

Eosinophils in atopic dermatitis^{*}

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The eosinophilic granulocyte is commonly associated with allergic inflammation. Although blood eosinophilia frequently accompanies atopic dermatitis, accumulation of tissue eosinophils is not prominent. Recent studies have elucidated the structure, content, and activities of the eosinophil. In addition, immunofluorescence localization of eosinophil granule proteins has shown that eosinophils disrupt in tissue depositing toxic granule proteins in several diseases. Here, evidence for eosinophil degranulation in atopic dermatitis is presented and mechanisms whereby eosinophil degranulation may mediate pathophysiologic effects in atopic dermatitis and related conditions are discussed.

The eosinophil was first identified by Paul Ehrlich in 1879 and was so named because of its intense avidity for acid dyes, particularly eosin (1). Although eosinophils are easily recognized in tissue, the role of the eosinophil in cutaneous disease is only now being elucidated. The eosinophil granule is composed of potent toxic proteins which are deposited in tissue as eosinophils disrupt in inflammatory reactions (2). These toxic cationic proteins mediate parasite killing in helminth infections and are a likely cause of tissue and cell damage in hypersensitivity diseases (2). Once eosinophils disrupt in tissue, their morphological characteristics are lost. Therefore, the participation of eosinophils in pathophysiologic processes cannot be determined simply by counting the number of cells.

Structural and functional characteristics of eosinophils

The human eosinophil is characterized morphologically by its characteristic granules containing an electron-dense core and an electron-radiolucent matrix (2). The granule core is a crystalline lattice composed of an intensely basic

protein called "major basic protein" (MBP). Several other cationic proteins are present in the granule matrix including eosinophil cationic protein, eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO) (2). A hallmark of tissue eosinophilia is the presence of the bipyramidal hexagonal Charcot-Leyden crystals distributed in tissue especially in areas of eosinophil degranulation; the Charcot-Leyden crystal protein possesses lysophospholipase activity and is localized to the non-crystalline containing granules (3).

The eosinophil has several surface receptors including IgG receptors, low-affinity IgE receptors, complement receptors for C3b and C4 and probably also IgA receptors (2, 4-8). Secretory IgA appears to be particularly effective in causing eosinophil degranulation (7). In patients with peripheral blood eosinophilia, including patients with atopic dermatitis (AD), a high proportion of eosinophils have densities lower than those in normal persons; these light density eosinophils have been termed hypodense (9-15). Whereas normal eosinophils have densities of approximately 1.082, hypodense eosinophils have densities between 1.075 and 1.077 (14). The numbers of hypodense eosinophils correlate with total peripheral eosinophil counts (14). Hypodense eosinophils probably represent activated cells and appear to be potent killer cells in

^{*} Supported in part by National Institutes of Health Grant AR-36008, the Mayo Foundation, Rochester, MN, and the Kieckhefer Foundation, Prescott, AZ, U.S.A.

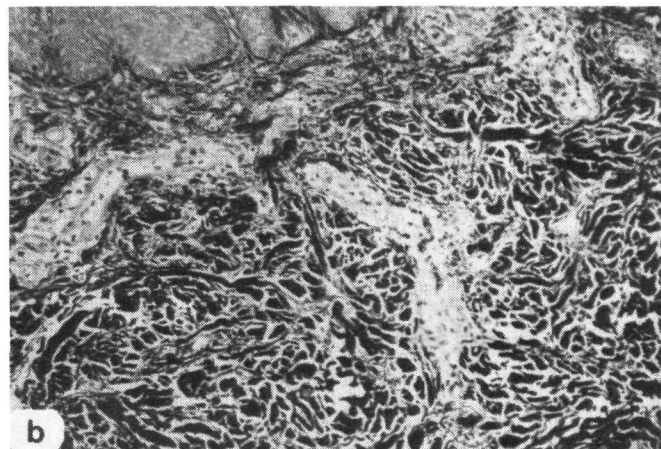
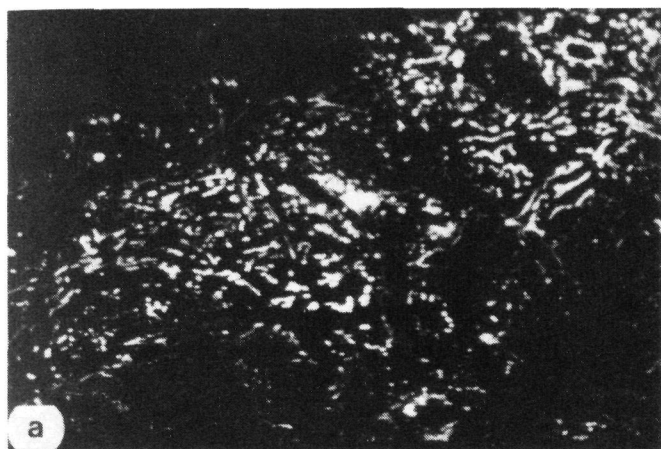


