

Eosinophils in atopic dermatitis

Kristin M. Leiferman, MD Rochester, Minn.

Atopic dermatitis is a common, chronic eczematous skin disease. Recent studies have shown that the prevalence of atopic dermatitis has increased during the past several decades and now affects 10% to 15% of the population at some time in life.¹ Atopic dermatitis is primarily a childhood disease²⁻³; the onset of the disease is usually at a very young age and the disease may improve with time. However, among patients with moderate or severe atopic dermatitis in their teens, 77% to 91% have persistent or frequently relapsing dermatitis throughout adulthood.⁴ Atopic dermatitis may occur with skin involvement only, but it occurs together with respiratory allergies approximately 50% of the time.⁵ Pruritus is the cardinal feature of atopic dermatitis.⁶ Although mild disease is usually easily treated, moderate to severe disease may be recalcitrant to therapy, responding only to immunomodulators that have adverse systemic effects. Several reviews have recently discussed the pathogenesis and therapy of atopic dermatitis.^{2, 6-9}

Atopic dermatitis is associated with several immunologic anomalies, including aberrant cell-mediated immunity and increased IgE production.⁶ The number of CD8-positive T cells in peripheral blood is lower in patients with atopic dermatitis than in healthy subjects.⁹ The skin lesions show predominant infiltration of activated T-helper (CD4-positive) lymphocytes.^{9, 10} Activated T cells produce various cytokines with different inflammatory and immunoregulatory properties, and the pattern in atopic dermatitis appears to be a Th2 response with interleukin (IL)-4, IL-5, and IL-10 production and downregulation of interferon

Abbreviations used

ECP:	Eosinophil cationic protein
EDN:	Eosinophil-derived neurotoxin
IFN:	Interferon
IL:	Interleukin
LPR:	Late-phase reaction
MBP:	Major basic protein

(IFN)- γ production.¹¹ Based on in vitro studies and murine models, the addition of IL-4 to lymphocytes undergoing activation results in downregulation of IFN- γ production with concomitant enhancement of IgE synthesis.¹² In atopic dermatitis, decreased IFN- γ production and increased IL-4 production have been found to promote IgE production.¹³

Langerhans' cells and macrophages that infiltrate atopic dermatitis skin lesions bear IgE receptors, both Fc ϵ RI and Fc ϵ RII,¹⁴⁻¹⁶ and IgE antibody on their cell surfaces, which strongly suggests that allergen presentation by epidermal Langerhans' cells in atopic dermatitis is mediated by IgE.¹⁷ Allergens stimulate IgE-bearing macrophages to synthesize and secrete leukotrienes, platelet activating factor, IL-1, and tumor necrosis factor,^{18, 19} and they in turn may regulate the local accumulation of inflammatory cells or preferentially activate T cells to show the Th2-type response. Patients with atopic dermatitis also have circulating autoantibodies to IgE that may activate IgE-bearing macrophages.^{9, 20} Therefore, the presence of IgE receptors on Langerhans' cells and macrophages¹⁴⁻¹⁶ and the ability of IgE-bearing Langerhans' cells to bind allergen and present it to T lymphocytes to induce an allergen-specific T-cell response and IgE synthesis²¹ indicate the likely importance of IgE in a Th2 response in atopic dermatitis. In addition to IL-4 production, Th2 expression includes IL-5 production. IL-5 induces eosinophilopoiesis,²² eosinophil activation,²³ and eosinophil chemotaxis,²⁴ which probably account for the common association of eosinophils with allergic inflammation, including atopic dermatitis.

From the Department of Dermatology, Mayo Clinic and Mayo Foundation.

Supported in part by grant Nos. AR 36008, AI 15231, and AI 34577 from the National Institutes of Health, the Mayo Foundation, and the Kieckhefer Foundation, Prescott, Az. Reprint requests: Kristin M. Leiferman, MD, Department of Dermatology, Mayo Clinic and Mayo Foundation, Rochester, MN 55905.

J ALLERGY CLIN IMMUNOL 1994;94:1310-7.

Copyright © 1994 by Mosby-Year Book, Inc.

0091-6749/94 \$3.00 + 0 1/0/59925

EARLY OBSERVATIONS

Peripheral blood eosinophilia is associated with many diseases, and for decades it has been recognized that peripheral blood eosinophilia accompanies atopic dermatitis.²⁵ The eosinophil contains several distinctive cationic granule proteins, including the major basic protein (MBP), the eosinophil peroxidase, the eosinophil cationic protein (ECP), and the eosinophil-derived neurotoxin (EDN). MBP, eosinophil peroxidase, and ECP are potent toxins to various targets including helminths, protozoa, bacteria, and mammalian cells.²⁶ In 1981 Wassom et al.²⁷ measured levels of MBP in patients with peripheral blood eosinophilia; included in this group were 16 patients with atopic dermatitis. Virtually all healthy controls showed normal numbers of eosinophils and normal MBP levels in the peripheral blood. In contrast, more than half of the patients with atopic dermatitis showed elevated MBP levels in the peripheral blood, some with normal eosinophil counts and some with elevated eosinophil counts. These results suggested that the toxic eosinophil granule MBP was "escaping" from eosinophils through degranulation, secretion, or another process.

