

Frequencies of circulating allergen-specific T cells temporally associate with longitudinal changes in severity of cutaneous atopic disease

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Summary

Increased levels of allergen-specific T-cells have been documented in the peripheral blood of patients with atopic dermatitis (AD) compared with nonatopic controls. However, little is known about how these relate to disease severity. This study sought to examine if frequencies of circulating allergen-specific T cells correlate with changes in clinical disease severity in a cohort of seven adults with AD who were positive for human leucocyte antigen DRB1*1501. We found that frequencies of allergen-specific CD4+ T cells across the study group were not significantly ($P > 0.05$) associated with clinical disease severity; however, longitudinal changes within an individual did correlate significantly ($P < 0.01$) with changes in disease severity. These findings support a role for allergen-specific T-cells in disease pathogenesis.

Atopic dermatitis (AD) or eczema is a chronic pruritic inflammatory skin disease that occurs in approximately 15–20% of children and 1–3% of adults.¹ It is a complex disease, with both genetic and environmental susceptibility factors. Although it is likely that many loci are involved, the recent association of filaggrin null mutations with AD has provided an important step forward in our understanding of disease pathogenesis.² However, there is wide clinical variability in people with filaggrin mutations, and at least 50% of people with AD do not have these mutations. It is therefore likely that other factors are also important. Genetic and functional studies both support a role for systemic bias towards T-helper (Th)2 immune responses in the disease pathogenesis. People with AD often have adaptive Th2 immune responses to common environmental allergens.³ In genetically predisposed people with a systemic Th2 bias,⁴ this may be manifested primarily as a Th2 allergen-specific response. The Th2 cells express

markers that are associated with the capacity to migrate to inflamed skin,⁵ where it is believed they are able to recognize allergens presented by keratinocytes.⁶

Class II tetrameric complexes have been developed over the past decade and provide a unique opportunity to study the frequency and phenotype of allergen-specific T-cells in atopic disease. Furthermore the human leucocyte antigen (HLA) DRB1*1501 Derp1 epitope is known to be an immunodominant epitope in people with AD.^{6,7} However, to date there has been limited investigation into how the immunological response relates to clinical disease severity. One case report used HLA tetrameric analysis to track the frequency and function of T-cell responses in a patient; the percentage of tetramer-positive T-cells was seen to correlate with clinical improvement on methotrexate treatment.⁸

In this study we sought to examine T-cell responses longitudinally in a population of patients with moderate to severe AD, to investigate in more detail how immune responses are related to clinical phenotype.

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Report

The study was approved by the Oxfordshire Research Ethics Committee, and all patients gave informed

consent. Peripheral blood mononuclear cells (PBMCs) of 40 adults with moderate to severe AD were screened for the presence of HLA-DRB1*1501 using sequence-specific PCR. We identified seven HLA DRB1*1501-positive adult patients with severe AD, who were followed up for 18 months. All patients fulfilled the diagnostic criteria for AD as defined by the UK diagnostic criteria, which are both specific and sensitive.⁹ Of the seven patients, three (43%) had ≥ 1 of the common filaggrin null mutations.² All patients had positive IgE to house dust mite [range 3 to > 100 kilounits of allergen (kUA)/L]. None of these patients was on systemic treatments during the period of study.

At the first appointment (baseline) and each subsequent appointment, a validated measure (SCORing Atopic Dermatitis; SCORAD) was used to assess disease severity. This has been shown to be the most reliable grading system for AD and the best at detecting changes in clinical severity. It includes patient assessment of severity with a grading of itch and sleep disturbance on a visual analogue scale (VAS). The scale ranges from 0 to 103, with moderate to severe disease considered to be > 25.¹⁰ All seven patients had moderate to severe AD with SCORAD ranging from 19 to 63, with an average of 36. All 7 patients were seen at least twice in this period; 2 were seen three times and 1 was seen four times (total of 20 samples). Appropriate topical treatments were already being used by patients, and there were no marked changes in therapy over the period.

PBMCs were cultured for 10 days in the presence of a specific peptide derived from Der p 1 that is known to be immunogenic in patients with AD who are positive for HLA-DRB1*1501.⁶ As previously described, we used Der p 1-peptide-specific ELISpot for interleukin (IL)-4 and interferon (IFN)- γ and HLA-DRB1*1501/Der p 1 tetrameric complex staining to analyse the frequency and functional phenotype of the Der p 1-specific CD4⁺ T cells.⁶

HLA-DRB1*1501/Der p 1 tetramer-positive populations (Fig. 1) were found to range from 0.01% to 12% of CD4⁺ T cells. The frequency of IL-4-producing cells specific to Der p 1 correlated significantly ($P < 0.05$) with tetramer staining, suggesting that the tetramer-binding cells were largely of the Th2 phenotype. Although we did observe IFN- γ -producing Der p 1-specific cells, these were significantly lower than the levels of IL-4-producing Der p 1-specific cells. The change from initial readings was measured in both clinical severity and number of tetramer-positive cells.

