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Editorial

What can we learn from studies of lymphocytes present in allergic-reaction sites?

Mononuclear cell infiltration has long been recognized as a feature of the pathology of asthma and dermatitis,^{1,2} and the role of T cells in regulating IgE production is well established. Recently, attention has focused on the possibility that these cells play a more direct part in the pathogenesis of allergic disease.^{3,4} Early histologic studies of allergic inflammation provided kinetic data on eosinophil and neutrophil accumulation but provided less attention to mononuclear cells.⁵⁻⁷ In part, this was due to the fact that conventional histologic techniques could barely distinguish monocytes from lymphocytes, much less provide any insight into the functional characteristics of infiltrating lymphocytes. The development of improved immunohistologic techniques has led to a reexamination of the role of mononuclear cells in allergic inflammation.³ Such studies have demonstrated a predominantly CD4⁺ T cell infiltrate in AD,⁸ in the allergen-induced late-phase reaction in man⁹ and in animals,¹⁰ and also in clinical asthma, whether of allergic, non-allergic or occupational etiology.^{11,12} In addition, these studies have provided clear histologic evidence of a relationship between CD4⁺ T cells and activated eosinophils in allergic-tissue reactions,^{9,11} which is probably mediated through the cytokine IL-5.¹³

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Abbreviations used

IL: Interleukin
MHC: Major histocompatibility complex
AD: Atopic dermatitis

The presence of T cells at sites of allergic disease and the recruitment of T cells in disease models both suggest that T cells are involved in the local expression of allergic inflammation. However, by themselves, such observations do not *prove* that the T cell is an active participant. To assess fully the role of the T cell in allergic disease, it is necessary to address the specificity of the recruited T cells and to consider whether the T cells are proinflammatory or perhaps playing a role in clearing up and/or suppressing the inflammatory process.

Interpretation of biopsy studies depends heavily on inductive logic; that is, investigators have to assume that T cells with a given phenotype in tissue behave like peripheral blood T cells of the same phenotype. During the past 5 years, it has become evident that the CD4⁺ T cell subset is, in fact, complex and functionally heterogeneous. CD4⁺ T cells are usually termed "helper/inducer" cells, but in some circumstances they may have a negative regulatory role.¹⁴ In the mouse, CD4⁺ T cells can be subdivided into two main groups, differing in their cytokine profiles. Th1 cells predominantly secrete IL-2 and interferon- γ , whereas Th2 cells primarily secrete IL-4 and IL-5.¹⁵ Depending on the experimental conditions, murine

T cell clones can also demonstrate an intermediate pattern of cytokine production.^{16, 17} The position in man is less clear, with most T cell clones producing a mixed profile of cytokines^{18, 19} that may change if the T cell is stimulated with agents that induce tolerance.¹⁹ Thus, identification of CD4⁺ T cells in a tissue infiltrate can no longer be equated directly with a particular functional phenotype. More precision can, however, be obtained through the use of a larger panel of monoclonal antibodies or if conventional phenotypic studies are combined with studies of cytokine expression with *in situ* hybridization²⁰ or clonal analysis.^{4, 21}

In a study published in this issue of the JOURNAL, Silvennoinen-Kassinen et al.^{21a} have used a broad panel of monoclonal antibodies to demonstrate that peripheral blood T cells that respond to nickel *in vitro* express a similar range of activation markers, as do the T cells that are identified in biopsy specimens of patch test reactions to nickel. There were some differences between the blood and skin T cell phenotypes in terms of transferrin receptor (CD71) expression, which was more frequently observed in the blood-derived cultures, and expression of CD30 (a marker of B- and T-lymphocyte activation) that was present in some biopsy specimens but in none of the blood cultures.

The main limitation of this approach is that T cells in normal skin often express activation markers,²² suggesting either that expression of activation markers has different significance in the skin or perhaps, more likely, that activated cells are preferentially extracted from the circulation into tissue caused by their increased expression of surface-adhesion molecules^{23, 24} and tissue-specific homing receptors.²⁵

How does an antigen-specific T cell get recruited into the inflammatory site? Are antigen-specific cells activated in lymph nodes before migration to the tissue site, or is the initial process antigen independent, with antigen-specific cells meeting antigen in the inflammatory site, being selectively activated and then preferentially retained? Given the low frequency of antigen-specific cells in peripheral blood, the latter mechanism would appear an efficient mechanism for the early recruitment of T cells, whereas, in more chronic forms of inflammation, T cells are probably activated in draining lymph nodes and then recruited into inflammatory sites through their increased expression of adhesion molecules.²⁴

The discovery that naive and primed T cells express different isoforms of the CD45 common leukocyte antigen^{26, 27} suggested that it would be possible to distinguish histologically between selective and non-selective T cell recruitment with the presence of CD45RO⁺ (primed/memory) T cells indicating a

more relevant process. *In vitro*, naive (CD45RA⁺) T cells have very precise requirements for antigen presentation, whereas primed (CD45RO⁺) T cells can recognize antigen presented by a wide range of cells bearing MHC molecules and will thus secrete their cytokines more rapidly after antigen exposure.^{28, 29} Consequently, recruitment of CD45RO⁺ T cells would be consistent with the direct participation of T cells in the inflammatory process, whereas it would be much harder to explain how CD45RA⁺ cells could contribute to the acute-phase response.

