

Eosinophils and Urticaria

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Abstract

Previously believed to have an exclusive role in the release of cytotoxic mediators in the defense against helminthic infections, eosinophils are now considered key players in inflammatory reaction and regulation of immune response. Through activation of a large variety of membrane receptors and production of various pharmacologically active mediators, eosinophils may exert a detrimental role in tissues in which they have been recruited and may contribute to the perennization of inflammatory processes. The crucial role of eosinophils has been documented in several eosinophilic skin diseases, such as hypereosinophilic syndrome and bullous pemphigoid, and the literature provides strong evidence for their role in urticaria. The aim of this article is to discuss the mechanisms of specific tissue recruitment of eosinophils, the factors of eosinophil activation, and the contribution of these cells to inflammation and immunoregulation in urticaria. Recent advances in the knowledge of eosinophils will certainly help toward developing new strategies for the management of antihistamines resistant to urticaria.

Index Entries

Eosinophil; urticaria; inflammation; mucosa; skin.

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Introduction

Urticaria is a common disorder, affecting as many as 20% of all people at some point during their lives. Nevertheless, the underlying pathogenesis of this syndrome has not been completely elucidated. The clinical phenotype is characterized by pruritic wheals (superficial urticaria) and/or by deep mucocutaneous swelling (angioedema). Both of these flare reactions result from the release of vasoactive mediators—mainly histamine—from mast cells and basophils. Various studies have indicated that urticaria has a complex physiopathology involving multiple inflammatory pathways (1). Affected skin or mucosal tissues demonstrate an increased number of several other cells that may also play a role in this disorder, including T-cells, macrophages, neutrophils, and eosinophils. The potential role of eosinophils in urticaria is supported by the observation of urticarial skin lesions in individuals with hypereosinophilic syndrome or Wells syndrome, and also by the detection of an eosinophilia in episodic angioedema. Expression by eosinophils of several membrane receptors renders these cells sensitive to a large variety of molecules and leads to the release not only of various pro-inflammatory mediators (e.g., cationic proteins and leukotrienes) but also of immunoregulatory mediators, conferring crucial roles in immunity and inflammation on eosinophils. Therefore, the function of eosinophils has been extended considerably from an exclusive protective role in defense against parasitic pathogens toward a key role in tissue damage and chronic inflammation in pathological conditions such as allergy and urticaria.

Recruitment Into Inflammatory Tissues and Activation of Eosinophils

In normal blood, eosinophils amount to about 0 to 3% of the white blood cells, with a normal range under 500 eosinophils/mm³.

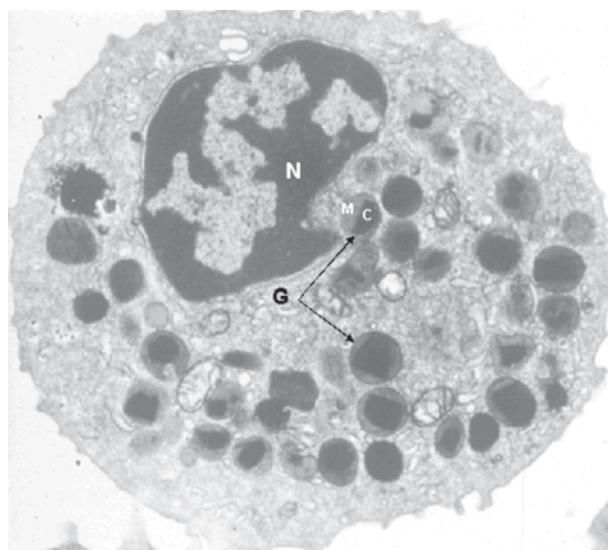


Fig. 1. Eosinophil viewed with electronic microscope. N, nucleus; G, mature granules; C, granule cristalloid; M, granule matrix.

Eosinophils are predominantly tissue cells, located mainly under physiological conditions in the gastrointestinal tract, where they play a protective role against pathogens. Eosinophil infiltration of other tissues is increased in inflammatory conditions and frequently occurs independently of eosinophilia in blood (2), thus suggesting the existence of specific mechanisms for eosinophil extravasation.