Fig. 1a. Photomicrograph ($\times 160$) of lesional AD skin biopsy specimen showing extensive extracellular fibrillar MBP deposition by immunofluorescence. Fig. 1b. Hematoxylin and eosin (H&E) counterstain ($\times 160$) of the identical field in Fig. 1a; extracellular MBP is not detectable on the H&E stained section. Negative control serial section stained with protein A purified normal rabbit IgG, showed no specific fluorescence.

parasite infection (12, 13). Leukotriene C₄, an arachidonic metabolite which promotes vaso-permeability, is preferentially produced by hypodense eosinophils (16–18). Eosinophils produce platelet activating factor which has vaso-permeability properties and is a powerful chemoattractant for eosinophils (19, 20) as well as substance P (21).

MBP, the protein comprising the crystalloid core of the eosinophil granule, is a 13.8 kD, arginine-rich basic polypeptide (2). In the guinea pig, MBP constitutes about 25% of the total cellular protein (2). MBP is a potent toxin for helminths and various mammalian cell types *in vitro*. Analysis of the cDNA of MBP has shown that MBP is translated as a preprotein with an acidic pro-portion (10). Because MBP is a potent toxin, its translation as a preprotein with an acidic pro-portion may neutralize the toxicity of the molecule and thus protect the eosinophil from damage as toxic MBP is processed through the endoplasmic reticulum to its sequestered site in the granule core. MBP deposition in tissue serves as a useful probe for assessing eosinophil degranulation in disease (1, 11–15). Two types of MBP staining are clearly discernible in tissue using indirect immunofluorescence with affinity-chromatography purified antibody to MBP: cellular staining of intact eosinophils presents as brightly fluorescent ovals, whereas extracellular MBP is distributed throughout tissue in granular, fibrillar and dif-

fuse staining patterns. Extracellular MBP, presumably from eosinophil degranulation, has been localized to damaged helminths and tissues in hypersensitivity diseases including asthma (1, 11–14). The observation that MBP is a toxin suggests that it causes damage to cells and tissues at sites where it is deposited. In addition to cytotoxic properties, MBP induces histamine release from mediator-containing cells including rat mast cells and human basophils (1). MBP and the other granule proteins also cause wheal-and-flare reactions when injected intradermally in human skin, further implicating them in direct effects on mast cells (15, 16).

The eosinophil as a parasite-killer cell

An association between peripheral blood eosinophilia and parasitism has long been recognized. Since 1970 abundant new information has demonstrated a role for the eosinophil as an effector cell in parasitic diseases. This information includes (1):

- Evidence showing that anti-eosinophil serum abolishes or reduces immunity to helminths and a tick;
- Studies demonstrating the direct killing of helminths by eosinophils;
- Further studies showing the direct killing of helminths by eosin granule proteins;

