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# T cell receptor $\gamma\delta$ bearing cells are decreased in the peripheral blood of patients with atopic diseases

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## SUMMARY

The biological role of T cell receptor (TCR) $\gamma\delta$ bearing cells is currently not fully understood. Recently, a monoclonal antibody (TCR$\delta$1) reacting against the whole molecule became available which facilitates the direct analysis of TCR-$\gamma\delta^+$ cells. We studied 11 children with atopic dermatitis, 20 children with atopic asthma, 18 adults with atopic dermatitis and 38 healthy age matched controls aged 4–51 years. Lymphocytes were isolated from heparinized peripheral blood and the proportion of TCR-$\gamma\delta^+$ lymphocytes was determined by FACS analysis. Patients with atopic diseases yielded a significantly ($P<0{\cdot}01$) lower proportion of TCR-$\gamma\delta^+$ cells compared with normal controls (median 4·8% *versus* 7·1%). The percentage of TCR-$\gamma\delta^+$ cells showed an age-dependent decline in both the patient group ($r=-0{\cdot}49$, $P<0{\cdot}01$) and the control group ($r=-0{\cdot}40$, $P<0{\cdot}01$). In addition, the proportion of cells which expressed CD8, TCR-$\gamma\delta$ or CD4, TCR-$\gamma\delta$ simultaneously was determined by double labelling immunofluorescence. Whereas CD4$^+$, TCR-$\gamma\delta^+$ cells could be identified in only a few individuals, CD8$^+$, TCR-$\gamma\delta^+$ cells were found in nearly all controls (median 2·4%, range 0·0–10·8%); atopic patients displayed significantly ($P<0{\cdot}01$) lower proportions of CD8$^+$, TCR-$\gamma\delta^+$ cells.

**Keywords** allergy atopic dermatitis asthma lymphocyte subsets

## INTRODUCTION

The vast majority of mature T lymphocytes in the peripheral blood and lymphoid organs bear the CD3-associated $\alpha\beta$ T cell receptor heterodimer which recognizes foreign antigen peptides only in the context of self MHC molecules. Recently, a small subpopulation of T cells was found to express a distinct T cell receptor (TCR) composed of $\gamma$ and $\delta$ subunits. Currently, the biological role and repertoire of TCR-$\gamma\delta^+$ cells are not fully understood (Brenner, Strominger & Krangel, 1988; Moretta *et al.*, 1989).

Parker *et al.* (1990) provided evidence for an extrathymic post-natal expansion of the TCR-$\gamma\delta^+$ cells found in the peripheral blood. Provided that they have a role in immunoregulation one would expect a perturbation of TCR-$\gamma\delta$ expression in immunoregulatory disorders. An increased frequency of TCR-$\gamma\delta^+$ cells was found in the peripheral blood and the joints of patients with rheumatoid arthritis (De Maria, Malnati & Moretta, 1987; Brennan *et al.*, 1989), in inflammatory lesions of patients with leprosy (Modlin, Pirmez & Hofman, 1989) and in the intestinal mucosa in coeliac disease (Halstensen, Scott & Brandtzaeg, 1989).

The most frequent immunoregulatory disorder in children and young adults is atopy. Atopic diseases are associated with markers of defective cell-mediated immunity. In addition, patients with severe atopic dermatitis display an increased susceptibility to infections with viruses and fungi and a depressed delayed type skin test reactivity to both recall antigens and neoantigens (McGeady & Buckley, 1975; Leung & Geha, 1986).

In this study we used the MoAb TCR$\delta$1 which has panreactivity with all TCR-$\gamma\delta$-bearing cells (Band *et al.*, 1987) to analyse directly the frequency of TCR-$\gamma\delta^+$ cells in atopic subjects and healthy controls.

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## MATERIALS AND METHODS

*Subjects*

The study group consisted of 20 children with atopic asthma (3–16 years of age, median 8·5), 11 children with atopic dermatitis (3–16 years of age, median 9·8) and 18 adult patients with atopic dermatitis (23–57 years of age, median 27 years). The asthmatic patients fulfilled the criteria of the American Thoracic Society. They suffered from an illness of recurrent attacks of diffuse expiratory wheezes audible on auscultation and accompanied by some respiratory distress. Depending on the severity of the disease, patients were treated with inhaled salbutamol, DSCG, beclomethasone or oral theophylline. Medication was withheld for at least 12 h before venous puncture. The diagnosis of atopic dermatitis was made according to the criteria of Hanifin (1984).

