

Chemokines and atopic dermatitis

Key words: Atopic dermatitis, chemokines

Atopic dermatitis (AD) is a common condition affecting children and adults. Although clinicians can usually identify the condition fairly easily, it is a very heterogeneous disorder and no absolute diagnostic criteria for AD have been described. The diagnosis is based on the presence of a cluster of major and minor features including pruritus, chronically relapsing dermatitis, the presence of a personal or family history of atopic disease, and a typical distribution affecting the facial and extensor surfaces in infants but flexural regions in older children and adults.¹ A number of lesser features have also been described, including alterations in resistance to cutaneous infections; sensitivity to environmental and emotional factors; intolerance to food, wool, and lipid solvents; and elevated serum IgE.

The histologic features of AD are as diverse as its clinical manifestations. The acute lesions of AD show intracellular and intercellular edema (spongiosis) of the epithelial prickle cell layer, which can enlarge to form intraepidermal vesicles. The damage to the prickle-cell layer leads to disturbed epithelial cell maturation and hence disordered keratinization, which is characterized by parakeratosis. The pure acute form of AD is very rare. Normally the appearances seen are a mixture of acute and subacute dermatitis, characterized by parakeratosis, spongiosis, acanthosis, and mononuclear cell inflammation. If the lesion persists, the epidermis thickens, resulting in the clinical appearance of exaggerated skin markings and lichenification. Chronic dermatitis is characterized by parakeratosis and acanthosis with little spongiosis and variable inflammatory cell inflammation. There is also fibrosis of the papillary dermis. Patients with AD have a very low itch threshold and altered vascular responses to scratching. Thus it would be fair to say that the histologic features represent a combination of the initial inflammatory response and the consequences of the scratching that occurs.

Because AD commonly coexists with other allergic conditions and the total serum IgE value is usually elevated, it has been suggested that the pathogenetic mechanisms of AD may share several features with other forms of allergic disease. One of the ironies of the asthma

revolution is that we feel we know more about the disease activity in asthma, a disease occurring at a remote site, than we do about the mechanisms underlying AD, a disease occurring at the surface of the body and therefore relatively accessible.

Although many patients with AD have elevated total serum IgE levels and most of these will also show positive skin test to house dust mite, there is considerable controversy about the possible role of house dust mite and other allergens in the causation of AD. Some investigators believe that cutaneous presentation of mite allergens may play an important role in exacerbating the condition, but others are skeptical.² Some support for the role of mite allergens in AD comes from the successful use of allergen avoidance measures to control AD.³ The atopy patch test has been used to explore these issues in greater detail.⁴ When relevant allergens are applied to the skin of patients with AD, eczematous responses may develop 24 to 48 hours later. These sites show dermal infiltration of lymphocytes and eosinophils. The T cells present in these biopsy specimens secrete IL-4, IL-5, and GM-CSF but little in the way of T helper type 1 (T_H1) cytokines. After 48 hours, the number of IL-4-producing T cells decreases and the response shifts toward a T_H1 response.^{5,6} Several clinical studies have suggested that eosinophils may play an important role in the clinical disease as well. Patients with AD show preactivation of eosinophils in the peripheral blood and there is a link between the degree of the eosinophil activation and disease activity.⁷ Moreover, histologic studies have shown widespread deposition of eosinophil granule proteins in biopsy specimens of lesional skin of patients with AD. Intact eosinophils may be less prominent and, when present, are mainly found associated with infiltrating mononuclear cells.⁸

In an article in this issue of the Journal, Taha et al (p 1002) present evidence describing the association of chemokine messenger RNA (mRNA) expression with eosinophil infiltration in skin biopsy specimens from patients with AD. The role of chemokines in the selective recruitment of eosinophils has provided a fertile ground for investigation and speculation in recent years. The list of chemokines has expanded enormously of late and as more has become known about the various subfamilies and their receptors, it has become increasingly difficult to achieve a synthetic view of their relevance and function.⁹ In the context of allergic disease, most interest has focused on those chemokines that are capable of inducing eosinophil migration. These include eotaxin-1, eotaxin-2, eotaxin-3, monocyte chemoattractant protein (MCP)-2, MCP-3, MCP-4, RANTES, macrophage-derived chemokine, and macrophage inflammatory protein-1.¹⁰⁻¹⁷ Some of these chemokines also act on basophils. The principal receptor involved in eosinophil attraction seems to be CCR-3, which has been shown to be responsible for the

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Received for publication Feb 8, 2000; revised Feb 17, 2000; accepted for publication Feb 18, 2000.

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J Allergy Clin Immunol 2000;105:892-4.

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0091-6749/2000 \$12.00 + 0 1/1/106570

doi:10.1067/mai.2000.106570

Abbreviations used

AD:	Atopic dermatitis
MCP:	Monocyte chemotactic protein
mRNA:	Messenger RNA
T _H 1:	T helper type 1

majority of the eosinophil chemotactic effect of chemokines.^{12,16,18} These observations have encouraged investigators to believe that targeting the CCR-3 receptor might offer a possible therapy for asthma and other allergic diseases that involve eosinophils.

