second patient, it restores sensitivity to low doses of conventional immunosuppressive drugs (unpublished) remains to be seen. Further studies to explore use of these mAbs in other autoimmune diseases seem warranted.

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T-cell-mediated response in Dupuytren's disease

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The cause of Dupuytren's disease is unknown, but inflammatory cells might have a role. Enzymatic digestion of diseased tissue permits identification and immunofluorescent labelling of a cell subset displaying inflammatory cell morphology. Cytofluorimetry of this cell population demonstrated the presence of CD3-positive lymphocytes and expression of major histocompatibility complex (MHC) class II proteins. These results raise the possibility that Dupuytren's disease is a T-cell-mediated autoimmune disorder. The development of medical treatment on this basis may reduce the need for surgery, with its associated morbidity and high recurrence rates.

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Individuals with Dupuytren's disease have a genetically-determined predisposition to the condition.[1] The precise aetiology remains unclear but fibroblasts and myofibroblasts are thought to have a central role,[2] and superoxide free radicals might be the stimulus to myofibroblast proliferation.[2] The condition may also occur in association with various other medical disorders.[3-5] The prevalence and clinical significance of inflammatory cells in diseased tissue is controversial.[6] Baird et al[7] have demonstrated cytokine expression in tissue from Dupuytren patients, and certain inflammatory cells are known to be potential sources of these intercellular signalling molecules. We have examined inflammatory cells in tissue from patient's with Dupuytren's disease by flow cytometry of digested specimens to reduce sampling errors in histological examination of this heterogeneous tissue.

Specimens of subcutaneous tissue were obtained from 13 male and 1 female (aged 43–77 years, mean 63) Dupuytren's patients undergoing palmar fasciectomy. Control tissue was obtained by combining palmar fascia specimens excised from 3 male and 3 female (aged 38–67, mean 58) patients having carpal tunnel decompression. These specimens were pooled to obtain adequate cell numbers for analysis. All operations were done under regional anaesthesia with exsanguination of the limb. Tissue was diced into 2 mm fragments and digested at 37°C in collagenase 0·1%/DNAse 0·01% for 3 h followed by trypsin 0·05%/edetic acid 0·02% for 1 h. Filtration yielded a cell suspension in which residual enzyme acivity was neutralised by washing and resuspending cells in Dulbecco's modified eagle medium containing 10% fetal calf serum (Gibco). Cells were plated out at a maximum initial density of 5×10^6 cells per dish and incubated overnight to allow separation of adherent cells (fibroblasts and macrophages) from inflammatory cells. This procedure also allowed regeneration of cell surface markers depleted by enzymatic activity. Non-adherent cells were harvested and samples of $2{\cdot}0–3{\cdot}0 \times 10^5$ cells were resuspended in 200 µl medium, then labelled with fluorescent monoclonal antibodies designed for flow cytometry (Dako). The panel of paired fluorescent antibody combinations used and the inflammatory cell subsets that they recognise were: CD45/14 (pan-leucocyte/monocyte); CD3/19 (pan T-cell/pan B-cell); CD3/4 (pan T-cell/T-helper cell); CD3/8 (pan T/cytotoxic T-cell); HLA-DR/CD3 (activated cells/pan-T); and CD16+56 (natural killer [NK] cells). Cells were labelled at room temperature for 10 min with 10 µl of neat antibody, washed with phosphate-buffered saline, fixed with 1% paraformaldehyde, and fluorescence was measured with the Lysys II programme on a Becton Dickinson FACScan flow cytometer. Dedicated software permitted statistical analysis of the data obtained. Cytospin preparations of both adherent and non-adherent cells were stained with Giemsa's stain for examination by light microscpy.

Light microscopy of adherent cells showed a homogeneous subpopulation of relatively large cells with foamy cytoplasm. By striking contrast the non-adherent subpopulation was heterogeneous, with many small, darkly-stained cells having the appearance of lymphocytes. The table shows the results from flow cytometry of non-adherent cells. 15–30% (mean 25%) of all Dupuytren cells in the lymphocyte gate were CD3-positive T-lymphocytes, compared with less than 0·5% of non-adherent cells from normal fascia. HLA-DR antigen was detected in 15–41% (mean 27%) of all gated cells from Dupuytren's patients, compared with 1·2% of controls. All other markers were expressed infrequently on Dupuytren cells, indicating a low prevalence of CD4 T-helper cells (1·6–4·3%) and CD8 cytotoxic T-cells (2·7–8·0%). Not shown in the table (because of uniformly low-labelling frequency) are CD16+56 NK cells (<1–1·1%), CD19 B-lymphocytes (<1–5·0%), and CD14 monocytes (<1%). Very low frequencies of antibody labelling were also demonstrated by the pooled control fascia cells (less than 1·5% in each case).