**Table 1.** Clinical features and γδ-receptor expression

| | Children with asthma | Children with atopic dermatitis | Adults with atopic dermatitis |
|---|---|---|---|
| Total IgE U/ml (mean ± s.d.) | 499 ± 612 | 1262 ± 2458 | 381 ± 405 |
| Therapy (topical) | | | |
| Topical | | | |
| none | 20/20 | 2/11 | 5/18 |
| hydrocortisone | 0/20 | 1/11 | 5/18 |
| lubricating ointment | 0/20 | 9/11 | 12/18 |
| Inhalation | | | |
| none | 1/20 | 7/11 | 0/18 |
| salbutamol | 19/20 | 4/11 | 0/18 |
| disodium chromoglycate | 7/20 | 1/11 | 0/18 |
| beclomethasone | 5/20 | 1/11 | 0/18 |
| Oral | | | |
| none | 5/20 | 11/11 | 12/18 |
| theophylline | 15/20 | 0/11 | 6/18 |
| Family history, positive/negative | 14/6 | 7/4 | 10/8 |
| Duration of disease, years (mean ± s.d.) | 6·0 ± 2·9 | 8·9 ± 2·8 | 23·8 ± 14·8 |
| Proportion of T-γδ cells (%) | 5·9 ± 2·9 | 7·1 ± 4·0 | 3·2 ± 2·5 |

At the time of investigation all patients were free of respiratory symptoms, infection or other diseases. Age, type of atopic disease, age of onset, family history, specific and total IgE and therapy of the individual patients in relation to expression of TCR-γδ are given in Table 1.

The control group consisted of 21 children aged 4–17 years (median 8·7 years) undergoing elective surgery and 17 adult volunteers 23–51 years of age (median 27·7 years). All controls had a negative personal and family history of atopy and a normal level of total serum IgE. In addition, no specific IgE to airborne allergens could be detected in the serum using the Phadiatop test (Pharmacia, Freiburg, Germany). Control and patient groups were age matched. Written consent was obtained from all patients and controls, or from their parents.

*Isolation of mononuclear cells*

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by means of Ficoll-gradient density centrifugation, washed twice and adjusted to a concentration of $1 \times 10^7$/ml; $5 \times 10^5$ cells were used for one test.

*Cell staining and flow cytometry*

Mononuclear cells were stained according to the manufacturers' recommendations. T cell subsets were enumerated by double labelling immunofluorescence with PE- or FITC-conjugated MoAbs: CD3 (Leu-4), CD8 (Leu-2a) (Becton Dickinson, Heidelberg, Germany), CD4 (T4) (Coulter Electronics, Krefeld, Germany) and TCRδ1 (T Cell Sciences, Cambridge, MA). The antibody TCRδ1 was shown to bind to all γδ-bearing T cell hybridomas tested but failed to react with TCR-αβ cells. In addition, the antibody was demonstrated to precipitate the TCR δ chain either expressed in cells or by *in vitro* translation from cDNA (Band *et al.*, 1987). Ten thousand events were counted using a FACS-Analyser (Becton Dickinson) interfaced with a Hewlett-Packard Consort 30 Computer. The mononuclear cells were gated by volume and light scatter criteria to exclude contaminating erythrocytes and debris.

*Statistical analysis*

Differences between the study groups were tested using the Mann–Whitney *U*-test. The relationship between age, results of pulmonary function tests and percentage of lymphocyte subsets was tested using the Spearman test.

## RESULTS

Peripheral blood lymphocytes of patients with atopic diseases yielded a significantly ($P < 0.001$) lower frequency of TCR-γδ bearing cells compared with normal controls (median 4·8% *versus* 7·1%). As shown in Fig. 1, the difference is highly significant in young children under 10 years of age ($P < 0.01$) and is still significant in older children (10–20 years, $P < 0.05$) and young adults (20–30 years, $P < 0.05$). However, there is no significant difference in individuals older than 30 years. In addition, there is a significant decline of the proportion of γδ cells with age in both the patient group ($r = -0.49$, $P < 0.01$) and the control group ($r = -0.40$, $P < 0.01$).

