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# T- AND B-LYMPHOCYTE SUBSETS IN PATIENTS WITH DUPUYTREN'S DISEASE

## Correlations with disease severity

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Previous reports have indicated that inflammatory mechanisms may be involved in the pathogenesis of Dupuytren's disease and it has even been suggested that this condition is a T-cell mediated autoimmune disorder. We investigated peripheral blood lymphocyte subsets from 21 patients with Dupuytren's disease and compared them with ten healthy blood donors. The Dupuytren's patients had an increase in DR+ T-cells compared with healthy controls. Furthermore, patients with both palmar and plantar involvement had a higher percentage of DR+ T-cells than those with only the palm affected. The percentage of circulating CD5+ B-cells was lower in the Dupuytren's patients compared with the control group; this feature was marginally significant for the whole group of Dupuytren's patients but was strongest in the group of patients with both palmar and plantar involvement. These findings support previous suggestions that immunological mechanisms, involving activated T-cells and probably also B-cells, are involved in the pathogenesis of Dupuytren's disease.

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Dupuytren's contracture is a chronic disease characterized by fibrosis, thickening and shortening of the palmar fascia producing flexion deformities of the fingers. It can in some cases also affect the plantar aponeurosis. The disease is mainly confined to Caucasians and is particularly prevalent among persons of northern European origin. It may be the most common heritable connective tissue disorder (McKusick, 1994) and may in some instances cause severe disability (Jensen et al. 1993; Watson and Fong, 1991).

Diseased tissue from patients with Dupuytren's disease has been reported to have substantially increased infiltration of T-lymphocytes (CD3+) compared with healthy tissues and the majority of these cells are activated, as judged by expression of HLA-DR antigens (Baird et al. 1993a). Therefore, it has been suggested that Dupuytren's disease may be a T-cell mediated autoimmune disorder (Baird et al. 1993a). There are also other reports indicating that dysregulation of the immune system may be of importance in the pathogenesis of Dupuytren's disease. Association with certain HLA-DR subclasses and high prevalence of collagen autoantibodies has been found (Neumuller et al. 1994). Furthermore, an increase in total IgM and IgA antibodies in affected tissues in Dupuytren's patients has been reported (Józsa et al. 1988). It has also been suggested that certain T-cell growth factors may have a role in the pathogenesis of Dupuytren's disease (Baird et al. 1993b; Kloen et al. 1995). A positive association with certain autoimmune diseases, such as diabetes mellitus, has been described (Heathcote et al. 1981; Ravid et al. 1977). Topical steroids and intralesional steroid injections can be an effective treatment for Dupuytren's disease in some cases at least (Aaron and Quesnel, 1978; Pentland and

Anderson, 1985; Shelley and Shelley, 1993). Furthermore, it has been reported that intralesional injections of gamma-interferon (IFN- $\gamma$ ) may have a beneficial effect on the palmar nodules and contractures of Dupuytren's disease (Pittet et al. 1994). These observations are consistent with the possibility that Dupuytren's disease is an autoimmune disorder. We therefore decided to analyse T- and B-lymphocyte subsets in the peripheral blood of a group of patients with Dupuytren's disease.

## MATERIALS AND METHODS

### Patients and samples

Blood samples were collected from 21 patients with Dupuytren's disease and ten matched healthy blood donors, aged 30 to 60 years. Table 1 shows in more detail the demographic and clinical characteristics of the Dupuytren's patients.

Table 1—Clinical characteristics of the 21 patients with Dupuytren's disease

Sex ratio, male/female	15 / 6
Age in years, mean (range)	46.3 (28–70)
Age at disease onset, number	
≤ 35 years	11/21
> 35 years	10/21
Clinical status, number	
Palmar involvement only	11/21
Palmar and plantar involvement	10/21
Had surgery due to contractures	9/21

### Flow cytometric analysis of peripheral blood lymphocyte subsets

The following monoclonal antibodies (Mabs) obtained from Beckton-Dickinson (Paramus, NJ, USA) were fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated: FITC labelled anti-CD4 with PE-labelled anti-CD8; FITC labelled anti-CD8; FITC labelled anti-CD19 and PE labelled anti-DR. DAKO (Glostrup, Denmark) provided the following Mabs: PE labelled anti-CD5; FITC labelled anti-CD3; PE labelled anti-CD45RO; and FITC labelled anti-CD4.