The article by Taha et al demonstrates the presence of eosinophils in AD lesions in most of the biopsy samples, with considerably more eosinophils in the acute lesions than in sites with chronic lichenification. Expression of mRNA for eotaxin and MCP-4 was also linked to the acute lesions. Interestingly, there was a clear association, both within groups and over the whole series of samples, between the level of eotaxin mRNA expression and the number of eosinophils present. However, there was no association between eosinophil numbers and MCP-4. The expression of MCP-4 mRNA was very tightly correlated to the number of CD68⁺ macrophages in the biopsy samples, reflecting the fact that MCP-4 is produced by macrophages and monocytes. This work raises a number of technical issues and a number of more general points. Assessing correlations between cell numbers and expression of chemokines is not evidence of causation. Thus the expression of MCP-4 is clearly linked to the number of macrophages present in the biopsy specimens. Although this did not correlate with eosinophil numbers, it may be that the factors recruiting macrophages and eosinophils are similar and hence there is a risk of finding a spurious correlation between a macrophage cell product and another cell type.

To understand the significance of these findings, we need to have a clearer view of the role of eosinophils in the pathogenesis of AD. Because we do not know the true pathogenesis of AD, it is tempting to speculate that the cellular infiltrate in biopsy specimens and chronic lesions must be causal, but we need to consider the possibility that these cells are recruited in response to injury rather than being part of the causative mechanisms. In either of these cases, preventing recruitment of eosinophils by a targeted approach against a particular chemokine or chemokine receptor might not prove helpful in controlling the disease. By the same token, it is now generally agreed that eosinophils are important in the pathogenesis of asthma and rhinitis, but the airways of patients with asthma show considerable heterogeneity in eosinophilia or eosinophil protein deposition.^{19,20} Although nobody would argue that eosinophils are not important in asthma, it is now becoming questioned whether eosinophils are the sole mechanism of asthma or indeed whether asthma has a single common pathogenesis. Moreover, even if eosinophils are involved in causing asthma, targeting eosinophils might not be sufficient to

restore the airway to normal. Some concerns have also been raised about whether targeting chemokines and their receptors would be a viable therapeutic approach, given the degree of redundancy between chemokines and their receptors. We believe that this approach deserves further study both in animal models and in man, but some caution is clearly needed.

Several studies have shown that airway epithelium is vulnerable to the effects of particles and other irritants, which induce chemokine expression in the epithelial cells.²¹⁻²³ Chemokine expression is also found in biopsy specimens from the airways of patients with asthma, especially RANTES,^{24,25} although others have not been able to confirm the disease specificity of RANTES expression.²⁶ A number of other chemoattractants such as IL-5, IL-16, and macrophage inflammatory protein-1 have been identified in the airways after allergen challenge.^{27,28} Enhanced expression of eotaxin and RANTES has been demonstrated in skin biopsy specimens from patients with AD, the eotaxin being mainly in mononuclear cells of the dermis.^{29,30} In addition, it has been shown that stimulation of dermal fibroblasts with TNF- α and IL-1 leads to the production of the chemokines RANTES and GM-CSF.³¹ Chemokine production by epithelial cells could thus act to recruit inflammatory leukocytes but is also a feature of a damaged epithelium. One way of clarifying this would be to look at a wide range of chemokines within the biopsy specimens. The study by Taha et al focuses on 2 chemokines that are known to be associated with eosinophil recruitment. The validity of their conclusions would have been enhanced if other chemokines had been studied and found not to be linked to eosinophil or other cellular recruitment. To cite but one example, in a mouse model of AD, expression of the chemokines TARC and MDC was found to be associated with the development of AD-like lesions,³² but that study did not address eotaxin because of lack of suitable reagents. On the other hand, statistical purists would argue that looking for a wide range of chemokines would devalue the statistical associations found with eotaxin by Taha et al. It is true that when multiple associations are sought there is a risk of finding a chance association and regarding it as significant, unless the significance level assigned to *P* values is corrected. Unfortunately, this can encourage investigators to limit their investigation to a prime suspect without considering the possibility of other molecules being involved. Statistical referees and the scientific community at large need to think this through carefully, especially in areas such as chemokine biology where there are so many candidate molecules and receptors. Another statistical issue in this type of study is the performance of correlation statistics with biopsy specimens from different types of lesions. When 3 different types of lesion are studied, there is an increased chance of seeing a difference between the cell numbers of chemokine expression levels in the various groups of biopsy specimens. However, to carry out correlation analysis, it has to be assumed that the samples are all broadly similar. If 2 or 3 distinct groups are included in a correlation analysis, then one will detect the fact that the

groups are different rather than the fact that there is a gradient within each group. Thus correlation statistics are valid when a random set of samples are obtained from lesions of different age or severity but can yield misleading correlations when groups of samples are clustered in some way (eg, by age of lesion or severity).

In summary, therefore we need to understand more about the biologic relevance of eosinophils in AD before we embark on a detailed analysis of how they get recruited. Because there is extensive overlap between various chemokines in terms of cells sources, receptor specificity, and function, we need to know about the full range of chemokines expressed in samples from diseased sites before we can judge the biologic relevance of individual observations. Further work in this area will help us to focus on the most likely targets for therapeutic intervention, but some caution is needed in interpreting the data that are currently available.

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