Analysis of co-expression of CD8 and the TCR-γδ complex revealed that in normal subjects between 1% and 10% of all lymphocytes were double positive. This pattern of distribution is consistently found in all age groups of healthy individuals (Fig. 2). In atopic patients, however, there is a decline in $CD8^+$, $\gamma\delta^+$ cells with age. A significantly lower proportion of CD8 γδ cells was found in children aged 11–20 years, young adults and, most obviously, in adults older than 30 years.

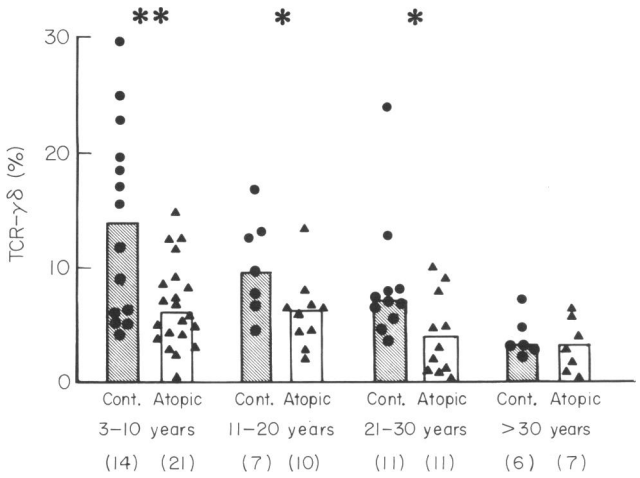


**Fig. 1.** The percentages of TCR-$\gamma\delta$ bearing T cells among the peripheral blood lymphocytes in atopic patients (□) and non-atopic subjects (▨) in different age groups. The bars represent the median value in each age group. *, $P<0{\cdot}05$; **, $P<0{\cdot}01$ compared with age matched healthy controls. The numbers in parentheses indicate the numbers of samples for each age group.

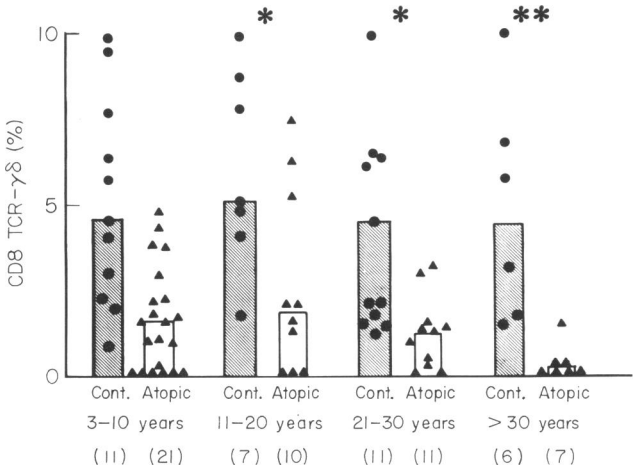


**Fig. 2.** The percentages of CD8, TCR-$\gamma\delta$ bearing T cells among the peripheral blood lymphocytes in atopic patients (□) and non-atopic subjects (▨) in different age groups. The bars represent the median value in each age group. *, $P<0{\cdot}05$; **, $P<0{\cdot}01$ compared with age matched healthy controls. The numbers in parentheses indicate the numbers of samples for each age group.

In some subjects low numbers (0·5–2%) of CD4, $\gamma\delta$ cells were identified. However, there was no age dependency or difference between patients and controls (data not shown).

## DISCUSSION

In this study we used the framework-specific antibody TCR$\delta$1 to examine the frequency of $\gamma\delta$ T cells in patients with atopic diseases and healthy controls. In both groups there was a gradual decline of the proportion of $\gamma\delta$ cells with age. This observation is consistent with results reported previously (Yachie *et al.*, 1989). Parker *et al.* (1990) found that after a rise from birth to 6 years of age the proportion of TCR-$\gamma\delta^+$ cells steadily declines with age. In the present study the youngest age group (3–10 years) had already reached maximal TCR-$\gamma\delta$ expression in both patients and healthy controls. It is remarkable that this age group showed the most striking difference in TCR-$\gamma\delta^+$ cell expression between atopic patients and controls. The peripheral blood of atopic patients yielded a significantly lower proportion of $\gamma\delta$ cells compared with controls. Along with the further decrease of $\gamma\delta$ cells with age the difference gradually diminished.