The correlation between the number of tetramer-positive cells and clinical disease severity over the entire population was not significant ($P > 0.05$; $R^2 = 0.01$) (Fig. 2), implying that across a cohort, the frequency of

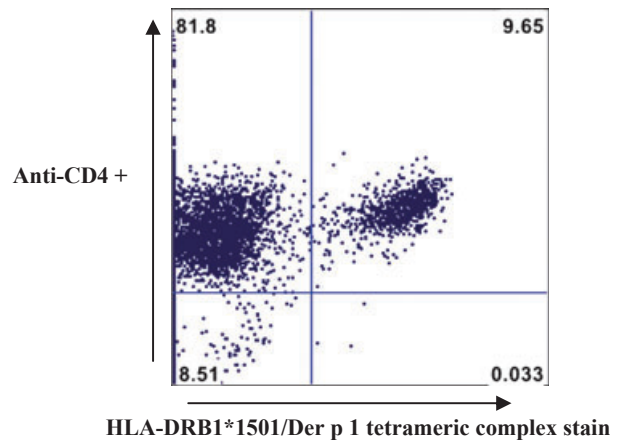


Figure 1 Representative HLA-DRB1*1501/Der p 1 tetrameric complex stain (x axis) with anti-CD4 (y axis).

Der p 1-specific DRB1*1501-restricted CD4⁺ T cells is not a useful population-based tool as a biomarker for disease severity. However, when we examined the changes within individuals over time, we found that the changes in frequency of tetramer-positive cells correlated significantly ($P < 0.05$, $R^2 = 0.67$) with changes in disease severity compared with initial baseline measurements (Fig. 3).

Although cutaneous atopic disease is known to associate with increased frequencies of circulating allergen-specific CD4⁺ T cells, there have been few studies that have related such frequencies to clinical disease severity. If we consider a disease model in which barrier dysfunction promotes epithelial penetration of allergen, leading to priming of allergen-specific T cells that mediate cutaneous inflammation, we would predict that frequencies of allergen-specific T cells would correlate with disease severity. This is an important concept for understanding of disease pathogenesis, yet

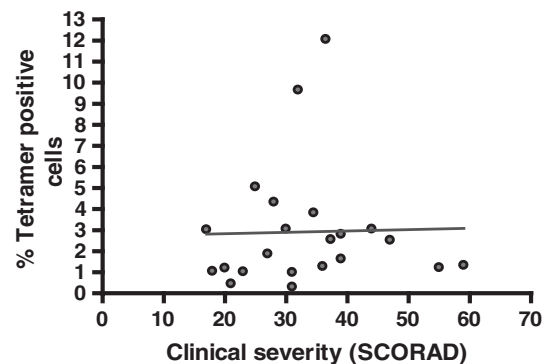


Figure 2 Clinical severity and tetramer-positive cells over all clinic visits ($P > 0.05$).

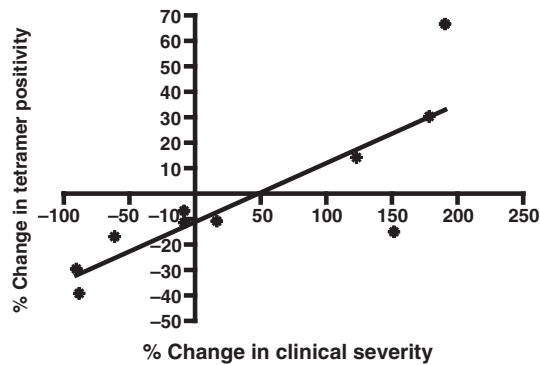


Figure 3 Change in clinical severity and change in percentage population of tetramer-positive cells from baseline (for all clinic visits for all patients) ($P < 0.05$, $R^2 = 0.67$). Each point indicates percent deviation from initial baseline measurement in subsequent clinic visits, and the line indicates the correlation trend.

has been poorly studied. In this study, we found that within an individual, changes in the frequency of circulating allergen-specific T cells associate with changes in disease severity, which is compatible with a role for such T cells in the pathogenesis of cutaneous atopic disease. It may be predicted that filaggrin mutations would predispose to an increased frequency of allergen-specific T cells, and this is currently under investigation.

By using a DRB1*1501 class II tetramer we necessarily restricted our sample to only 7 patients from our initial cohort of 40, a limitation of our study. We now need to extend these findings to other HLA allele-restricted responses and to other allergens.

In summary, our data support a model in which Der p 1-specific CD4⁺ T cells are important for the development of cutaneous atopic inflammation, and relevant opportunities to identify potential treatment targets within such a pathway should be pursued.

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