evident. The perivenular and intervenular infiltrate now contained many eosinophils and a few basophils in addition to the lymphocytes observed before. Numerous intact and degenerating eosinophils were noted, and large numbers of eosinophil granules, free in the dermis, were present. Basophils, present in small quantity, were variably hypogranulated.

Formation of the Blister

Separation at the dermal-epidermal junc-

tion was associated with necrosis of basal cells at the edge and roof of the bulla; the bullous cavity contained fibrin, lymphocytes, eosinophils, basophils, and monocyte-macrophages. Many young mast cell forms were present; some showed the configuration of motility. The perivenular infiltrate contained lymphocytes, and eosinophils were distributed about and between venules. Venular alterations were most marked. Severe endothelial cell damage was evidenced by sloughing, with accumulation of debris in lumens.

Ultrastructural Correlates of the Development of Clinical Lesions

Ultrastructural examination of lesions at each stage of development confirmed and expanded the appreciation of morphologic alterations detected by refined light microscopy.⁵ The ultrastructural detection of mast cells with pseudopod-like projections, a finding typical of motile cells, lends support to the redistribution of mast cells noted during lesion development. The apparent mast cell hypogranulation noted by light microscopy was reflected ultrastructurally by focal loss of electron-dense material in intact granules. These studies demonstrated that the eosinophil granules free in the dermis were the result of cell death rather than secretion. The free granule was intact and membrane-coated; disrupted cells with pyknotic nuclei were observed, while degranulating cells were never seen. The basophils, when noted, exhibited piecemeal degranulation and were associated with a fibrin gel. The progressive destruction of endothelial cells was confirmed, and basement membrane zone reduplication was clearly demonstrated.

Potential Functional Correlates of Light Microscopic and Ultrastructural Abnormalities

Morphologic information suggest that mast cell and eosinophil participation are critical to the pathogenesis of lesion formation in bul-

lous pemphigoid. In addition, the data are consistent with the concept that blister formation may result from the concerted action of these two cell types. To understand the contribution of mast cells and eosinophils, the potential effector function of each cell type will be discussed in depth.

The Mast Cell

Characterization of Mast Cells

First identified over a century ago, mast cells constitute one of the two classes of IgE-bearing mediator-generating cell types. The basophilic polymorphonuclear leukocytes (basophils) constitute the other.

In the past few years, it has become apparent that mast cells are a heterogeneous cell type. Two basic types of mast cells, the connective tissue and the mucosal, have been recognized.⁶ These mast cell subtypes are most clearly recognized in the rodent but are represented in humans as well. One, the mast cell termed connective tissue type or typical, is prominent in loose connective tissue, on serosal surfaces, and in skin. The other, the mucosal mast cell (also termed atypical), is richly represented in gastrointestinal mucosa and perhaps in other mucosal and epithelial locations as well. The connective tissue mast cell is large, contains numerous small (0.1–0.4 μ) granules that stain intensely with metachromatic dyes due to their content of heparin,⁷ stain less intensely with metachromatic dyes. Precursors for this mast cell type arise in the bone marrow and then populate various target tissues in which they then differentiate into mast cells under the influence of the T-lymphocyte-derived lymphokine interleukin 3.⁸

Although typical connective tissue mast cells and atypical mucosal mast cells can be differentiated by microscopic and biochemi-

cal techniques, their relationship is unknown. It is possible that these are merely different forms of a single cell type expressed at different times in cell maturation. However, current opinion favors the concept that these cells differ either in their progenitor cell or in the specific growth and differentiation factors that lead to their final expression.

Mast cells occur throughout the body, and they are especially prominent in the skin,⁹ upper and lower respiratory tract,¹⁰ and gastrointestinal tract mucosa.¹¹ In the skin, the lungs, and the gastrointestinal tract, mast cell concentrations approximate 10,000–20,000 cells/mm³.^{1,10,11} They also exist free in the bronchial lumen and in loose connective tissue around small blood vessels and nerves. Mast cell numbers are increased in bone marrow in association with malignant disorders,¹² in the gut in inflammatory and parasitic diseases, and in the lung in asthma and fibrotic diseases.