A possible explanation for the reduced proportion of $\gamma\delta$ cells might be an enhanced influx of these cells into the site of allergic inflammation. This hypothesis seems to be attractive in view of the prominent role of TCR-$\gamma\delta$ bearing cells in murine epithelia (Steiner *et al.*, 1988; Tigelaar, Lewis & Bergstresser, 1990). However, analysis of healthy human skin and lungs did not reveal a prevalent tropism of TCR-$\gamma\delta^+$ cells for human epithelia (Groh *et al.*, 1989; Bos *et al.*, 1990; Foster *et al.*, 1990). Moreover, acute inflammatory infiltrates including those from patients with atopic dermatitis lacked TCR-$\gamma\delta^+$ cells (Dupuy *et al.*, 1990).

Alternatively, our findings can be interpreted as a result of a defective expansion of the TCR-$\gamma\delta^+$ T cell pool. Currently TCR-$\gamma\delta$ bearing cells are thought to expand within the thymus as well as extrathymically (Moretta *et al.*, 1989). Parker *et al.* (1990) provided evidence for an extrathymic post-natal expansion of TCR-$\gamma\delta^+$ cells found in the peripheral blood. This increase is due to an expansion of the cells expressing the variable gene V$\gamma$2 and the CD45RO antigen and might be inhibited in atopy. On the other hand we can not exclude that thymic expansion is reduced since not only TCR-$\gamma\delta^+$ cells as a whole but also the CD8, TCR-$\gamma\delta$ double positive subpopulation is reduced in atopy. CD8$^+$, TCR-$\gamma\delta^+$ cells were shown to belong to the $\delta$-TCS1 reactive V$\gamma$1-bearing subtype which resides predominantly in the thymus in the intestinal epithelium but not in the peripheral blood (Mingari *et al.*, 1988).

At present, the hypothesis of a defective $\gamma\delta$ cell expansion in atopy remains a matter of speculation. Further studies are needed to show whether there is a defect in TCR-$\gamma\delta^+$ cell growth *in vitro*, and in particular whether there is a reduction in V$\gamma$1 or of V$\gamma$2, CD45RO-bearing TCR-$\gamma\delta^+$ cells in the peripheral blood of atopic patients.

However, it is tempting to speculate that a reduced TCR-$\gamma\delta^+$ cell activity could be involved in the pathophysiology of other immunological deviations observed in atopic diseases. A large proportion of TCR-$\gamma\delta^+$ cells has been shown to respond specifically to mycobacterial antigens without MHC restriction (Holoshitz *et al.*, 1989) and an increase of TCR-$\gamma\delta^+$ cells was observed in close association with tuberculous lymphadenitis and in granulomatous reactions of leprosy (Modlin *et al.*, 1989). These observations gave rise to the speculation that TCR-$\gamma\delta^+$ cells may play a role in the initiation of delayed type reactions against mycobacteria (Janis, Kaufmann & Hofman, 1989). Interestingly, atopic patients display a diminished delayed type reaction to mycobacterial antigens (Leung & Geha, 1986). Moreover, TCR-$\gamma\delta^+$ cell clones are able to secrete interferon-$\gamma$ (Kozbor *et al.*, 1989) which suppresses IgE formation (Pene *et al.*, 1988). Thus, it is conceivable that the reduced number of TCR-$\gamma\delta$ bearing cells and perhaps their reduced activity contribute to IgE formation in atopic disease. It is interesting to note that patients with ataxia teleangiectatica, who have been shown

to have an increased proportion of peripheral TCR-$\gamma\delta^+$ cells (Carbonari *et al.*, 1990) have a defective IgE-production (Waldmann *et al.*, 1986). However, since there is a broad overlap in TCR-$\gamma\delta$ expression between healthy individuals and atopic patients, certainly not all immunologic features of atopy can be explained by a defect in TCR-$\gamma\delta^+$ cell activation.

In conclusion, we have shown a reduced proportion of TCR-$\gamma\delta^+$ cells in the peripheral blood of patients with atopy which might be due to a defect in TCR-$\gamma\delta^+$ cell expansion and at least in part be responsible for some immunologic abnormalities found in atopic diseases.

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