

Autoreactive CD4⁺ T cells in patients with atopic dermatitis

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Both environmental and genetic factors are known to contribute to the onset of atopic dermatitis (AD). Multiple genes are positively associated with AD, primarily implicating innate and adaptive immunity and epidermal barrier functions as key components of susceptibility.¹ Against this backdrop of genetic risk, multiple environmental factors, including exposure to allergens and infectious organisms, have been implicated as disease triggers.^{2,3} In cases of extrinsic AD, patients have IgE responses to food allergens, aeroallergens, and microorganisms, which correlate with disease severity. Among several microorganisms associated with AD, colonization with *Malassezia sympodialis* is commonly observed. However, recent reports suggest that AD might have an autoreactive component. A recent study documented the presence of numerous specific IgEs, which cross-recognize self-antigens in patients with AD.⁴ In addition, patients with AD with *M sympodialis* colonization were shown to have IgE against *M sympodialis* thioredoxin (Mala s 13) that cross-recognize human thioredoxin.⁵ These reports suggest that self-reactive IgE appears to play an important role in the exacerbation of AD. Emerging evidence suggests that T cells that cross-recognize self-proteins might also play an important role in AD. A 2005 study published in this journal demonstrated both IgE and T-cell recognition of human manganese superoxide dismutase in patients with AD who were sensitized to *Malassezia* species.⁶ However, although this work correlated sensitization to human manganese superoxide dismutase with *M sympodialis* manganese superoxide dismutase, it was not possible to directly demonstrate T-cell cross-recognition because of the methodology used.

In this issue of the *Journal*, Balaji et al⁷ demonstrated that Mala s 13-specific CD4⁺ T cells from *Malassezia* species-sensitized patients with AD were cross-reactive with human thioredoxin at a clonal level. These T-cell clones were cutaneous lymphocyte-associated antigen-positive (implying skin homing), secreted inflammatory cytokines, and could be isolated from both peripheral blood and skin, reinforcing their disease relevance. Most likely, these cross-reactive T cells arise through molecular mimicry. Mimicry has been implicated in other areas of allergic disease, including oral allergy syndrome, in which sensitization by

aeroallergens elicits food-reactive IgE and T cells.⁸ The study by Balaji et al⁷ represents the best evidence that molecular mimicry between non-self-antigens (*Malassezia* species proteins) and homologous self-antigens (human thioredoxin) plays a role in AD at the T-cell level. Notably, the vast majority of individual clones responded more vigorously to non-self-antigens, implicating *Malassezia* species proteins as their primary antigen. Furthermore, Balaji et al⁷ observed that T cells that recognized human thioredoxin could only be detected in patients with AD with *Malassezia* species infection, implying that *Malassezia* species-specific responses are required to elicit self-reactivity.

In detailed cytokine studies with thioredoxin-specific CD4⁺ T cells derived from skin biopsy specimens and peripheral blood, Balaji et al⁷ demonstrated antigen-specific T_H1/T_H2 profiles, which is in agreement with the inflammatory/atopic nature of the disease. In addition, thioredoxin-specific cells produced IL-17 and IL-22, cytokines shown to upregulate antimicrobial peptide in keratinocytes, which plays a role antifungal immunity. T_H17 and T_H22 cells have recently been detected in the skin lesions of patients with AD.⁹ IL-17 is a proinflammatory cytokine capable of causing tissue damage. IL-22 is involved in epidermal remodeling and can lead to hyperplasia of keratinocytes in the epidermis.¹⁰ The detection of thioredoxin-specific IL-17 and IL-22 production, as shown by Balaji et al,⁷ underscores the potential importance of these cells in the disease process of AD. In addition, these cytokines were elicited through stimulation with human thioredoxin, indicating that these cytokines are a component of the self-reactive response.

Thioredoxin is a ubiquitous cellular protein, and thioredoxin-reactive T cells are present in peripheral blood. Therefore it is of interest that no tissue or organ other than skin is affected by thioredoxin-specific T-cell responses in these subjects. Even in healthy subjects the human peripheral T-cell repertoire includes cells that recognize self-antigens. In noninflamed environments self-reactive T cells are typically held in check by regulatory T cells and other tolerance mechanisms.¹¹ However, tolerance can be broken as a result of innate and adaptive immune responses against infectious organisms, such as skin infection by *M sympodialis*. Cytokines, chemokines, and other inflammatory mediators released in this context also increase the apoptosis and necrosis of epithelial cells, releasing additional intracellular antigens. An analogous release of self-antigens from keratinocytes after administering exogenous IFN-γ was demonstrated by Balaji et al⁷ in their current article. These self-antigens are processed by activated dendritic cells and presented to autoreactive T cells, releasing additional mediators and leading to a self-perpetuating cycle of inflammation. In this way the presence of the cross-reactive T cells in the lesion could lead to chronic inflammation, even after clearance of the infection. Such involvement of autoreactive T cells would contribute to the T_H1-type immune responses seen in the chronic phase of AD. Impaired infiltration of forkhead box protein 3-positive regulatory T cells into AD skin lesions could also contribute to this autoimmune process.¹²

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Despite the promise of this interesting work, unanswered questions remain. For example, because the amino acid sequence homology between human and *Malassezia* species thioredoxin is limited (approximately 50%), it is unclear whether cross-reactivity is due to highly homologous conserved epitopes or whether certain sequences are cross-recognized in spite of having dissimilar amino acid sequences. Therefore it would be interesting to define precise epitopes within human and *Malassezia* species thioredoxin.

It will also be of interest to examine autoreactive T cells in patients with AD without *Malassezia* species infection. Patients with AD without *Malassezia* species infection might be sensitized to other microorganisms, food allergens, or aeroallergens, giving rise to T cells that cross-recognize self-antigens in skin lesions. Based on the diversity of IgE-binding self-antigens,⁴ it is likely that T cells infiltrating the lesions of patients with AD recognize multiple self-antigens (in addition to thioredoxin and manganese superoxide dismutase), perhaps because of chronic inflammation. This would agree with the concept of antigenic epitope determinant spreading, as first proposed by Sercarz,¹³ which has been verified in multiple experimental models. Cumulatively, those studies have shown that self-reactive T-cell responses, which initially recognize a single individual epitope, eventually spread to other epitopes within the same antigen and subsequently to determinants within other antigens. Therefore it would be of great interest to determine whether additional self-reactive IgE targets, which have been detected in patients with AD, are also targeted by T cells.

It has long been speculated that infectious agents act as triggering agents in autoimmunity through molecular mimicry. For example, multiple sclerosis has been linked to EBV infection, whereas type 1 diabetes has been linked to enterovirus infection.^{14,15} However, it is difficult to prove whether the infection actually causes the autoimmune disease because the viruses that have been implicated in the diseases are also prevalent in the general population. Likewise, because *Malassezia* species colonization is common in both patients with AD and patients without AD, it will be difficult to definitively prove that *Malassezia* species infection triggers the initial onset of AD in susceptible patients. However, by using the atopy patch test, Balaji et al⁷ demonstrated that Mala s 13 directly induced erythema, T-cell infiltrates, papules, and vesicles. Thus there seems to be little doubt that *Malassezia* species infection can lead to disease exacerbation and that because of the cross-reactivity between Mala s 13 and human thioredoxin, there is an autoimmune component of AD,

which plays a role in the chronic inflammatory stage of the disease. Observations from the current study also raise the possibility that autoreactive T cells can be present in a chronically inflamed environment. The broader implication is that any chronic allergic disorders could have an autoimmune component. These aspects of autoimmunity in patients with AD and other chronic allergic disorders clearly warrant further study.

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