The common presence of peripheral blood eosinophilia in atopic dermatitis, the typical histologic picture in atopic dermatitis in which tissue eosinophils are found but are not prominent, and the finding that a granule protein was elevated in peripheral blood prompted studies to determine whether eosinophils in tissue might have degranulated. The tissue deposition of eosinophil MBP was studied as a marker of eosinophil degranulation.²⁸ Nineteen biopsy specimens of affected atopic dermatitis skin showing chronic dermatitis and one of unaffected skin were obtained from 18 patients who met the diagnostic criteria for atopic dermatitis.²⁹ Biopsy specimens were taken simultaneously from the affected and unaffected skin of one patient. Two biopsy specimens from the affected skin of another patient were obtained 2 years apart. The biopsy specimens were stained for MBP by indirect immunofluorescence. All 20 biopsy specimens from patients with atopic dermatitis showed extracellular MBP localization. In 18 specimens, the dominant pattern was fibrillar fluorescence in the upper dermis; 12 of these 18 specimens also showed extracellular granules scattered in the dermis. Two specimens showed only intact eosinophils and a few extracellular granules without evidence of fibrillar fluorescence. The specimen from unaffected skin showed minimal fluorescence, with fine fibrils in the upper dermis

staining faintly with antibody to MBP; by contrast, a specimen of affected skin showed marked upper dermal fibrillar fluorescence. In the other case in which two specimens of affected skin were obtained 2 years apart, both showed marked upper dermal fibrillar fluorescence.²⁸

The most striking observation in the atopic dermatitis tissues was the extensive extracellular deposition of MBP with a paucity of intact eosinophils. The few identifiable eosinophils were located predominantly within the foci of mononuclear cell infiltrates. Yet in many of the biopsy specimens, MBP staining was prominent throughout the entire upper dermis. The prior study showing increased levels of MBP in the peripheral blood²⁷ along with another study showing peripheral activation of eosinophils in atopic dermatitis³⁰ suggested that dermal MBP deposition in atopic dermatitis was the result of deposition from the circulation. However, the focal patchy localization principally in the upper dermis and the minimal staining of uninvolved skin argue against this possibility and strongly suggest that eosinophils deposit granule proteins directly in the skin in this disease.

CORRELATION OF EOSINOPHILS AND DISEASE ACTIVITY

Since the initial observation showing marked extracellular eosinophil granule MBP deposition in lesions of atopic dermatitis, 22 additional patients have been studied to further define the role of eosinophils in atopic dermatitis.³¹ Skin biopsy specimens were obtained from all 22 patients; blood and urine were collected the same day as the biopsies in 19 and 14 patients, respectively. Blood eosinophil counts were calculated by multiplying the total white blood cell count by the percentage of eosinophils, and body surface involvement was estimated by the "rule of nines."³² Indirect immunofluorescence was performed to detect the presence of MBP, EDN, and ECP in formaldehyde-fixed paraffin-embedded skin biopsy specimens. MBP, EDN, and ECP were measured in serum and urine by immunoassay. Relationships among the various parameters were statistically analyzed, including tissue deposition of MBP, EDN, and ECP, serum levels and urine levels of MBP, EDN, and ECP, serum IgE levels, calculated eosinophils, and the percentage of body surface involvement in all possible combinations.

Fifteen of the 22 patients demonstrated prominent extracellular deposition of at least one eosinophil granule protein in lesional skin specimens;

TABLE I. Significant correlations in patients with atopic dermatitis by Spearman's rank test*

Parameters compared†		N	r_s	p
Serum MBP	Serum EDN	19	0.64	<0.007
Serum EDN	Serum ECP	19	0.63	<0.008
MBP deposition	EDN deposition	22	0.76	<0.0005
MBP deposition	ECP deposition	22	0.67	<0.003
EDN deposition	ECP deposition	22	0.68	<0.002
Urine MBP	Serum EDN	14	0.60	<0.04
Body surface involvement (%)	Serum MBP	19	0.59	<0.02
Calculated eosinophils	MBP deposition	19	0.49	<0.04

From Ott et al. J ALLERGY CLIN IMMUNOL 1994;94:120.

*Abacus Concepts, StatView II (Abacus Concepts, Inc., Berkeley, Calif., 1987).