As described in detail elsewhere 50  $\mu$ l of EDTA blood samples were incubated on ice with 6  $\mu$ l of conjugated Mabs for 30 minutes (Arinbjarnarson et al. 1997). After 10 minutes in lysing solution the cells were washed and fixed with 0.5% paraformaldehyde and then analyzed by flow cytometry (FACScan, Beckton-Dickinson). When enumerating double positive cells (i.e. CD3+DR+ or CD19+CD5+) samples stained only for CD3+ or CD19+, respectively, were used to act as the threshold for DR+ and CD5+ lymphocytes. Results are expressed as percentages of each lymphocyte subset, i.e. the percentage of CD3+ T-cells that were also DR+ or B-cells (CD19+) that were also CD5+.

### Statistical evaluation

Results of the flow cytometric analysis are expressed as box plots with medians and the 25th and 75th percentiles (boxes) and 10th and 90th percentiles (error bars). Individual patients falling outside the 10th to 90th percentiles are indicated by circles. Statistical significance between groups was calculated with the Mann-Whitney U-test and correlations with the Spearman's rank correlation coefficient. The level of significance was set at  $P < 0.05$ .

### RESULTS

Figure 1 shows that the patients with Dupuytren's disease had an increased ratio of activated DR+ T-lymphocytes in the peripheral blood compared with the healthy control group (median 15% vs 6% respectively;  $P = 0.008$ ). Furthermore, it can be seen that patients with both palmar and plantar involvement had a higher percentage of DR+ T-cells than those who only had palmar involvement (median 18% vs 12%).

In contrast, Figure 2 shows that the patients with Dupuytren's disease had a decrease of CD5+ B-cells in peripheral blood compared with the healthy controls (median 38% vs 20%;  $P = 0.056$ ). Patients with both palmar and plantar involvement had less CD5+ cells than patients with only palmar involvement or the healthy controls (median 31% vs 15%;  $P = 0.021$ ).

No significant differences were found between patients and controls regarding CD45RO+ T-lymphocytes (memory) or the CD4+/CD8+ helper/suppressor

ratio (data not shown). However, in the patients a significant positive correlation was observed between the CD45RO+CD4+ T-cells and DR expression ( $r = 0.59$ ,  $P = 0.031$ ) and the CD45RO+CD8+ T-cells and DR expression ( $r = 0.65$ ,  $P = 0.011$ ). In contrast, for the control group no significant associations were found between CD45RO+CD4+, CD45RO+CD8+ and DR expression by T-cells ( $r = 0.10$ , NS and  $r = 0.35$ , NS respectively).

### DISCUSSION

The principal finding of this study is that the patients with Dupuytren's disease had a significant increase in activated peripheral blood T-lymphocytes (CD3+ DR+) compared with the control group. This supports a previous observation indicating that activated T-cells may be important in the pathogenesis of Dupuytren's disease (Baird et al. 1993a) and shows that this increase is also present in the peripheral blood. Strikingly, this deviation was greatest in patients with severe disease, i.e. involvement of both the palmar and plantar aponeurosis.

In the patients, but not the controls, we observed a significant positive correlation between DR positivity and both the CD4+CD45RO+ and CD8+CD45RO+ (memory) T-cell subsets. This might indicate that it is mainly the CD45RO+ T-cell population that is of importance in the pathogenesis of Dupuytren's disease, but in this context it should be noted that the occurrence of

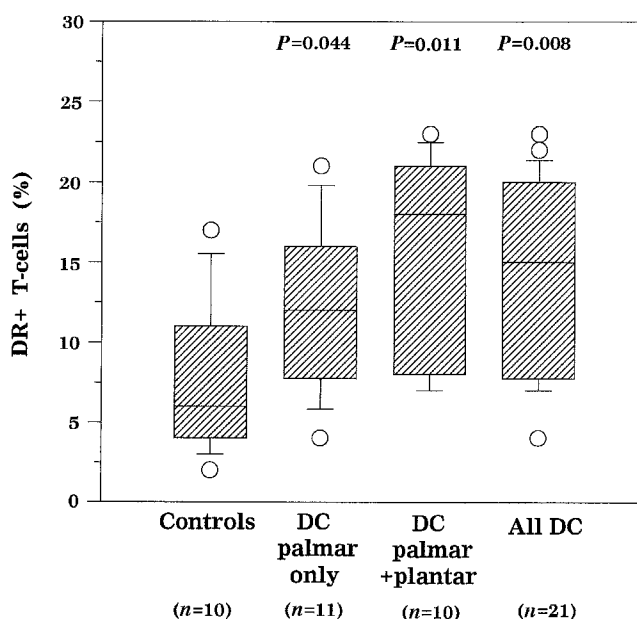


Fig 1 The proportion of T-cells that were DR+ in the control group and patients with Dupuytren's disease affecting only the palmar aponeurosis (DC palmar only), both palmar and plantar aponeurosis (DC palmar+plantar) and the whole group of Dupuytren's patients (All DC). Significance as compared to the control group using the Mann-Whitney U-test.