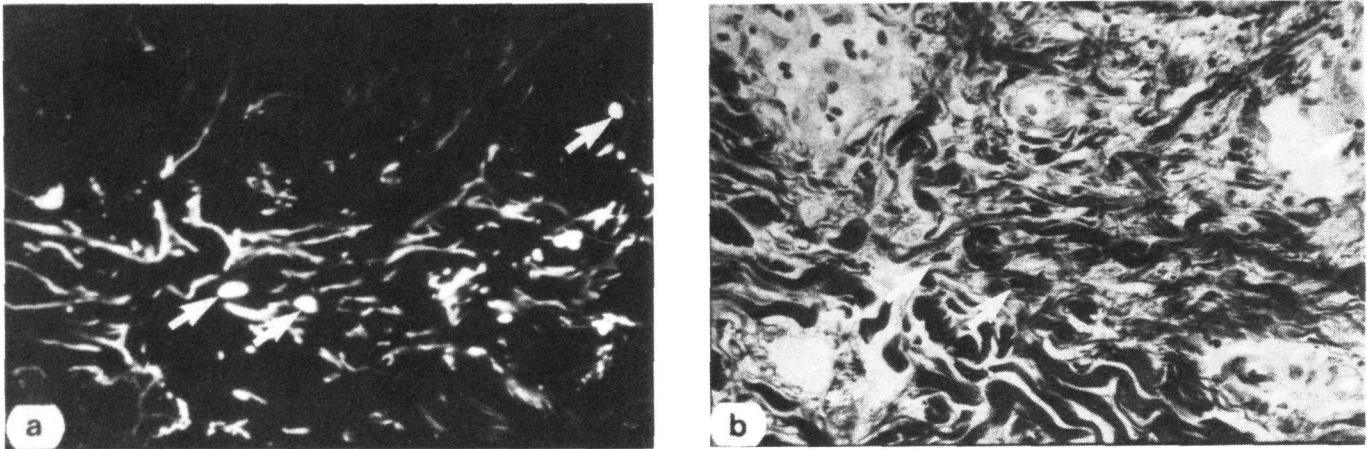


Fig. 2a. Higher power view of Fig. 1 ($\times 400$) showing extensive extracellular fibrillar fluorescence in upper dermis. There are a few infiltrating eosinophils indicated by arrows. Fig. 2b. H&E counterstain ($\times 400$) of the identical field in Fig. 2a; intact eosinophils identified by arrows, extracellular MBP not detectable. In the negative control serial section stained with protein A purified normal rabbit IgG, no specific fluorescence was observed.

- *In vitro* demonstration of eosinophil infiltration around and degranulation onto degenerating parasites.

Parasite damage is probably caused both by cationic proteins and toxic oxygen metabolites elaborated by eosinophils. The ability of eosinophils to kill parasites is also enhanced by activating factors derived from T cells, monocytes and mast cells (reviewed in ref. 1).

Eosinophils and atopic dermatitis

Atopic dermatitis is associated with patchy, characteristically distributed areas of cutaneous

eczema, with intense itching and subsequent lichenification of the skin. Skin lesions histologically show nonspecific inflammatory changes. Although tissue eosinophilia is not prominent in atopic dermatitis, peripheral blood eosinophilia is common.

Because previous studies had shown that eosinophil disruption in tissue with loss of morphological identity had occurred (1, 11–15), atopic dermatitis lesional skin was studied to determine if eosinophils degranulate in skin in this disorder (13). Initially, 19 biopsies of lesional AD skin and 1 biopsy of unaffected skin from 18 patients who satisfied the criteria for the diagnosis of AD were studied by indirect immu-

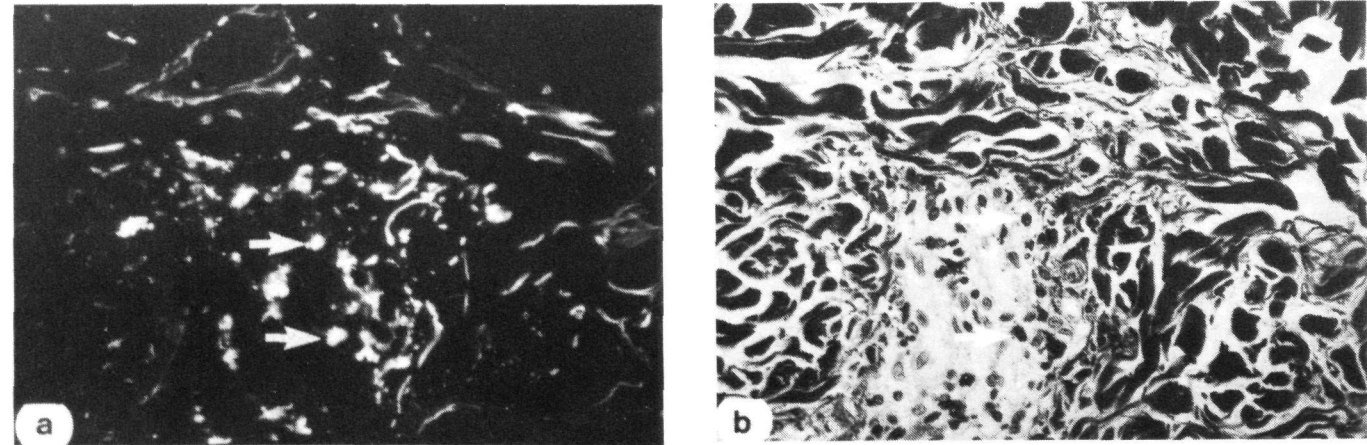


Fig. 3a. Photomicrograph ($\times 400$) of lesional AD skin showing extracellular granular and fibrillar MBP deposition by immunofluorescence. Intact eosinophils indicated by arrows. Control serial section stained with normal rabbit IgG was negative. Fig. 3b. H&E counterstain ($\times 400$) of the identical field in Fig. 2a. Eosinophils identified by arrows; extracellular MBP is not detectable here.