†Statistical comparisons were performed on all the following parameters in all possible combinations: serum MBP levels, serum EDN levels, serum ECP levels, tissue MBP deposition, tissue EDN deposition, tissue ECP deposition, urine MBP levels, urine ECP levels, urine EDN levels, serum IgE, calculated eosinophil counts, and percentage of body surface involvement. Only significant positive correlations are indicated.

tissue deposition of the eosinophil granule proteins was markedly out of proportion to the few infiltrating cells. Four of 19 patients in whom blood differential counts were available had peripheral blood eosinophilia (>760 cells/ μ l); serum levels of MBP, EDN, and ECP were elevated in 15 of 19, 14 of 19, and 10 of 19 specimens, respectively. Urine levels of MBP, EDN, and ECP were elevated in 3 of 14, 7 of 14, and 0 of 14 specimens, respectively.³¹

Eight significant positive correlations emerged from the statistical comparisons of eosinophil granule proteins and are shown in Table I. Serum levels of MBP and EDN were correlated ($r_s = 0.64$, $p < 0.007$) and serum levels of EDN and ECP were correlated ($r_s = 0.63$, $p < 0.008$). Tissue deposition of MBP and EDN was correlated ($r_s = 0.76$, $p < 0.0005$), as was tissue deposition of MBP and ECP ($r_s = 0.67$, $p < 0.003$), and tissue deposition of EDN and ECP ($r_s = 0.68$, $p < 0.002$). One of the most interesting correlations to emerge from the studies was the relationship between body surface involvement and serum MBP levels ($r_s = 0.59$, $p < 0.02$) (Fig. 1).

In the past few years, several studies have reported relationships between eosinophil numbers and/or ECP levels with atopic dermatitis disease activity. The reports have shown increased numbers of eosinophils with severe disease^{33, 34} and decreased numbers of eosinophils with clinical improvement after therapy.³⁵⁻³⁷ Other reports have shown a relationship between ECP levels and disease activity,^{33, 37-43} which indicates that blood levels of eosinophil granule proteins correlate with disease activity in atopic dermatitis.

PATHOPHYSIOLOGY OF ATOPIC DERMATITIS

The histopathologic changes in atopic dermatitis are nonspecific and contribute little to understanding the pathogenesis of the disease.²⁵ Acute lesions show epidermal spongiosis and dermal perivascular inflammatory cell infiltration. Chronic atopic dermatitis lesions show thickening of the epidermis with elongation of the rete ridges and fibrosis around the rete ridges. Langerhans' cells and mast cells may be increased in number, and endothelial cells are enlarged. Most lymphocytes in biopsy specimens are T cells possessing the CD4 phenotype.^{9, 10} Langerhans' cells and macrophages infiltrating atopic dermatitis skin lesions show surface-bound IgE molecules.¹⁴⁻¹⁶

The late phase of the IgE-mediated reaction has been proposed as a model for chronic allergic inflammation, including that seen in atopic dermatitis.^{44, 45} Immediate hypersensitivity reactions in the skin are characterized by the development of a wheal-and-flare that peaks in 10 to 20 minutes. This may be followed by a late inflammatory reaction at the same site. Pathologic examination of the cutaneous late-phase reaction (LPR) has shown infiltration with mononuclear cells, neutrophils, basophils, and eosinophils, along with edema and mast cell degranulation.⁴⁶⁻⁴⁸ Studies have shown extracellular deposition of eosinophil and neutrophil granule proteins in the LPR beginning 1 hour after antigen challenge and persisting up to 56 hours.⁴⁹ Electron microscopy has also shown evidence for eosinophil disruption in the LPR with fragments of eosinophils and free granules in interstitial spaces.⁴⁹ Studies from allergen-induced pulmonary late-phase inflamma-

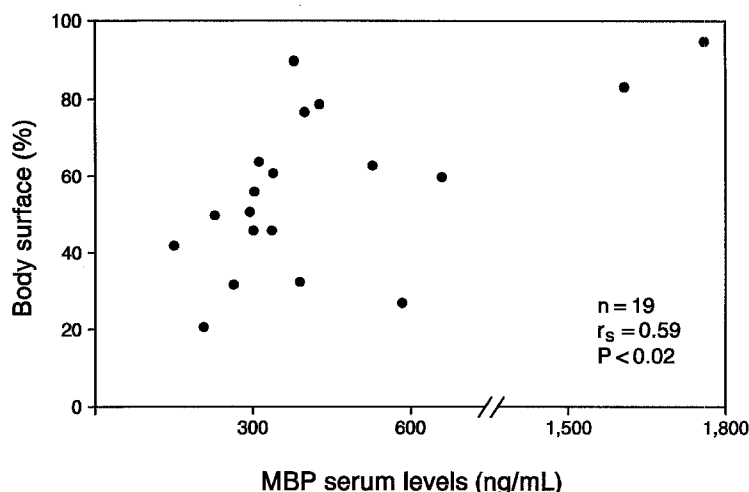


FIG. 1. Correlation of serum MBP levels with extent of disease. Serum MBP levels as measured by immunoassay correlated with extent of body surface involvement with atopic dermatitis lesions as determined by the "rule of nines," $r_s = 0.59$, $p < 0.02$.