However, when a wide range of inflammatory skin conditions were investigated, CD45RO⁺ cells were preferentially recruited in virtually all inflammatory dermatoses,³⁰ including the allergen-induced late-phase skin reaction.³¹ Enhanced recruitment of CD45RO⁺ T cells is probably due to the greater expression of CD11/CD18 adhesion glycoproteins on CD45RO⁺ cells compared with CD45RA⁺ T cells.²⁴ Thus, the driving force for local recruitment of CD45RO⁺ T cells would appear to be up regulation of the appropriate endothelial ligands.^{32, 33} Enthusiasm for the identification of CD45 isoforms in tissue sections has been dampened by recent studies that CD45RO expression may be less stable than previously suggested³⁴ and may identify T cell subsets with varying functional phenotypes.³⁵

Silvennoinen-Kassinen et al.^{21a} studied CD45 isoform expression during *in vitro* culture and found increased CD45RO expression in nickel-reactive blasts. This finding is consistent with previous studies of CD45 isoform expression on cultured cells^{26, 27} but may not be all that relevant to what actually happens *in vivo*³⁶ in which recruitment of memory cells is probably more important than local activation of naive cells and their later transformation into memory cells.

An additional complication has been introduced by the discovery of tissue-specific homing receptors. Within the pool of circulating T cells, there are specialized T cell subsets that home preferentially to the gut, skin, or other organs.²⁵ At the present time, such subsets cannot readily be identified *in vitro*, but clearly this is an important issue when attempts are made to extrapolate results from *in vitro* studies of blood T cells to the skin.

Silvennoinen-Kassinen et al.^{21a} also report the absence of any significant response to nickel (*in vivo* or *in vitro*) by subjects without overt nickel allergy. These authors argue that this finding implies the absence of specific suppressor mechanisms in the allergic subjects, but their results could equally reflect the low frequency of nickel-reactive cells in the non-allergic individual. Other authors investigating this area have been able to isolate nickel-specific T cell

clones from nonallergic subjects and have found little difference in the cytokine profile of nickel-specific T cell clones from nonallergic as opposed to nickel-allergic subjects.⁴ In evaluating such studies, it is important to recognize that the induction of antigen tolerance in vitro is associated with a shift in the profile of cytokine production.¹⁹ The allergen-reactive state may thus represent loss of nickel tolerance rather than abnormalities in cytokine secretion or loss of nickel-specific suppressor cells.⁴

It is clear from the results of Silvennoine-Kassinen et al.^{21a} that their assay preferentially activates CD4⁺ T cells. Evidence from other sources indicates that both CD4⁺ and CD8⁺ nickel-specific T cells are involved in the pathogenesis of contact sensitivity.³⁷⁻³⁹ Lack of response in CD8⁺ cells may well reflect the differing antigen presentation requirements of CD4⁺ and CD8⁺ T cells. In vivo, nickel is believed to act as a hapten and could easily be presented in association with MHC class I molecules, whereas proliferation assays reflect mainly CD4⁺ T cells stimulated by antigen-presenting cells expressing MHC class II molecules.⁴⁰

The extent of cellular activation in allergic inflammation can also be assessed by in situ hybridization. Kay et al.²⁰ studied cytokine expression in the allergen-induced late-phase skin response and found evidence of a Th2-like pattern of cytokine expression at 24 hours after allergen challenge. A similar pattern of cytokine expression has also been reported in bronchial biopsy specimens from atopic subjects with asthma.⁴¹ Such studies need to be reinforced by assessment of the soluble gene products, since the time course and duration of cytokine activity may not directly reflect the amount of cytokine mRNA expression.⁴²

Cytokine-expression studies do not address how many of the activated cells were antigen specific. Cloning out cells from affected tissues offers an alternative approach to this problem and has been successfully used to analyze allograft rejection and tuberculin hypersensitivity.^{43, 44} One of the striking observations in acute allograft rejection is that only a small proportion of the T cells present in the graft actually recognize and respond to the offending allogeneic determinants.⁴³ What then are the other T cells doing there? Are they simply innocent bystanders, recruited because of the up regulation of endothelial adhesion proteins but unable to participate because they do not recognize any of the foreign antigens? Are they autoreactive T cells, recognizing self-antigens? Or are they active participants in the response, activated by cytokines released locally by antigen-specific T cells and then amplifying the response

to assist the antigen-specific cells in dealing with the perceived threat? The recent identification of T cell-derived chemotactic cytokines (e.g., IL-8 and platelet factor 4) support a role for T cells in recruiting other inflammatory cells to tissue sites.⁴²