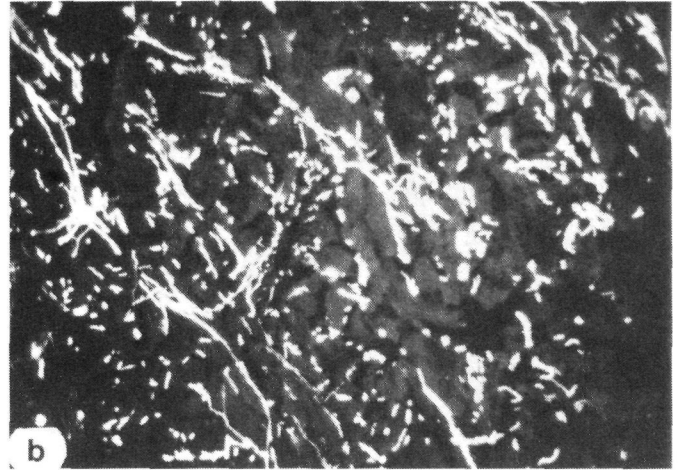
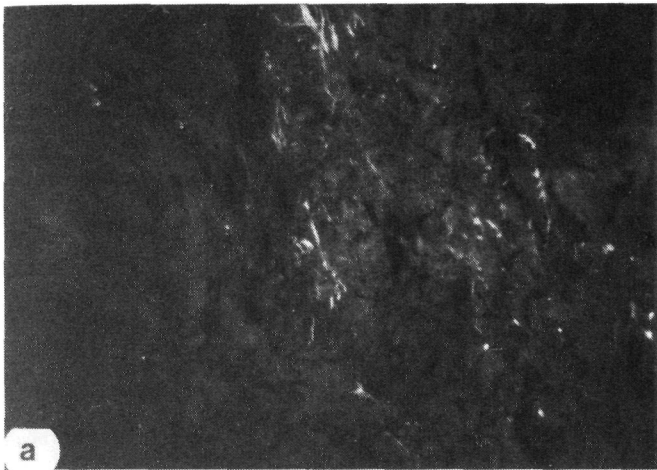


Fig. 4a. Photomicrograph ($\times 250$) of MBP deposition in unaffected skin from an AD patient; minimal fine fibrillar staining is present. Control serial section stained with protein A purified normal rabbit IgG was negative. *Fig. 4b.* Photomicrograph ($\times 250$) of MBP deposition in affected skin from same patients as in *Fig. 3a*; extensive extracellular MBP deposition is observed. Control serial section stained with normal rabbit IgG was negative.

nofluorescence for extracellular MBP localization. Single biopsies of lesional skin were obtained from 16 atopic dermatitis patients. Biopsy specimens were taken simultaneously of affected and unaffected skin from 1 patient, and 2 biopsies of affected skin were obtained from another patient 2 years apart.

Immunofluorescent staining demonstrated extracellular MBP deposition in all 20 specimens and two patterns of MBP deposition were observed (13). The dominant pattern consisted of fibrillar fluorescence in the upper dermis which was seen in 18 of the 20 biopsies (Figures 1 and 2). The other pattern consisted of scattered granules in the dermis (Fig. 3) observed in 12 of the 18 specimens (which also showed fibrillar fluorescence); 2 specimens showed scattered intact eosinophils and scattered extracellular granules without evidence of fibrillar fluorescence. The specimen from unaffected skin showed minimal fine fibrillar MBP deposition in the upper dermis, in marked contrast to affected skin from the same patient which showed extensive fibrillar MBP staining (Fig. 4). The fibrillar extracellular MBP staining in AD was deposited in the same distribution as elastic tissue including arborizing fibrils at the dermal-epidermal junction resembling oxytalan fibers (13). Although fluorescing fibrils could not be identified by counterstaining with connective tissue stains, serial sections of tissue

stained for MBP and Smith's elastin Giemsa demonstrated that the fluorescing fibrils corresponded almost identically to the staining pattern of elastic tissue (Fig. 5).

The most striking observation in these AD tissues was the extensive deposition of MBP in the absence of many tissue eosinophils, suggesting that eosinophils degranulate in the skin. Prior studies have shown both increased levels of MBP in the peripheral blood and peripheral activation of eosinophils in AD (6, 17). Although it is possible that dermal MBP in AD is the result of MBP deposition from the circulation, the focal patchy localization principally in the upper dermis and the minimal staining of uninvolved skin argue against this possibility.