tion have shown increased IL-5 levels in concert with increased levels of leukotriene C4 and increased release of eosinophil granule proteins.⁵⁰ Other studies of the cutaneous LPR have shown increased messenger RNA expression of the cytokine gene cluster, IL-3, IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor.⁵¹ IL-3 and granulocyte-macrophage colony-stimulating factor, in addition to IL-5, strikingly enhance eosinophil degranulation in vitro.⁵²

To determine whether the pathologic changes in atopic dermatitis resembled those of the cutaneous LPR, the lesional atopic dermatitis specimens in which eosinophil granule proteins had been localized were also studied for neutrophil degranulation by indirect immunofluorescence of neutrophil elastase and lactoferrin. Neutrophil elastase is present in azurophilic granules and lactoferrin is present in specific granules of neutrophils. Immunoassays of serum were also performed for neutrophil elastase. Minimal or no neutrophil elastase or lactoferrin deposition was found in the atopic dermatitis specimens, and no patient had elevated levels of neutrophil elastase in the serum.³¹ Therefore, in atopic dermatitis lesions, prominent dermal extracellular eosinophil granule protein deposition is present but dermal neutrophil granule protein deposition is not, which is in contrast to the IgE-mediated LPR in which both eosinophil granule protein and neutrophil elastase deposits are prominent in the dermis. Overall, the results indicate that eosinophils actively participate in the pathogenesis of atopic dermatitis but, unlike the IgE-mediated

LPR, neutrophils do not appear to play a role in established lesions of atopic dermatitis.³¹

Several observations suggest that IgE and allergens contribute to the pathogenesis of atopic dermatitis. Serum IgE levels are elevated in approximately 80% of patients with atopic dermatitis and are highest in patients with coexisting respiratory allergies. A correlation between serum IgE level and extent of skin involvement has been reported.⁵³ When prick or intradermal skin tests are performed, patients with atopic dermatitis often show wheal-and-flare responses to multiple allergens, and they frequently have elevated serum IgE antibody levels to multiple allergens. Controlled studies have shown that ingestion of foods by sensitive children with atopic dermatitis provoked cutaneous pruritus and erythema, which led to scratching and subsequent eczematoid lesions.⁵⁴ Furthermore, these exacerbations were associated with significant increases in plasma histamine concentrations after food challenges, which suggests that at least in a subset of patients, repeated ingestion of food and the reactions that result from such exposure contributes to the development of atopic dermatitis skin lesions. Interest in the potential role of inhalant allergens in atopic dermatitis has long existed. Several decades ago Walker⁵⁵ described several patients who experienced flaring of atopic dermatitis after exposure to horse dander or ragweed pollen. Tuft et al.^{56, 57} observed that patients with atopic dermatitis had pruritus and eczematoid skin lesions after inhalation of mold or ragweed pollen. Additional studies have focused on the role of direct

contact by inhalant allergens in provoking atopic dermatitis lesions. Mitchell et al.⁵⁸ induced cutaneous eczematoid lesions in patients with atopic dermatitis by applying aqueous allergens to superficially abraded skin. Clark and Adinoff⁵⁹ subsequently demonstrated eczematous reactions to a variety of inhalant allergens on nonabraded skin of patients with atopic dermatitis. Platts-Mills et al.⁶⁰ have reported that avoidance of aeroallergens that elicit eczematous reactions at patch test sites or cause immediate hypersensitivity reactions results in improvement of atopic dermatitis.

Recent observations on staphylococcal enterotoxins suggest new mechanisms by which *Staphylococcus aureus* could exacerbate atopic dermatitis.⁶¹ Approximately 50% of patients with chronic atopic dermatitis have circulating IgE directed to staphylococcal toxins present on their skin and implicate additional allergen mechanisms at the skin surface level.⁶¹ Staphylococcal enterotoxins are superantigens capable of stimulating T cells in a class II major histocompatibility complex-dependent yet unrestricted manner. Therefore, staphylococcal enterotoxins probably exacerbate atopic dermatitis in two additional ways. First, staphylococcal enterotoxins secreted at the skin surface could penetrate inflamed skin and engage human leukocyte antigen-DR on macrophages or Langerhans' cells to stimulate the production of IL-1 and tumor necrosis factor; both cytokines have potent proinflammatory activities. Second, staphylococcal enterotoxins may stimulate T cells via their T-cell receptor β -chain to divide and produce cytokines that modulate tissue inflammation.⁶¹