Eosinophilopoiesis and Morphology of Eosinophils

Eosinophils are mononucleated cells, differentiated from bone marrow precursors under the influence of the growth factors and cytokines interleukin (IL)-3, IL-5, and granulocyte macrophage–colony-stimulating factor (GM-CSF). As examined under microscope after hematoxylin-eosin staining, mature eosinophils are characterized by a two-lobed nucleus (which is stained in blue by hematoxylin) and granules in the cytoplasm (which take up the red eosin). These granules contain cationic proteins that are ready for secretion from the activated cell. Observation with electronic microscope (Fig. 1) shows that the mature granules are

comprised of a central cristalloid that contains major basic protein (MBP) and a peripheral matrix, which includes eosinophil peroxidase, eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN). Smaller granules contain the proteolytic enzymes lysophosphatase, galectin 10, aryl sulfatase, and acid phosphatase. Activated eosinophils release the granule contents by an active process (degranulation). Distinct eosinophil populations have been characterized on the basis of discontinuous Percoll density gradients: normodense nonactivated eosinophils and hypodense eosinophils, with morphological characteristics consistent with an activated state that induces degranulation (3).

Eosinophil Recruitment Into Tissues

Recruitment of eosinophils from the blood into the skin or the mucosae involves two distinct steps. The first step consists of eosinophil adhesion to endothelial cells through adhesion molecules. The participation of these molecules in eosinophil is complex and probably depends on the organ system involved. Eosinophil adhesion to the endothelium in skin and mucosae primarily involves adhesion of $\beta 1$ - and $\beta 2$ -integrins, expressed by eosinophils, to E- and P-selectins at the surface of endothelial cells. For a second time, interaction between vascular cell adhesion molecule-1 and the ligand very late antigen-4, which is specifically expressed on eosinophils, is required. Vascular cell adhesion molecule-1 is upregulated on endothelial cells by IL-4, a cytokine produced by activated eosinophils (4).

The second step of the migration of eosinophils into tissues is controlled by chemotactic factors, such as cytokines and chemokines, whose receptors are expressed on eosinophils constitutively or after activation. IL-5 is selectively involved in the recruitment and activation of eosinophils (5). Eosinophils also express receptors for various chemokines that contribute to their recruitment in inflammatory tis-

sues: macrophage inflammatory protein (MIP)-1 α , monocyte chemoattractant protein (MCP)-1 and MCP-3, regulated on activation normal T-cell excreted and secreted, and eotaxin (a chemoattractant of high efficacy on eosinophils) (6,7). Finally, eosinophils express various pharmacological receptors that confer a sensitivity to nonspecific pro-inflammatory molecules with a potential chemoattractant effect, including complement anaphylatoxins C3a and C5a and arachidonic acid-derived mediators such as leukotrienes, prostaglandins (PGD₂), and platelet-activating factor (PAF)-acether (8,9).

Activation of Eosinophils

During inflammatory reactions, eosinophils are subjected to a process of cellular activation, which leads to degranulation and mediator production. Several pathways are involved in eosinophil activation. The cytokines IL-3, IL-5, and GM-CSF not only control eosinophilopoiesis but also increase membrane receptor expression and the release of granule proteins. Eosinophils may also be activated by various cytokines produced during the inflammatory response, such as IL-2, IL-4, IL-10, interferon (IFN)- γ , and tumor necrosis factor (TNF)- α (10). Besides cytokines, immune complexes are also efficient activators of eosinophils. Antibody-mediated eosinophil activation, leading to the release of cytotoxic proteins in antibody-dependent cellular cytotoxicity mechanisms, was first demonstrated in helminthic infections. Later, the demonstration that eosinophils can express receptors for immunoglobulin (Ig)E high- and low-Fc ϵ RII or CD23 affinity receptor for IgE (11), as well as for IgA and its secretory component (12) indicated a direct activation of eosinophils by IgE and IgA, strengthening the evidence of a significant role for eosinophils located in the cutaneous and mucosal tissues in allergic reactions. Therefore, similarly to mast cells and basophils, eosinophils may be activated by allergens through

crosslinking of cell-surface-bound IgE when an allergic cause of chronic urticaria (CU) can be identified; nevertheless, this situation does not appear to explain most cases of CU, which are classified as idiopathic when allergic, infectious, drug-induced, or physical causes cannot be identified.