Subsequent to the initial study (13), 22 biopsy specimens from 20 additional cases of AD have been studied. In addition to determining MBP deposition in tissue, biopsy specimens from certain patients were examined for EDN deposition and by electron microscopy; blood specimens were obtained for the determination of peripheral MBP and EDN levels. Two biopsy specimens from 2 patients with quiescent AD who developed eczematous reactions following food challenge (supplied by H. A. Sampson) showed extensive extracellular fibrillar MBP deposition in the lesional areas. The remaining 20 specimens confirmed and extended previous findings: 19 specimens showed fibrillar

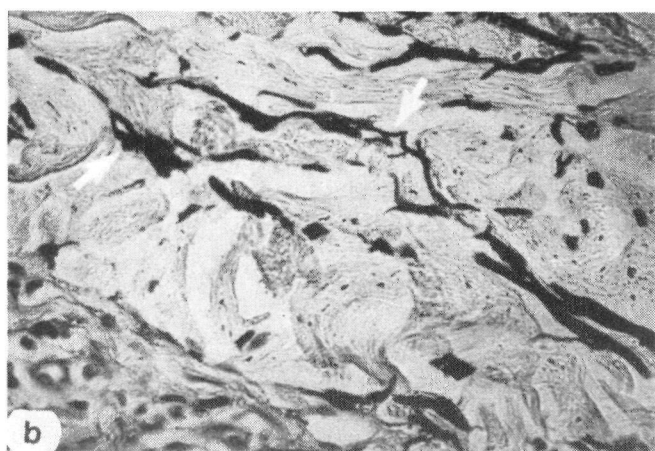
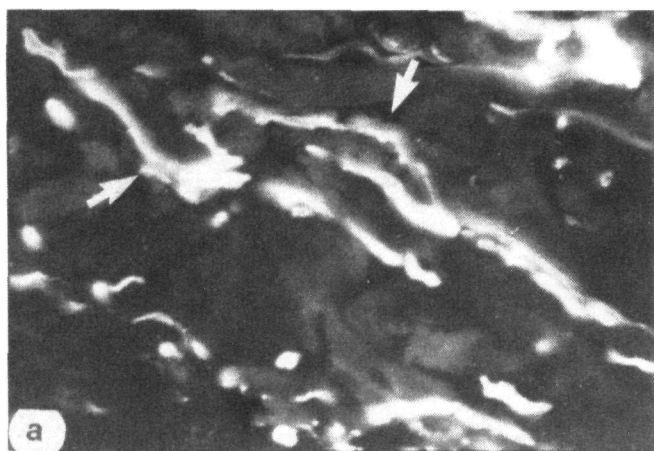


Fig. 5a. Photomicrograph ($\times 400$) of lesional AD skin biopsy showing fibrillar extracellular MBP deposition; fluorescing fibers indicated by arrows. Control serial section was negative. Fig. 5b. Serial section to 5a stained with Smith's elastin Giemsa ($\times 400$); elastic fibers indicated by arrows correspond to those staining with MBP.

extracellular MBP deposition in the upper dermis; 4 also showed granular MBP deposition in the dermis. Six specimens had minimal extracellular MBP fluorescence; 4 of these specimens were from patients who were being treated with systemic steroids and 2 were from patients who were hospitalized for treatment several days prior to biopsy. These findings suggest that MBP deposition may reflect disease activity.

Lesional tissue from 5 AD patients was studied for EDN deposition by indirect immunofluorescence using rabbit antisera to EDN. Similar to MBP localization, EDN in intact eosinophils appeared as brilliant fluorescent ovals and extracellular EDN deposition was granular, diffuse or fibrillar. All 5 AD specimens showed extensive extracellular EDN deposition in the upper dermis. Granular extracellular EDN deposition was most common although fibrillar staining was prominent in 2 of the 5 biopsies (unpublished observations). The deposition of 2 eosinophil granule proteins, EDN and MBP, in AD lesions provides further evidence for eosinophil degranulation in the disease.

Examination of 2 AD specimens by electron microscopy showed eosinophil degeneration and disruption with many free granules in the dermis (unpublished observations). Elastic fibers appeared normal. Thus, the results from electron microscopy corroborate the findings from immunofluorescence and provide yet additional evidence for eosinophil disruption in AD.