Other allergen models have been proposed to explain the pathogenesis of atopic dermatitis and establish further links between eosinophils and pathophysiology. For example, biopsies of aeroallergen patch testing in patients with atopic dermatitis show that lymphocytes and eosinophils infiltrate the dermis 2 to 6 hours after allergen application. Twenty-four to 48 hours after patch testing, eosinophils release ECP, and at this point in time eosinophils have infiltrated the epidermis.⁶² In another study in which house dust mite antigens were applied to the skin of patients with atopic dermatitis, activated eosinophils were present in the dermis in close contact with antigen-presenting cells in the epidermis and dermis.⁶³ Furthermore, eosinophil granules were present outside of intact cells, and ECP was present in macrophage-like dermal cells.⁶³ Studies of eosinophil chemotaxis have shown that eosinophils from patients with atopic

dermatitis have significantly increased migratory responses toward certain chemotaxins,⁶⁴⁻⁶⁶ and comparisons of platelet activating factor injection and allergen injection in atopic dermatitis skin showed a much stronger eosinophilic infiltrate at allergen-injected sites.⁶⁷ In vascular models of transendothelial migration, eosinophils from patients with atopic dermatitis spontaneously migrated through endothelial cells; eosinophils from nonallergic persons were unable to transmigrate until primed with cytokines.⁶⁸ Taken together, these studies show a relationship between eosinophil activity and atopic dermatitis and suggest that the mechanism of the cutaneous eosinophilia in atopic dermatitis may be allergen-induced.

Although the immunohistochemical findings show differences between the IgE-mediated LPR and atopic dermatitis, the possibility cannot be excluded that they are part of the same mechanism. Repeated antigen exposure may alter inflammatory reactivity to enhance eosinophil participation while neutrophil involvement diminishes, as has been seen in the monkey model of antigen challenge in the lung.⁶⁹ Furthermore, in atopic dermatitis, exposure to allergen may be through a transepidermal route with Langerhans' cell involvement; this is different from the intradermally administered allergen in the LPR. Therefore, disease expression may be modified depending on antigen presentation by immunocompetent cells in various skin compartments. Finally, the LPR studies were performed in patients with allergic rhinitis. Therefore, the possibility exists that antigen exposure in atopic dermatitis induces a different inflammatory response than cutaneous antigen expression in patients with respiratory allergies. If so, this would suggest constitutive cutaneous abnormalities in atopic dermatitis. A recent report suggests that the LPR is inducible only in patients with atopic dermatitis who have respiratory allergies and is not inducible in patients with atopic dermatitis alone.⁷⁰ Therefore, different reaction patterns may be seen in atopic dermatitis skin.

FATE OF EOSINOPHILS IN ATOPIC DERMATITIS

The striking extracellular localization of eosinophil granule proteins in lesions of atopic dermatitis and elevations of granule protein in peripheral blood suggest that these granule-associated proteins have escaped from the eosinophil as part of the disease process. In the IgE-mediated LPR, extracellular eosinophil granule protein deposi-

tion is associated with electron microscopic findings of prominent eosinophil disruption with fragments of eosinophils and free granules in interstitial spaces.⁶⁹ In electron microscopic studies of biopsy specimens from 10 patients with atopic dermatitis, seven of the specimens had identifiable eosinophils or eosinophil granules.⁷¹ In these seven specimens, however, no normal eosinophils were found and various stages of eosinophil degeneration were observed, including intact eosinophils with granule abnormalities, intact eosinophils with granule abnormalities and pseudopod-like extensions, eosinophils with degenerating cell membranes and/or nuclear membranes, and free eosinophil granules in the dermis near or in the absence of degenerating eosinophils. The granules showed lucency and loss of eosinophil granule core density both within eosinophils and in free granules scattered in the tissues. The study showed a progression of abnormalities by electron microscopy in atopic dermatitis, ranging from marked granule abnormalities in the eosinophils to complete disruption of cells with free granules in contact with extracellular space. No electron microscopic evidence for any other form of degranulation was observed. On the basis of these findings, it appears likely that eosinophil degeneration with deposition of granules and granule proteins occurring in lesional atopic dermatitis skin is the result of a cytolytic process.

In summary, studies strongly support an active role for eosinophils in the pathogenesis of atopic dermatitis. The induction of a Th2 type of response probably initiates an inflammatory cascade in the skin that includes activation of eosinophils, degranulation of eosinophils, release of cytotoxic granule proteins, and, ultimately, the disease expression of atopic dermatitis. How these granule proteins affect the skin and influence the expression of the disease is under investigation.