Exploration of this area requires tremendous dedication and technical expertise. In another article in this issue of the JOURNAL, Sager et al.^{44a} report the results of studies in which they have cloned T cells from patch test sites in two patients with AD. The technique used was to clone T cells directly with *Der-matophagoides pteronyssinus* extract and IL-2⁴⁵ rather than use a nonspecific mitogen to clone all available T cells²¹ and then assess the proportion that were *D. pteronyssinus* specific. Extrapolating from T cell counts, their cloning success rate varied between 1 in 22 and 1 in 95 T cells. Based on previous cloning studies,⁴⁵ it might have been expected that T cell clones of Sager et al.^{44a} would be exclusively antigen specific, but in fact, only half of their clones proliferated when they were subcultured with *D. pteronyssinus*. Sager et al.^{44a} attributed this finding to the presence in the biopsy specimens of T cells that were already nonspecifically activated and were expressing IL-2 receptors. Such cells might thus be able to respond to exogenous IL-2. Against this suggestion, their cultures with IL-2 alone demonstrated much lower cloning rates.

Given the short time frame of these experiments and studies of long-term T cell clones, it appears most unlikely that these "nonspecific" T cell clones were initially *D. pteronyssinus* specific and then lost their ability to respond to *D. pteronyssinus*. In contrast, T cells and clones fed exclusively with IL-2 or mitogens are known to down regulate their surface expression of T cell antigen receptor and associated CD3 and CD4 molecules,⁴⁶ which in turn will lead to decreased antigen responsiveness.⁴⁷

Notwithstanding these reservations, Sager et al.^{44a} have demonstrated elegantly that patch testing with *D. pteronyssinus* leads to a local increase in the proportion of T cells that are *D. pteronyssinus* specific. This finding could represent efficient recruitment and preferential retention of circulating antigen-specific T cells or else result from local proliferation of a small number of cells. It appears likely that both processes contribute to the pathology, but analysis of T cell antigen-receptor gene rearrangements would be one step forward. In addition, these studies support the concept that some of the T cells recruited to patch tests are either nonspecifically activated and acting as "bystander amplifiers," or else they may be responding to, as yet unidentified, antigens (e.g., self-antigens).

Analysis of surface-marker expression in this study indicated that several of these T cell "clones" were, in fact, derived from more than one T cell. In limiting dilution studies, there is always a compromise to be made between the desirability of securing truly monoclonal cultures and the costs of setting up excessive numbers of infertile cultures. Clonality can be readily confirmed by subcloning⁴⁸; alternatively, T cell antigen receptor gene rearrangements or cytokine profiles can be studied.

An important unanswered question is the relevance of the *D. pteronyssinus* patch test to the clinical disorder of AD. When Sager et al.^{44a} analyzed the cytokine-secretion pattern of their T cell clones, most of the clones from *D. pteronyssinus* patch tests were found to be Th2-like, and as might be expected,⁴⁹ a selected panel of IL-4 producers were able to support IgE production in vitro.

Is it then safe to assume that these Th2-like cells are also the effector cells for the lesions of AD? Evidence from other studies suggests that caution is needed in interpreting these findings. Two factors may lead to shifts in the relative proportions of Th1 and Th2 cells obtained by limiting dilution-cloning analysis. First, Th1 and Th2 cells differ in their requirements for antigen presentation.⁵⁰ Second, cloning with IL-2 preferentially stimulates Th2 cells, as compared to cloning in the presence of interferon- γ .⁵¹ In classic contact dermatitis, Th1-like CD4⁺ cells dominate and amplify the generation of antigen-specific CD8⁺ cytotoxic cells that cause the eczematous lesions.^{39, 40, 52} The additional recruitment of Th2-like cells probably reflects the fact that most *D. pteronyssinus*-reactive cells in the peripheral blood of atopic subjects are Th2-like.⁵³ It is also noteworthy that in atopic subjects without AD, Th2-like cells can be recruited and activated after *D. pteronyssinus* skin testing without any sign of dermatitic lesions.^{9, 18}

Perhaps the aeroallergen patch test actually represents a hybrid phenomenon in which both Th1-like and Th2-like cells are recruited, the Th1-like cells inducing the eczematous lesions, as in contact dermatitis, whereas the Th2-like cells control the production of IgE and the eosinophilic infiltrate, as in atopy without eczema. In this case, the difference between AD and atopy without dermatitis may lie, not in the Th2-like subset, but rather in the 15% of clones that were Th1-like. Clearly, the cytokine profile of T cells in lesional skin will reveal some clues as to the true answer. It will also be instructive to examine the clonal frequency of *D. pteronyssinus* reactivity in T cells from spontaneous lesions of AD.

In conclusion, the past 5 years have revealed a revolution in our understanding of the allergic re-

sponse, with increasing emphasis on the role of allergen-specific T cells and their products in controlling both IgE production and the expression of allergic-tissue inflammation.^{3, 54} Much of this finding stems from phenotypic studies of lymphocytes in tissue samples, interpreted in the light of in vitro studies of lymphocyte function. The arrival of new techniques for functional analysis of cells obtained directly from allergic-reaction sites is an important step forward and will undoubtedly help to shed more light on the pathogenesis of allergic disease, provided that the results of these complex studies are carefully interpreted.

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