Activation of Mast Cells

Numerous agents have been demonstrated to induce mast cells to generate and secrete mediators.¹³ This process is noncytolytic and, in the case of IgE mediated signals, is due to cross-linking of cell surface bound IgE molecules. In addition to antigens recognized by cell-bound IgE, activators of mast cells include highly charged molecules, peptides such as opiates, neurotensin, and substance P, ATP, divalent cation ionophores, anaphylatoxins C3a and C5a, and many drugs and complex carbohydrates. The mechanisms by which these processes induce activation are complex and include alterations in intracellular calcium concentrations, changes in transmembrane calcium flux, metabolism of phospholipids to generate inositol polyphosphates and diacylglycerol, increases in cAMP, utilization of several protein kinases, phosphorylation of cellular proteins, solubilization and swelling of granules, fusion of granules with cell membranes, and eventual liberation of granule contents. These processes occur essentially simultaneously and their inter-relationships, sequence, and, in

fact, true relevance to mediator generation and secretion remain to be fully elucidated.

Whatever the precise biochemical processes might be, their successful completion is accompanied by release of the constituent amines, peptides, enzymes and proteoglycans of the granules and by the generation of purine nucleosides, prostanoids and complex lipids.

Bioactive chemicals generated and released by mast cells have been termed mediators. A variety of biologic activities are available, and mediators may be classified as vasoactive, chemotactic, enzymatic, and structural. Several of these may account for pathologic events that characterize the development of bullous pemphigoid lesions.

Histamine. Histamine is the only preformed, granule-associated mediator in this class. It is formed by the decarboxylation of histidine and is stored bound to the protein-proteoglycan backbone of mast cell and basophil granules (5 and 1 $\mu\text{g}/10^6$ cells, respectively). Histamine is released when granules are exposed to cations and circulates in blood at concentrations of 100–300 pg/ml.¹⁴ The biologic activities of histamine are a consequence of its interaction with H_1 and H_2 receptors.¹⁵ H_1 receptors predominate in the skin and smooth muscle and are inhibited by classic antihistamines. H_2 receptors are found in the skin, the lungs, the stomach, and a variety of leukocytes, and are selectively inhibited by cimetidine and ranitidine. The biologic response to histamine reflects the ratio of these receptors activated by histamine in a given tissue. H_1 -mediated effects are contraction of respiratory and intestinal musculature, enhancement of vascular permeability, pulmonary vasoconstriction, and nasal mucus production. Conversely, relaxation of respiratory smooth muscle, increased airway mucus production, suppression of lymphocytotoxicity, and increased suppressor T-cell function are mediated by histamine action on H_2 receptors. Together, H_1 and H_2 receptor activation are responsible for cutaneous vasodilation, cardiac irregularities, and pruritus.¹⁶

Platelet-activating Factor. Platelet-acti-

vating factor (PAF) is an unstored lipid whose structure is 1-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine.¹⁷ It is generated by human mast cells after IgE-dependent activation.

Initially, PAF was identified as a molecule capable of aggregating human platelets.¹⁸ PAF also causes a wheal-and-flare permeability response,¹⁹ contracts pulmonary and intestinal musculature, and induces vasoconstriction. It is a hypotensive agent capable of causing cardiovascular collapse and death, and may alter coronary blood flow and lead to cardiac dysrhythmia.²⁰ PAF also can cause pulmonary artery hypertension, pulmonary edema, rapid shallow breathing, an increase in total pulmonary resistance, and a decrease in dynamic compliance.