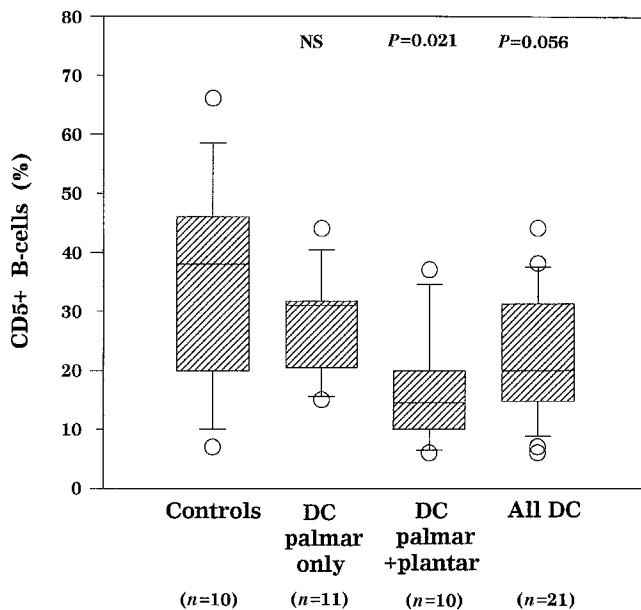


Fig 2 The proportion of B-cells that were CD5+ in the controls and patients with Dupuytren's disease affecting only the palmar aponeurosis (DC palmar only), both palmar and plantar aponeurosis (DC palmar+plantar) and the whole group of Dupuytren's patients (All DC). Significance as compared to the control group using the Mann-Whitney U-test.

CD45RO+ T-cells has never been analysed in diseased tissues from Dupuytren's patients.

A significant reduction in CD5+ B-cells in the blood of patients with Dupuytren's disease was also observed. Such a reduction of CD5+ B-cells has, to our knowledge, not been reported before in patients with Dupuytren's disease. Patients with involvement of both the plantar and palmar aponeurosis showed greater deviations from normal than those who had involvement of only the palmar aponeurosis. The significance of this finding is still not clear. However, it should be noted that patients with certain autoimmune diseases characterized by increased production of autoantibodies, such as rheumatoid arthritis, have elevation of CD5+ B-cells (Arinbjarnarson et al. 1997). The CD5+ B-cells are generally believed to be involved in the production of autoantibodies, including 'natural' autoantibodies. In this context it should also be noted that it has been reported that Dupuytren's disease is very uncommon in patients with rheumatoid arthritis (Arafa et al. 1984).

Intralesional injections of IFN- $\gamma$ , a Th1 (T1 helper cell) derived cytokine, have been found to have beneficial effects on the contractures of Dupuytren's patients (Pittet et al. 1994). Therefore, it is tempting to speculate that a Th1/Th2 imbalance might have a role in the pathogenesis of this disease. Injections of IFN- $\gamma$  might be able to correct such a possible Th1/Th2 imbalance. It should,

however, be noted that at this stage we do not have any experimental data to support this suggestion.

Increased expression of transforming growth factor-beta (TGF- $\beta$ ) has been found in diseased tissues from Dupuytren's patients and TGF- $\beta$  stimulates both proliferation of fibroblasts and collagen production (Baird et al. 1993b; Kloen et al. 1995). In this context it is also relevant that TGF- $\beta$  is the only known cytokine that promotes class switching of B-cells to IgA production (Kim and Kagnoff, 1990; Van Vlasselaer et al. 1992) and interestingly, increased concentration of IgA antibodies has been reported in diseased tissues from Dupuytren's patients (Józsa et al. 1988). The exact antigen specificity of these IgA antibodies is to our knowledge not known, but autoantibodies to collagen types I to IV have been found in Dupuytren's patients (Neumuller et al. 1994).

We conclude that dysregulation of the immune system involving both T- and B-lymphocytes may be important in the pathogenesis of Dupuytren's disease.

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