second patient, it restores sensitivity to low doses of conventional immunosuppressive drugs (unpublished) remains to be seen.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. The precise aetiology remains unclear but fibroblasts and myofibroblasts are thought to have a central role, and superoxide free radicals might be the stimulus to myofibroblast proliferation. The condition may also occur in association with various other medical disorders. The prevalence and clinical significance of inflammatory cells in diseased tissue is controversial. Baird et al 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 exanguination 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 activity 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 × 10⁶ 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 2-3 × 10⁶ 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 recognised were: CD4/14 (pan-leucocyte/monocyte); CD3/19 (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 N1 of neat antibody, washed with PBS, neutralised by washing and resuspending cells in PBS, and then labelled with fluorescent monoclonal antibodies designed for flow cytometry. This panel allowed detection 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.

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.
subset of T-lymphocytes, such as the recently described population of "double-negative" T-cells found in epidermis, 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. 8

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 tissue. 9

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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Chorionic villus sampling and amniocentesis have disadvantages. In 100 women undergoing termination of pregnancy, chorionic 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 chorionic 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.