REFERENCES

- Larsen FS, Holm NV, Henningsen K. Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. *J Am Acad Dermatol* 1986;15:487-96.
- Sampson HA. Pathogenesis of eczema. *Clin Exp Allergy* 1990;20:459-67.
- Krafchik BP. Atopic dermatitis. *Pediatr Clin North Am* 1983;30:669-85.
- Forsten Y. Prognosis of atopic dermatitis: a prospective study in early adulthood. *Int J Dermatol* 1991;30:563-8.
- Diepgen TL, Fartasch M. Recent epidemiological and genetic studies in atopic dermatitis. *Acta Derm Venereol (Stockh)* 1992;13(suppl 176):13-8.
- Hanifin JM. Atopic dermatitis: new therapeutic considerations. *J Am Acad Dermatol* 1991;24:1097-101.
- Cooper KD. Atopic dermatitis: recent trends in pathogenesis and therapy. *J Invest Dermatol* 1994;102:128-37.
- Dahl MV. Flare factors and atopic dermatitis: the role of allergy. *J Dermatol Sci* 1990;1:311-8.
- Leung DYM. Immunopathology of atopic dermatitis. *Springer Semin Immunopathol* 1992;13:427-40.
- Lever R, Turbitt M, Sanderson A, MacKie R. Immunophenotyping of the cutaneous infiltrate and of the mononuclear cells in the peripheral blood in patients with atopic dermatitis. *J Invest Dermatol* 1987;89:4-7.
- Romagnani S. Human TH1 and TH2 subsets: doubt no more. *Immunol Today* 1991;12:256-7.
- Vercelli D, Jabara HH, Lauener RP, Geha RS. IL-4 inhibits the synthesis of IFN- γ and induces the synthesis of IgE in human mixed lymphocyte cultures. *J Immunol* 1990;144:570-3.
- Juho K, Renz H, Abe J, Gelfand EW, Leung DYM. Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. *J ALLERGY CLIN IMMUNOL* 1992;90:323-31.
- Vercelli D, Jabara HH, Lee B, Woodland N, Geha RS, Leung DYM. Human recombinant interleukin-4 induces Fc ϵ R2/CD23 on normal human monocytes. *J Exp Med* 1988;167:1406-16.
- Bieber T, de la Salle H, Wollenberg A. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E. *J Exp Med* 1992;175:1285-90.
- Wang B, Rieger A, Kilgus O. Epidermal Langerhans' cells from normal human skin bind monomeric IgE via Fc ϵ RI. *J Exp Med* 1992;175:1353-65.
- Mudde GC, Van Reijssen FC, Boland GJ, DeGast GC, Bruijnzeel PLB, Bruijnzeel-Koomen CAFM. Allergen presentation by epidermal Langerhans' cells from patients with atopic dermatitis is mediated by IgE. *Immunology* 1990;69:335-41.
- Fuller R, Morris P, Richmond R, et al. Immunoglobulin E-dependent stimulation of human alveolar macrophages: significance in type 1 hypersensitivity. *Clin Exp Immunol* 1986;65:416-26.
- Rouzer CA, Scott WA, Hamill AL, Liu F-T, Katz DH, Cohn ZA. Secretion of leukotriene C and other arachidonic acid metabolites by macrophages challenged with immunoglobulin E immune complexes. *J Exp Med* 1982;156:1077-86.
- Quinti I, Brozek C, Geha RS, Leung DYM. Circulating IgG antibodies to IgE in atopic syndromes. *J ALLERGY CLIN IMMUNOL* 1986;77:586-94.
- Fokkens WJ, Bruijnzeel-Koomen CAFM, Vroom TM, et al. The Langerhans' cell: an underestimated cell in atopic disease. *Clin Exp Allergy* 1990;20:627-38.
- Silberstein DS, et al. Hemopoietins for eosinophils; glycoprotein hormones that regulate the development of inflammation in eosinophilia-associated diseases. *Hematol Oncol Clin North Am* 1989;3:511-33.
- Rothenberg ME, Peterson J, Stevens RL. IL-5 dependent conversion of normodense human eosinophils to the hypodense phenotype using 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody dependent cytotoxicity. *J Immunol* 1989;143:2311-6.
- Yamaguchi Y, Hayashi Y, Sugama Y. Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. IL-5 as an eosinophil chemotactic factor. *J Exp Med* 1988;167:1737-42.