Eicosanoids. Several mediators in this class are products of arachidonic acid metabolism. Arachidonic acid is a 20-carbon fatty acid present in all mammalian cells as one of the fatty acid constituents of membrane phospholipids. The metabolic fate of arachidonic acid is quite varied. At least 20 potential end-products can be generated from this substrate, but no one cell type generates them all. Two enzymes, lipoxygenase and cyclooxygenase, regulate the pattern of metabolites generated from arachidonic acid. Mast cells generate a restricted pattern of arachidonic acid metabolites representing products of each of these enzymes.

Human mast cells preferentially generate prostaglandin D_2 along with lesser amounts of thromboxane²¹ as products of cyclooxygenase action. PGD₂ is a potent inducer of wheal-and-flare responses that are more persistent than those caused by histamine and that may be accompanied by perivascular neutrophil accumulation.²² This prostaglandin is felt to be responsible for attacks of flushing and hypotension seen in some patients with mastocytosis.

The substitution of hydroperoxy groups to arachidonic acid is catalyzed by a family of enzymes termed lipoxygenases. Each lipoxygenase adds the hydroperoxy group to a specific position on the arachidonic acid molecule, and, in the mast cell, it is the 5-lipoxygenase

enzyme that is most active. The action of this enzyme produces an unstable intermediate 5-HPETE (hydroperoxyeicosatetraenoic acid), which can be further metabolized to 5-HETE or, as in the mast cell, to leukotriene A. This latter product is then further metabolized by the addition of the tripeptide glutathione (cys-gly-glu) to generate the sulfidopeptide leukotriene C₄.²³ Removal of the terminal glutamic acid generates LTD₄ and removal of the glycine residue from LTD₄ produces LTE₄.²⁴ These three products (LTC₄, D₄, and E₄) comprise what was once termed slow-reacting substance of anaphylaxis (SRS-A).

IgE-mediated activation of human mast cells leads to generation of substantial quantities of LTC₄.²⁵ The sulfidopeptide leukotrienes are potent inducers of wheal-and-flare responses accompanied by local discomfort.²⁶ They also induce constriction of gastrointestinal and respiratory smooth muscle, induce hypotension directly and via cardiodepression, and may inhibit lymphocyte function.

Neutrophil-Directed Activities. Several molecules generated during mast cell activation are capable of altering neutrophil migration. Some, including PGD₂, leukotriene B₄, several monohydroxy fatty acids, and platelet-activating factor are chemotactic or chemokinetic but act upon a variety of migratory cell types.

A high-molecular weight (660-KD), neutral isoelectric point protein (HMW-NCF) has been identified in blood of patients with antigen- or exercise-induced bronchospasm²⁷ or experimentally induced physical urticaria.²⁴ Other specific neutrophil-directed chemotactic activities, termed inflammatory factors of anaphylaxis, have been partially isolated from the granules of rodent mast cells.²⁸

Eosinophil-Directed Activities. Factors capable of attracting the directed migration of eosinophils have been identified in human lung mast cells¹⁰ and isolated from the blood of patients experiencing antigen-induced bronchospasm and experimentally induced physical urticaria²⁹ and from bullous fluid of patients with bullous pemphigoid.³ At least

three such factors exist. Other factors including LTB₄, monohydroxy fatty acids, PGD₂, and histamine can also modulate eosinophil responsiveness but are not selectively active on this cell type.

Other Chemotactic Activities. Factors capable of attracting lymphocytes, mononuclear leukocytes, and basophils have been found to be released after IgE-mediated activation of mast cells in various model systems. The presence of these activities in humans remains to be investigated.

Several enzymes have been identified in isolated human or rodent mast cells by their extraction from these cell types or by their identification in supernatant fluids after IgE-mediated cell activation. These have not been studied extensively in human disease; however, they are of potential importance in the mediation of tissue damage that characterizes bullous pemphigoid.