The observation of a wheal-and-flare response following intradermal injection of autologous serum in some patients with CU and the capacity of their serum to induce marked histamine release from basophils from a donor led to the discovery of the presence of histamine-releasing IgG auto-antibodies directed against the α -subunit of Fc ϵ RI in the blood of these patients (13). Therefore, auto-immunity may be an important mechanism in the pathogenesis of some types of CU. Finally, eosinophils may be activated by receptors for pharmacological ligands. Recent studies have shown that histamine may play a critical role for the chemotaxis and the activation of eosinophils through binding on histamine receptors involved in the oxidative metabolism—particularly histamine receptor 1—(14) and a more recently isolated receptor, histamine receptor 4, which regulates intracellular mobilization of calcium and cytoskeletal changes in eosinophils (15). As mentioned previously, eosinophils express receptors for complement anaphylatoxins C3a and C5a, which are produced after activation of the complement pathway by bacterial endotoxins and *Hymenoptera* venoms.

Eosinophil Function in Urticaria

After recruitment and activation, eosinophils can contribute to the pathogenesis of urticaria, not only by releasing tissue damaging proteins, but also by producing many other pro-inflammatory and immunoregulatory molecules.

Cytotoxic Functions of Eosinophils

Activation of eosinophils leads to degranulation, consisting of the extracellular release of the cationic proteins contained in granules. The

type of cationic proteins released by eosinophils is selectively dependent on the activated receptor (mechanism of “peace meal” degranulation; ref. 16). The cellular targets of these proteins are tissue-specific. MBP selectively and directly damages epithelial cells of upper respiratory mucosa, gastrointestinal tract, and epidermis; ECP and EDN exert a neurotoxic effect. Deposition of MBP and ECP has been observed outside of eosinophils by indirect immunofluorescence on skin specimens from individuals with chronic idiopathic urticaria, delayed pressure urticaria (17), and solar urticaria (18), as seen on bronchial tissue obtained from asthmatic individuals or on lesional skin from patients with atopic dermatitis. These deposits are localized in the dermis, preferentially into the small vessels walls or the conjunctive tissue (19). Eosinophil granule proteins may also be found in nonlesional skin from patients with a history of CU and delayed pressure urticaria (17). In patients with severe CU, the detection of high levels of MBP and ECP and hypodense eosinophils in the serum and the presence of necrotic eosinophils in the dermis suggest an abnormal activity of eosinophils, which is responsible for a massive and rapid degranulation (20). Furthermore, the deposition of granule proteins is vastly disproportionate to the number of identifiable cells and indicates that eosinophil involvement in urticaria cannot be judged by the number of intact eosinophils in the tissue. Cationic proteins may also contribute to tissue damage in an indirect way by amplifying the capacities of mast cells and basophils to release histamine and by inducing the oxidative metabolism of neutrophils (21).

Pro-Inflammatory Functions of Eosinophils

Activation of eosinophils induces the synthesis of various pharmacologically active substances, which may amplify the inflammatory response. These pro-inflammatory molecules are toxic oxygen radicals produced by oxidative metabolism that contribute to tissue dam-

