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Antibodies to Collagen Types I-VI in Dupuytren's Contracture

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Sera from 16 patients with Dupuytren's contracture were tested for IgG and IgM antibodies to native and denatured human collagen types I, II, III, IV, V and VI. IgG antibody to at least one collagen type was found in 11/16 (69%) of these patients, compared with 27/96 (28%) normal adult blood donor controls. The prevalence of antibody to denatured type II collagen was raised, and although there was no overall increase in HLA-DR4 compared with a control population, this antibody was associated with HLA-DR4 in this patient group.

The abnormal palmar fascia of the hand which characterises Dupuytren's contracture contains increased amounts of type III collagen (Bailey, 1977; Bazin, 1980). The primary cause of the contracture is unknown, and the disease has been related to a high alcohol intake with liver disease (Wolfe, 1956), epilepsy (Hueston, 1963), diabetes mellitus, tuberculosis, Peyronie's disease (Billig, 1975) and manual work, especially with vinyl chloride (Bennett, 1982).

The condition may be familial (Manson, 1931). An early study showing a lack of association with HLA-A,B,C (Aron, 1977) was followed by an American study which also showed no association (Hunter, 1981) and there is a suggestion of an association with HLA-B12 (Tait, 1982) and HLA-DR4, with a raised overall prevalence of the HLA-A1-B8-DR3 haplotype (Spencer, 1984), although these associations did not reach statistical significance. HLA-DR4 is associated with rheumatoid arthritis (Dobloug, 1980; Karr, 1980), in which the prevalence of serum antibody to collagen type II is raised; in animal models, injection of this collagen type can induce arthritis (Trentham, 1978; Morgan, 1980; Stuart, 1982) and this response is associated with immunogenetic markers (Griffiths, 1981).

The suggestion that Dupuytren's contracture might be associated with an auto-immune response to collagens was made by Gay (1972). Assays for serum antibodies to collagen types I and III by Menzel (1979) confirmed that a proportion of patients had antibodies, particularly to type I collagen, with 7/24 patients positive.

Serum antibodies to collagens may be measured by a variety of assays (Clague, 1983). ELISA methods have the merit of high sensitivity, but may be prone to non-specific interactions between the solid phase and serum proteins, particularly fibronectin (cold-insoluble globulin) (Beard, 1979; Adelman, 1980). In order to avoid the precipitation and interaction of serum fibronectin with the assay plate, the serum incubation was performed at room temperature; in addition, serum fibronectin levels were measured independently by gel diffusion for comparison.

This study set out to investigate serum antibodies to currently available purified and characterised human collagens, using a sensitive and specific ELISA method, in a group of patients who had been tissue-typed in a previous study (Spencer, 1984). The aim was both to explore the prevalence of such antibodies and to assess the degree of immunogenetic influence on their incidence in this disease.

Materials and Methods

All patients were typed for HLA-A, B, and DR locus antigens with sera standardised against the Eighth histocompatibility workshop sera. Lymphocytes were prepared from ten millilitres of blood taken into an equal volume of 0.5% EDTA. Separation of B and T cells and the HLA typing methods used have been described previously (Welsh, 1978).

Collagen antibodies

Serum antibodies to collagens were measured by an ELISA technique. Flat-bottom microtitre plates ('Immulon', Dynatech) were coated at 4°C with 200ng/well of purified human collagen. Human collagens types I-V were provided by the A.F.R.C. Food Research Institute, Langford, U.K.; purified collagen type VI was supplied by Dr. Steffen Gay, University of Alabama, Birmingham, Alabama, U.S.A. Collagens were used both in native form and after being denatured by heating at 56°C for 30 minutes, and were dissolved for coating in 0.05M bicarbonate buffer pH 9.6. After coating overnight, wells were washed with phosphate-buffered saline pH 7.4 with added 0.05% Tween 20 ('PBS-Tween'). Test sera were diluted 1:100 in PBS-Tween and 200µl/well was added; the plates were then incubated for four hours at room temperature. Wells were washed twice with PBS-Tween. The conjugate was then added as 200µl/well of alkaline-phosphatase labelled rabbit anti-human IgG (Sigma) diluted 1:1000 in PBS-Tween with 0.1% bovine serum albumin. The plates were incubated overnight and wells were washed twice with PBS-Tween. Substrate was added as 200 µl/well of p-nitrophenyl 1 phosphate (Sigma) 1mg/ml in 0.1M glycine buffer pH 10.4, with 0.001M MgCl₂ and 0.001M ZnCl₂. After incubation for thirty minutes in the dark at room temperature, the