Neutral Proteases. The most abundant human mast cell granular protein is the tetrameric, 144,000-MW enzyme tryptase. This enzyme is tightly bound to heparin and comprises nearly half of the mast cell granular protein content. It is released from the mast cell upon IgE-dependent activation. Mast cells located in both connective tissue and mucosal sites possess this enzyme. Tryptase has a tryptic specificity, and its function is enhanced and its stability improved by its interaction with heparin. Tryptase is not known to be inhibited by plasma antiproteases. A second neutral protease with chymotryptic specificity has also been identified in isolated human lung mast cells.³⁰ This enzyme was first noted in human skin, and its mast cell origin was surmised from the fact that its content was greatly increased in the lesions of mastocytosis.³¹ It is prominent in mast cells of the skin and those present in loose connective tissue. This enzyme binds to the epidermal-dermal junction of human skin and induces separation of this area at the lamina lucida.³²

Acid Hydrolases and Carboxypeptidase. Beta-hexosaminidase, arylsulfatase, beta-glucuronidase, myeloperoxidase, superoxide dismutase, and carboxypeptidase enzymes

have all been noted in mast cells from rodents or humans. Some of these enzymes are released upon IgE-mediated mast cell activation, but their persistence or role outside the cell remains speculative.³³

The major proteoglycan of the human mast cell³⁴ is heparin. This molecule confers metachromasia and is released on cell activation. It is an anticoagulant, promotes angiogenesis and bone remodeling and inhibits complement activation.

The Eosinophil

The appearance of eosinophils in bullous pemphigoid lesions has been clearly documented.³ Recent evidence indicates that this association has pathogenetic implications.⁵

Growth and Differentiation

Eosinophils develop in the bone marrow from precursors that have common lineage with neutrophils and basophils.³⁵ Eosinophil production is regulated by T-lymphocyte factors,³⁶ and the cells mature in the marrow and circulate for only a few hours before appearing at tissue sites to which they have been directed. The vast majority of eosinophils are in the tissue or bone marrow and less than 1/200 are in the circulation.

Eosinophil Constituents

Eosinophils contain granules and a characteristic crystalline core structure as viewed under the electron microscope. The granules are comprised of a variety of enzymes, peptides, and proteins, and it is this combination of factors, together with the specificity of eosinophil chemotactic responses, which is felt to give this cell its special function as an effector cell of tissue injury.

Of the enzymes present in the eosinophil granules, peroxidase, arylsulfatase B, phospholipase D, and histaminase are the ones that distinguish this cell from other granulocytes. The peroxidase is distinguishable immunologically and functionally from myeloperoxidase and is capable of inducing mast

cell degranulation when H₂O₂ and halide anion are present.³⁷ Arylsulfatase B can bind sulfidopeptide leukotrienes; phospholipase D is capable of inactivating platelet-activating factor; and histaminase can degrade histamine.

Several peptides and proteins unique to the eosinophil have been characterized and may be responsible for the adverse effects of this cell type. Eosinophil major basic protein is a peptide of approximately 11,000 daltons with an isoelectric point of 10. It comprises the majority of the core granule protein.³⁸ This peptide is toxic to tracheal epithelium, can cause mast cell and basophil degranulation, and is thought important in parasite killing. A functionally related molecule, eosinophil cationic peptide³⁹ is somewhat larger (MW ~17,000) than major basic protein, but it too is toxic to parasites. It has potent actions on the lymphocyte and upon the proteins in the clotting and fibrinolytic cascades. Another peptide, termed eosinophil-derived neurotoxin⁴⁰ of MW ~18,000 has been shown capable of damaging the neurons of the central nervous system. Its role in disease is speculative. Finally, an eosinophil membrane-associated enzyme with lysophospholipase activity has been identified.⁴¹ Aggregates of this protein comprise the Charcot-Leyden crystals so characteristic of eosinophilic inflammatory processes. Both the Charcot-Leyden crystal protein and major basic protein are present, albeit in much reduced amounts, in basophils. In addition to these preformed constituents, activation of eosinophils by immune complexes or by divalent cation ionophores can lead to the production of the sulfidopeptide leukotriene LTC₄,⁴² while chemotactic factors cause this cell to generate platelet-activating factor.⁴³