25. Rajka G. Atopic dermatitis. *Major Probl Dermatol* 1975; 3:42.
26. Gleich GJ, Adolphson CR, Leiferman KM. The eosinophil. In: Gallin JI, Goldstein IM, Snyderman R, eds. *Inflammation: basic principles and clinical correlates*. 2nd ed. Raven Press, 1992:663-700.
27. Wassom DL, Loegering DA, Solley GO, et al. Elevated serum levels of the eosinophil granule major basic protein in patients with eosinophilia. *J Clin Invest* 1981;67:651-61.
28. Leiferman KM, Ackerman SJ, Sampson HA, Haugen HS, Venencie PY, Gleich GJ. Dermal deposition of eosinophil-granule major basic protein in atopic dermatitis: comparison with onchocerciasis. *New Engl J Med* 1985;313: 282-5.
29. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980;92:44-7.
30. Miyasato M, Iryo K, Kasada M, Tsuda S. Varied density of eosinophils in patients with atopic dermatitis reflecting treatment with anti-allergic drug [Abstract]. *J Invest Dermatol* 1988;90:589.
31. Ott NL, Gleich GJ, Peterson EA, Fujisawa T, Sur S, Leiferman KM. Assessment of eosinophil and neutrophil participation in atopic dermatitis: comparison with the IgE-mediated late phase reaction. *J ALLERGY CLIN IMMUNOL* 1994;94:120-8.
32. Burke JF, Bondoc CC. Burns: the management and evaluation of the thermally injured patient. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. *Dermatology in general medicine*. 2nd ed. St. Louis: McGraw-Hill Book Co., 1979:932.
33. Tsuda S, Kato K, Miyasato M, Sasai Y. Eosinophil involvement in atopic dermatitis as reflected by elevated serum levels of eosinophil cationic protein. *J Dermatol* 1992;91: 208-13.
34. Uehara M, Izukura R, Sawai T. Blood eosinophilia in atopic dermatitis. *Clin Exp Dermatol* 1990;15:264-6.
35. Hanifin J, Schneider LC, Leung DY, et al. Recombinant interferon gamma therapy for atopic dermatitis. *J Am Acad Dermatol* 1993;28:189-97.
36. Walker C, Kagi MK, Ingold P, et al. Atopic dermatitis: correlation of peripheral blood T cell activation, eosinophilia and serum factors with clinical severity. *Clin Exp Allergy* 1993;23:145-53.
37. Kagi MK, Joller-Jemelka H, Wuthrich B. Correlation of eosinophils, eosinophil cationic protein and soluble interleukin-2 receptor with the clinical activity of atopic dermatitis. *Dermatology* 1992;185:88-92.
38. Wuthrich B, Kagi MK, Joller-Jemelka H. Soluble CD14 but not interleukin-5 is a new marker for clinical activity in atopic dermatitis. *Arch Dermatol Res* 1992;284:339-42.
39. Czech W, Krutmann J, Schopf E, Kapp A. Serum eosinophil cationic protein (ECP) is a sensitive measure for disease activity in atopic dermatitis. *Br J Dermatol* 1992; 126:351-5.
40. Krutmann J, Czech W, Diepgen T, Niedner R, Kapp A, Schopf E. High-dose UVAI therapy in the treatment of patients with atopic dermatitis. *J Am Acad Dermatol* 1992;26:225-30.
41. Juhlin L, Venge P. Eosinophilic cationic protein (ECP) in skin disorders. *Acta Derm Venereol (Stockh)* 1991;71:495-501.
42. Paganelli R, Fanales-Belasio E, Carmini D, et al. Serum eosinophil cationic protein in patients with atopic dermatitis. *Int Arch Allergy Appl Immunol* 1991;96:175-8.
43. Kapp A. The role of eosinophils in the pathogenesis of atopic dermatitis—eosinophil granule proteins as markers of disease activity. *Allergy* 1993;48:1-5.
44. Sampson HA. Late-phase response to food in atopic dermatitis. *Hosp Prac* 1987;15:111-28.
45. Gleich GJ. The late phase of the immunoglobulin-E mediated reaction: A link between anaphylaxis and common allergic disease? *J ALLERGY CLIN IMMUNOL* 1982;70: 160-9.
46. Charlesworth EN, Hood AF, Soter NA, Kagey-Sobotka A, Norman PS, Lichtenstein LM. Cutaneous late-phase response to allergen: mediator release and inflammatory cell infiltration. *J Clin Invest* 1989;83:1519-26.
47. Solley GO, Gleich GJ, Jordon RE, Schroeter AL. The late phase of the immediate wheal and flare skin reaction: its dependence upon IgE antibodies. *J Clin Invest* 1976;58: 408-20.
48. Dolovich J, Hargreave FE, Chalmers R, Shier KJ, Gaudie J, Bienenstock J. Late cutaneous allergic responses in isolated IgE-dependent reactions. *J ALLERGY CLIN IMMUNOL* 1973;52:38-46.
49. Leiferman KM, Fujisawa T, Gray BH, Gleich GJ. Extracellular deposition of eosinophil and neutrophil granule proteins in the IgE-mediated cutaneous late phase reaction. *Lab Invest* 1990;62:579-89.
50. Sedgwick JB, Calhoun WJ, Gleich GJ, et al. Immediate and late airway response of allergic rhinitis patients to segmental antigen challenge: characterization of eosinophil and mast cell mediators. *Am Rev Respir Dis* 1991; 144:1274-81.
51. Kay AM, Ying S, Varney. Messenger RNA expression of cytokine gene cluster, interleukin 3 (IL-3), IL-5, and granulocyte/macrophage colony-stimulating factor in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Exp Med* 1991;173:775-8.
52. Fujisawa T, Abu-Ghazaleh R, Kita H, Sanderson CJ, Gleich GJ. Regulatory effect of cytokines on eosinophil degranulation. *J Immunol* 1990;144:642-6.
53. Stone SP, Muller SA, Gleich GJ. IgE levels in atopic dermatitis. *Arch Dermatol* 1973;108:806-11.
54. Sampson HA. The immunopathogenic role of food hypersensitivity in atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1992;176:34-37.
55. Walker IC. Causation of eczema, urticaria, and angio-neurotic edema by proteins other than those derived from foods. *JAMA* 1918;70:897-900.
56. Tuft L, Tuft HS, Heck VM. Atopic dermatitis. I. An experimental clinical study of the role of inhalant allergens. *J Allergy* 1950;21:181-6.
57. Tuft L, Heck VM. Studies in atopic dermatitis. IV. Importance of seasonal inhalant allergens, especially ragweed. *J Allergy* 1952;23:528-40.
58. Mitchell EB, Crow J, Chapman MD, Jouhal SS, Pope FM, Platts-Mills TAE. Basophils in allergen-induced patch test sites in atopic dermatitis. *Lancet* 1982;1:127-30.
59. Clark R, Adinoff A. The relationship between positive aeroallergen patch test reactions and aeroallergen exacerbations of atopic dermatitis. *Clin Immunol Immunopathol* 1989;53:S132-40.
60. Platts-Mills TAE, Chapman MD, Mitchell B, Heymann PW, Deuell B. Role of inhalant allergens in atopic eczema. In: Ruzicka T, Ring J, Przybilla B, eds. *Handbook of allergens in atopic eczema*. Heidelberg: Springer-Verlag, 1991:192-203.