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reaction was stopped using 50µl saturated NaOH. The optical density at 410nm was read with a manual plate-reading spectrophotometer (MiniReader II, Dynatech), with computerised output for statistical analysis.

Sera from the sixteen patients described was tested in duplicate at 1:100 dilution, and the results compared with a panel of 24 normal adult sera. The results were expressed as a ratio ('ELISA ratio') of the optical density (O.D._{410nm}) of the test sample to the arithmetic mean O.D. of twenty four normal control samples on the same test plate, all at a serum dilution of 1:100. The twenty-four normal controls were randomly selected from a set of ninety-six blood transfusion donor samples which were also simultaneously tested and the means compared. Samples with an ELISA ratio which was more than 1 s.d. higher than the normal mean were considered positive for antibodies to that collagen type.

The probability that patients with each HLA type should have serum antibody to each collagen type was tested using Fisher's exact test.

Fibronectin assay

Fibronectin levels in the serum samples were measured by radial immunodiffusion. 100µl of rabbit IgG antibody to human fibronectin (Dakopatts) was added to 12 ml 1% agarose containing 0.02M barbitone and 0.04M EDTA buffered to pH 8.6. Wells were filled with neat serum which had been thawed rapidly with

shaking; replicate standard serum dilutions were included on the plate. After diffusion for twenty-four hours at room temperature, the plate was dried, washed and stained with Coomassie Blue. Fibronectin concentrations were measured by comparison of precipitate diameters with the standard curve.

Results

Collagen antibodies

The majority of patients showed IgG antibody activity to a variety of collagen types; none showed significant IgM antibody (data not presented). The number of subjects with IgG antibody to each collagen type, with prevalence in normal controls, is shown in Figure 1. Fibronectin levels were all in the 'normal' range for serum (fibronectin levels are normally measured on plasma samples, since variations tend to occur with clotting); there was no statistical correlation between fibronectin levels as measured and the IgG antibodies to any collagen type.

The prevalence of antibodies to denatured type II collagen was raised overall, but not significantly compared with a control population. However, when the HLA type of the individuals was taken into account, a significant increase in HLA-DR4 in those patients with antibody to collagen type II was found, using Fisher's Exact test. This was not seen with HLA-DR7 or other combinations of HLA type and collagen antibody.