The mechanism of release of eosinophil granular constituents was long debated, but it now appears to be a noncytolytic process that is enhanced in states of eosinophil activation. Such granule release is associated with the presence in the circulation of eosinophils with reduced buoyant density bearing new antigenic determinants and which are metabolically activated.⁴⁴

Functional Contribution of Cells to Development of Clinical Lesions

Mast Cell Products

Morphologic data suggest that mast cells participate early in the development of lesions. Mast cell granules contain vasoactive and smooth muscle contracting factors, enzymes, proteoglycans, and chemotactic factors. Mast cell-derived eosinophilotactic factors include the acidic tetrapeptides ala-gly-ser-glu and val-gly-ser-glu, which have been designated eosinophil chemotactic factor of anaphylaxis (ECF-A). Analysis of pemphigoid blister fluid for chemotactic factors after gel filtration revealed preferential activity for neutrophils in the exclusion volume and two regions of preferential eosinophilotactic activity of approximately 1500–3000 MW and 300–1000 MW. The eosinophilotactic activity in the lower-size range filtered with ^{14}C -val-gly-ser-glu and eluted from anion exchange chromatography at pH 2.2–3.5, indicating an acidic peptide reminiscent of the acidic tetrapeptides attributed in origin to human lung mast cells.³ Thus, mast cell participation suggested by the morphologic changes is confirmed by the detection of a mast cell product in bullous fluid. In addition, this activity may partially account for the attraction of eosinophils into lesions.

Although never clearly documented, it is possible that mast cell-derived enzymes may contribute to separation of the epidermal-dermal junction at the lamina lucida. Human mast cell chymase binds to and cleaves the lamina lucida *in vitro*, an activity that may at least partially account for blister formation.

Eosinophil Products

Eosinophil infiltration occurs just prior to blister formation. Eosinophil major basic protein (MBP) comprises the majority of material in the crystalline core of the eosinophil granule. The 11,000 MW, cationic material is cytotoxic to spleen, mononuclear cells, intestine, and epidermis. The presence of free eosinophil granules in the papillary dermis of erythematous plaques and the loss

of electron lucency of the crystalline core of the eosinophil granule suggest that eosinophil MBP may be released during lesion development.⁵ This possibility was confirmed by the detection by radioimmunoassay of a twofold to threefold elevation of eosinophil MBP in bullous pemphigoid blister fluid.⁴⁵ Thus, the morphologic and functional evidence suggest that the eosinophil may be an effector cell of tissue injury in bullous pemphigoid.

An Integrated View

An attractive but speculative pathologic sequence can be offered that would encompass the data to implicate complement, mast cells, eosinophils, and lymphocytes and account for the presence of an autoantibody. In this scheme, an undefined initiating event, such as the development of an autoantibody or a basement membrane chemical alteration, would activate the complement pathway. This, in turn, would result in the formation of any enzyme capable of cleaving C3 so as to deposit its major fragment C3b, at the site of activation. As the result of C3b bioactivity, migration of mast cells to the epidermal-dermal junction and release of mast cell-derived eosinophilotactic factors and enzymes would occur so as to direct the influx of eosinophils and cleave proteins at the lamina lucida.

Cleavage products of the complement component system would also account for the early influx of lymphocytes. The presence of activated lymphocytes responding either to complement products or to antigen would offer an alternative mechanism for the presence of these cells. The presence in bullous fluid of a cleavage fragment of the fifth complement component, C5a, could also contribute to the mast cell activation through its anaphylatoxic activity. The late eosinophil infiltration associated with eosinophil cell death and release of cytotoxic MBP precedes formation of the bullous lesion.

Finally, formation of a bullous lesion probably results from complex interactions of multiple bioactive molecules released by

mast cells and eosinophils at the site of lesion formation—the lamina lucida of the epidermal-dermal junction.

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