Plasma levels of MBP were measured in 10 atopic dermatitis patients by double antibody radioimmunoassay; 4 had elevated levels. Plasma levels of EDN were also measured in 9 of these patients; 8 showed elevated EDN levels. These results suggest that peripheral blood levels of EDN may be a more sensitive marker of local tissue eosinophil degranulation in AD than peripheral blood levels of MBP. Further studies are needed to determine if peripheral blood levels of eosinophil granule proteins correlate with tissue deposition and with disease activity.

Eosinophils and the IgE-mediated late-phase reaction

The pathophysiology of AD is not understood, although evidence exists that IgE mechanisms are operative in some cases. Atopic dermatitis is associated with increased serum IgE concentrations – which are often dramatically increased – and increased numbers of mast cells possessing high affinity IgE receptors are found in lesional skin. In some cases, challenge with food allergens provokes the disease and causes an increase in plasma histamine levels (13). Epicutaneous contact with allergens to which patients show immediate hypersensitivity results in an eczematous lesion which histologically shows a predominance of T cells of the help-

er/inducer phenotype; activated eosinophils which have lost granular contents are also seen in these lesions (18). Therefore, AD may be mediated, in part by IgE.

The immediate IgE-mediated wheal-and-flare reaction is often followed by a late phase reaction (LPR) which peaks 6–12 h after challenge and persists for at least 24 h (19). The LPR in the skin is much larger than the initial wheal-and-flare reaction and is characterized by edema, erythema, pruritus and tenderness. The mast cell is central to the development of the LPR (19). Eosinophil degranulation is prominent in the LPR with extensive deposition of MBP and EDN throughout the dermis (20). Ultrastructural changes in eosinophils in the LPR include alterations in granule structure, disruption of eosinophils and deposition of free granules outside of broken cells and dispersed between collagen bundles (20). In contrast to the striking fibrillar upper dermal MBP deposition in atopic dermatitis, MBP deposition in the LPR is mainly granular and deeper, probably reflecting the depth of the intradermal injection (20). The pattern of MBP deposition in the LPR resembles MBP deposition in urticarial and angiodematous syndromes (12). The granular extracellular MBP deposition in the LPR and its temporal appearance show a striking similarity to MBP deposition in solar urticaria and delayed pressure urticaria (21, unpublished observations). This finding is consistent with recent studies showing histological similarities between the LPR and delayed pressure urticaria and suggests that mechanisms other than the LPR are functioning in AD.

Extracellular MBP deposition has been found in several cutaneous syndromes showing urticaria and angioedema (12, 14, 15, 21). These include chronic urticaria, solar urticaria, delayed pressure urticaria, facial edema and episodic angioedema with eosinophilia. Although previously it was postulated that the eosinophil's function was to neutralize mast cell mediators, it has become apparent that the eosinophil possesses mediators that provoke tissue edema and tissue damage and that these mediators may directly act on mast cells potentiating inflammatory reactions involving mast cells (1,

11, 12). The functional interactions between mast cells and eosinophils have been further defined by recent studies which have shown that mast cells probably endocytose eosinophil granule proteins including EPO and MBP (22, unpublished observations). This may be a mechanism whereby mast cells sequester cationic proteins to reduce their toxic effects on tissues (22). Although it is not known if these pathophysiological interactions are playing a role in atopic dermatitis, the prominence of mast cells and eosinophil degranulation in lesions suggest that they may be.

Eosinophils in onchocerciasis and prurigo nodularis

Onchocerciasis is a helminth infection with *Onchocerca volvulus* and is characterized by markedly pruritic cutaneous lesions showing erythema and edema followed by lichenification (23). IgE is usually strikingly elevated and peripheral-blood eosinophilia is present. Prurigo nodularis is characterized by discrete hyperkeratotic nodules developing in areas of extreme pruritus. Serum IgE levels are frequently elevated and peripheral blood eosinophilia may be seen. In lesions of both onchocerciasis and prurigo nodularis, extensive fibrillar extracellular MBP deposition is present similar to that observed in atopic dermatitis (13) suggesting common pathophysiological mechanisms. In onchocerciasis, release of toxic eosinophil proteins may be part of the host defense against the parasite (1, 23) and these proteins may incite further inflammation in several ways including the induction of histamine release from mediator-containing cells. Similarly, eosinophil degranulation may promote cutaneous inflammation and provoke lesions in other diseases such as AD and prurigo nodularis. A feature common to these three diseases is the striking extracellular fibrillar deposition of MBP in the presence of few tissue eosinophils.

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