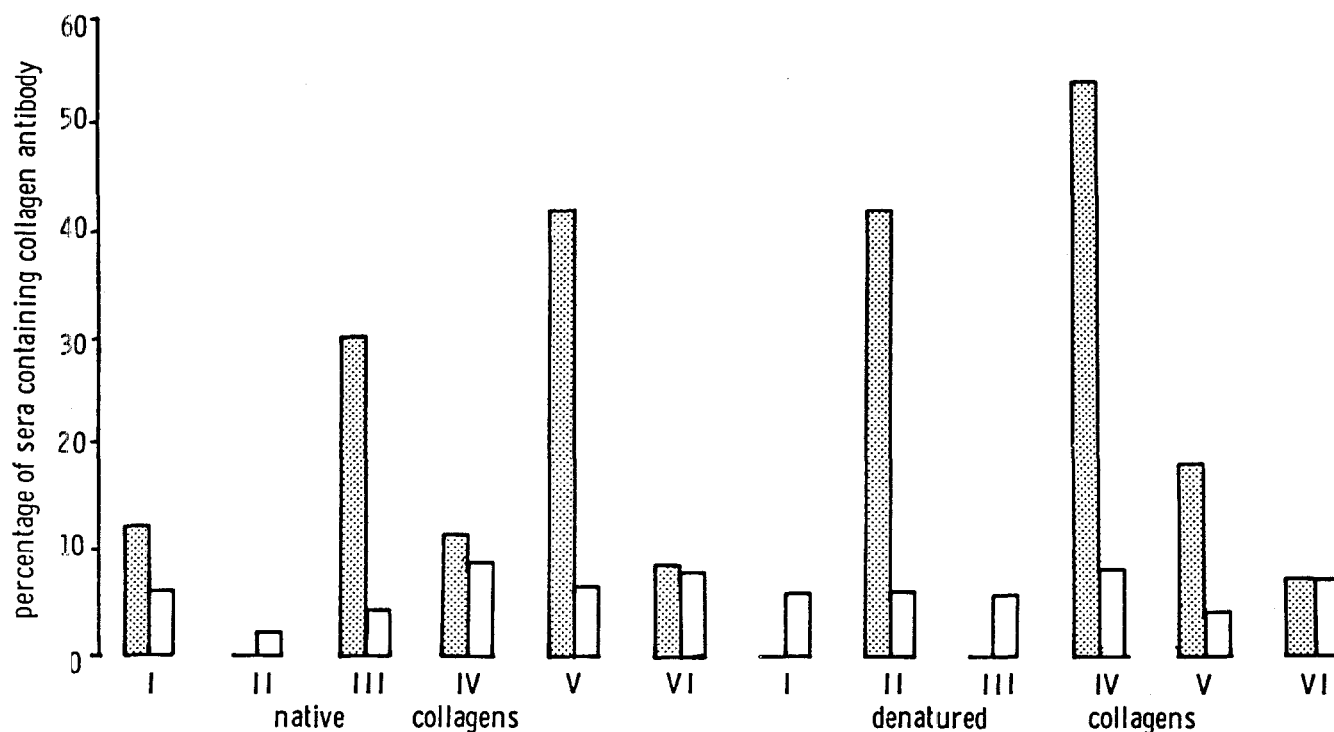


Fig. 1 Numbers of subjects with antibodies to each collagen type. Hatched columns: Dupuytren's contracture (16 subjects); open columns: Normal adult controls (96 subjects).

Discussion

The results confirm an earlier report (Menzel, 1979) that there is an increased prevalence of collagen antibodies in patients with Dupuytren's contracture, and extends the study to consider antibodies to other collagen types and possible immunogenetic associations. The increase in significantly raised antibodies to collagen type I was not as marked as previously reported (12% as compared with 29%). This may be due to our method of expressing the data as 'ELISA ratios', comparing antibody levels to those found in a normal control population, since normal controls may have 'natural' antibodies to type I collagen.

The raised incidence of antibodies to native type III collagen may result from the considerable increase in new type III collagen production which is found at the site of the disease (Bailey, 1977). Antibodies to types IV and V collagens probably reflect a non-specific cross-reaction between antibodies to collagens; this pattern is also shown in normal subjects with collagen antibodies. The raised prevalence of antibodies to denatured type II collagen is intriguing, since antibodies to this collagen type (in its native form) have been associated with connective tissue diseases, particularly rheumatoid arthritis. The association of antibodies to collagen type II with HLA-DR4 (but not with HLA-DR7 or DR3) differentiates this phenomenon from that seen in rheumatoid arthritis, in which the reverse association has been found (Klimiuk, 1984). While there is no suggestion that Dupuytren's contracture is associated with rheumatoid disease, these findings place the condition firmly in the spectrum of connective tissue disorders in which collagen auto-immunity is found.

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