61. Leung DYM, Harbeck R, Bina P, et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis: evidence for a new group of allergens. *J Clin Invest* 1993;92:1374-80.
62. Bruijnzeel PL, Kuijper PH, Kapp A, Warringa RA, Betz S, Bruijnzeel-Koomen CA. The involvement of eosinophils in the patch test reaction to aeroallergens in atopic dermatitis: its relevance for the pathogenesis of atopic dermatitis. *Clin Exp Allergy* 1993;23:97-109.
63. Maeda K, Yamamoto K, Tanaka Y, Anan S, Yoshida H. The relationship between eosinophils, OKT6-positive cells and house dust mite (HDM) antigens in naturally occurring lesions of atopic dermatitis. *J Dermatol Sci* 1992;3:151-6.
64. Bruijnzeel PL, Kuijper PH, Rihs S, Betz S, Warringa RA, Koenderman L. Eosinophil migration in atopic dermatitis. I: Increased migratory responses to N-formyl-methionyl-leucyl-phenylalanine, neutrophil-activating factor, platelet-activating factor, and platelet factor 4. *J Invest Dermatol* 1993;100:137-42.
65. Kapp A, Zeck-Kapp G. Interleukin-5-induced granulocyte activation in atopic patients. *Br J Dermatol* 1991;125:108-15.
66. Morita E, Schroder JM, Christophers E. Chemotactic responsiveness of eosinophils isolated from patients with inflammatory skin diseases. *J Dermatol* 1989;16:348-51.
67. Bruijnzeel-Koomen C, Storz E, Menz G, Bruijnzeel P. Skin eosinophilia in patients with allergic and nonallergic asthma and atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1992;89:52-9.
68. Moser R, Fehr J, Bruijnzeel PL. IL-4 controls the selective endothelium-driven transmigration of eosinophils from allergic individuals. *J Immunol* 1992;149:1432-8.
69. Gundel RH, Gerritsen ME, Gleich GJ, Wegner CD. Repeated antigen inhalation results in a prolonged airway eosinophilia and airway hyperresponsiveness in primates. *J Appl Physiol* 1990;68:779-86.
70. Oyama K. Cutaneous late-phase reaction to environmental antigen in patients with atopic dermatitis. *Dermatology* 1993;187:182-5.
71. Cheng J, Ott N, Peterson E, George T, Gleich GJ, Leiferman KM. Dermal eosinophils in atopic dermatitis undergo cytolytic degeneration [Abstract]. *J Invest Dermatol* 1993;100:545.