ages and eicosanoid mediators generated from arachidonic acid, such as leukotrienes C₄, PGD₂, PGE₂, and PAF-acether, which may attract other cells at the inflammatory sites and participate in the wheal-and-flare reaction by inducing vasodilatation (22). These molecules may play a critical role in urticaria when a nonimmune cause is suspected—particularly physical urticaria, such as primary cold, solar, or delayed pressure urticaria. In solar urticaria, the number of eosinophils rapidly increases in the dermis after ultraviolet exposure (18). In cold urticaria, eosinophils release high amounts of PAF-acether, suggesting a key role of this pro-inflammatory mediator in the pathogenesis of this disease (23). The frequent unresponsiveness to antihistamines of delayed pressure urticaria raises the hypothesis that the role of histamine may be less important than lipidic mediators, which are released in massive doses in the skin of patients with symptoms of delayed pressure urticaria (1). Similarly, aspirin-induced urticaria and angioedema results from inhibition by acetylsalicylic acid of the cyclo-oxygenase pathway, leading to an overproduction of pro-inflammatory leukotrienes through the lipo-oxygenase pathway. Finally, in response to histamine, prostaglandins, and leukotrienes, activated eosinophils are able to secrete neuropeptides, such as substance P, which binds to the neurokinin-1 receptor to exacerbate histamine release by mast cells and vasodilatation. The pro-inflammatory role of substance P is documented in patients with asthma, who present a hyperresponsiveness to substance P, as well as an overexpression of neurokinin-1 receptor in their bronchi (24); however, when stress is identified as a trigger factor, there are strong evidences for the key role of neuropeptides in inflammatory skin conditions with a neurogenic component, such as atopic dermatitis and urticaria.

Immunoregulatory Functions of Eosinophils

Eosinophils are not only effector cells in immunity and inflammation but are also in-

involved in the regulation of the immune response through the synthesis of various mediators. Activated eosinophils have a crucial role in the enhancement and the perennization of the inflammatory response by producing growth factors with autocrine and paracrine activities that are able to increase eosinophil survival; chemotaxis; effector functions, such as IL-3, IL-5, and GM-CSF (25); chemokines (regulated on activation normal T-cell excreted and secreted [RANTES], eotaxin, and MIP-1 α); cytokines involved in inflammation (IL-1, IL-8, and TNF- α) and the development of fibrosis (transforming growth factor- α and - β 1); and immunomodulatory cytokines (including both T-helper [Th]1 cytokines, such as IL-2, IFN- γ , and IL-12, and Th2 cytokines, such as IL-4 and IL-10) (26–28). The key role of eosinophils in sustaining the inflammatory response is suggested by the presence of a perivascular infiltration, predominantly of eosinophils, in the dermis, during the late-phase reaction observed in particular forms of urticaria, such as CU characterized by eruption lasting longer than 24 h, without evidence of vasculitis, and delayed pressure urticaria. This eosinophilic infiltration is associated with deposits of cationic proteins (MBP and EDN) and high levels of leukotrienes C₄ and PAF-acether in the lesional skin (29). This histological condition is similar to the allergic skin late-phase reaction observed in atopy patch-tests but appears to have a different profile of cytokines production, with a Th0 profile (increased production of Th2 cytokines, such as IL-4 and IL-5, and Th1 cytokines, such as IFN- γ) rather than a Th2 cytokine profile (30).

Conclusion

Eosinophils can contribute to the pathogenesis of urticaria not only by releasing tissue-damaging basic proteins but also by producing many other pharmacologically active mediators involved in the regulation of the immune response and the perennization of the inflammatory reaction. These mediators may represent new therapeutic targets for this disorder

when antihistamines remain ineffective. Many clinical trials and case reports have suggested important new applications of leukotriene blockers, which are currently used for the treatment of asthma, in several other diseases in which leukotrienes play a pathogenic role, such as chronic idiopathic urticaria (31). Furthermore, cytokine and anticytokine therapies, like neutralizing anti-IL-5 antibody, represent a promising strategy in the treatment of disorders in which eosinophils exert a key role, including hypereosinophilic syndrome with eosinophilic dermatitis (32). This treatment may be potentially useful for patients with atopy—particularly atopic dermatitis (33)—and urticaria. However, such narrowly focused approaches often are inadequate to cure such multifactorial pathologies. A better knowledge of the factors involved in eosinophil recruitment and activation will certainly help toward developing new strategies for the management of antihistamine-resistant urticaria.

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