Tissue in Dupuytren's disease contains substantial numbers of CD3-positive T-cells, suggesting that they are important mediators in the pathogenesis of this condition. The low prevalence of CD4, CD8, and CD16+56 antigens indicates that most of these cells may belong to a further

PERCENTAGE OF POSITIVELY-LABELLED CELLS

Specimen	CD45 pan leucocyte	CD3* pan T-cell	CD4 T-helper cells	CD8 cytotoxic T-cells	HLA-DR activated cells
Pooled control fascia	<0·5	<0·5	NT	NT	1·5
Patient					
1	30	27	NT	NT	25
2	16	15	1·9	2·7	29
3	25	23	1·3	4·1	15
4	21	26	2·8	5·0	30
5	26	26	2·2	5·0	30
6	18	26	4·3	6·0	27
7	21	28	NT	NT	26
8	37	25	NT	NT	26
9	14	30	NT	NT	NT
10	15	27	NT	NT	NT
11	22	17	NT	NT	NT
12	14	22	NT	NT	NT
13	34	23	1·8	NT	NT
14	30	29	NT	NT	26

Results >1% rounded to 2 significant figures NT = not tested.
*Several antibody combinations included CD3; results are mean values derived from all available CD3 results for that specimen.

subset of T-lymphocytes, such as the recently described population of "double-negative" T-cells found in epidermis;[8] this population needs to be defined. The increased frequency of HLA-DR-positive cells in Dupuytren's disease indicates expression of major histocompatibility complex (MHC) class II molecules, and the potential ability of these cells to present antigen to T-lymphocytes. HLA-DR is generally recognised as an indicator of cell activation, and activated T-cells also release cytokines that upregulate expression of MHC class II proteins encoded by genes of the HLA-DR locus. These findings are consistent with the inappropriate expression of fibroblast-stimulating cytokines reported in this disorder.[7]

Although T-lymphocytes probably act as mediators in the pathogenesis of Dupuytren's disease, we do not know whether they act as regulator or effector cells; nor has any specific antigen been identified. The precise role of the T-cells requires definition, and further studies are underway to characterise the other cells present in diseased issue (eg, macrophages and their interactions with lymphocytes). Dupuytren's disease might be triggered by the interaction of environmental factors with the primary genetic defect. One hypothesis consistent with our findings is that the defect might occur in genes coding either for MHC proteins or for T-cell receptor proteins. T-lymphocytes are certainly implicated in the pathogenesis of autoimmune disorders, several of which are associated with Dupuytren's disease. The HLA-antigen status of Dupuytren's patients has been recorded,[8] and at least one possible pattern of expression has emerged. Type I diabetes is associated with HLA-DR3 and DR4, and up to 30% of diabetics also have Dupuytren's disease. Conversely, there is a negative correlation between Dupuytren's disease and rheumatoid arthritis, a condition known to have strong association with HLA-DR4. Dupuytren's disease arises in 36% of patients infected with HIV,[4] and subcuticular fibrosis nodules with histological appearances similar to Dupuytren's nodules have been reported in simian acquired immune deficiency syndrome.[9] In alcoholic hepatic cirrhosis, T-cells are thought to support the production of cytokines by liver macrophages and these factors then regulate the fibrotic process.[10] Finally, the onset of Dupuytren's disease following injury in genetically-susceptible individuals[5] might be related to the large pool of activated T-cells and macrophages present in the wound.

The subcutaneous nature of the disorder allows its natural history to be followed with ease; if we can confirm that the disease is caused by cell-mediated immune mechanisms, medical therapy might be developed as an adjunct or alternative to surgery, allowing a more conservative surgical approach with the prospect of reduced postoperative morbidity and recurrence.

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Coelocentesis: a new technique for early prenatal diagnosis

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Chorionic villus sampling and amniocentesis have disadvantages. In 100 women undergoing termination of pregnancy, coelomic fluid was successfully aspirated in 96% of cases at 6–10 weeks' gestation, 42% at 11, and 10% at 12 weeks. Cytogenetic analysis always failed with coelomic fluid, but fetal sexing was always successful with fluorescence in-situ hybridisation and polymerase chain reaction, and the results agreed with those obtained from chorionic villi and amniotic fluid in all cases. Coelocentesis may be suitable for prenatal diagnosis in the first trimester.

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