COLLAGEN TYPES AND ANTICOLLAGEN-ANTIBODIES IN DUPUYTREN'S DISEASE

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SUMMARY

The relative proportion of collagen type I and type III in the aponeurosis of twenty-four patients with Dupuytren's disease was determined and compared with the aponeurosis of normal persons. The presence of considerable amounts of type III collagen was found in the Dupuytren's disease patients. The sera of the patients were screened for circulating anti-collagen antibodies using a sensitive radioimmunoassay. In seven out of the twenty-four patients low concentrations of these antibodies were found.

INTRODUCTION

Dupuytren's disease is characterized by progressive irreversible contracture of one or more fingers (Dupuytren, 1831; Calnan, 1977). In spite of many hypotheses (Millesi, 1965), both the aetiology and the pathogenesis of this frequent hereditary connective tissue disease remains obscure. Chemical studies centering on the collagen of Dupuytren's contracture have been notably few (Hunter, 1975; Bazin, 1977; Bailey, 1977). It was Bailey's group who demonstrated that the contractures and even the apparently unaffected aponeuroses from patients with Dupuytren's disease contained significantly higher amounts of type III collagen than the aponeurosis of normal persons.

Millesi (1959) was the first to discuss an autoimmunological mechanism as trigger for the cell proliferation following the primary step of Dupuytren's disease, namely thickening and coalescence of collagen fibrils. Collagen as a possible autoantigen in Dupuytren's disease has been suggested by Gay (1972). In this paper we report the results of several immunologic investigations such as radioimmunoassay for anticollagen antibodies in sera of patients with Dupuytren's disease together with some data on the presence of type III collagen in the contractures of these patients as compared to normal adult subjects.

MATERIALS AND METHODS

Materials: Specimens were surgically excised from twenty-four patients with Dupuytren's disease (twenty-two men, two women; ages 23-77 years). Samples of aponeurosis from six normal persons (four men, two women; ages 16-63 years) were obtained as controls. All specimens were stored at —70°C. Sera were also stored at —70°C.

Collagen extraction and separation of collagens type I and III: The tissues were cut into small pieces (3.5 mm^2) and defatted by extraction with ether:ethanol (1:1) for 20 h at 4°C. The dry residue was homogenized in phosphate-buffered saline and stirred for two days at 4°C. Then the tissue was extracted in 0.5 M acetic acid (2d, 4°C). The insoluble residue was resuspended in 0.5 M acetic acid (50 ml per gram of original

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TABLE 1
RELATIVE CONTENT OF COLLAGENS TYPE I AND TYPE III IN DUPUYTREN'S CONTRACTURES

Patient*	Age	Duration of disease	Stage**	Localisation	Type I (%)	Type III (%)
1	55	0.5	—I	r	61.6	38.4
2	51	1	45°II	5 l	85.2	14.8
3	77	1	30°I	4.5 r	55.5	44.5
4	35	2	45°II	3 r	68.2	31.8
5	64	2	90°III	4 r	62.4	37.6
6	56	3	30°I	4 r	61.9	38.1
7	68	4	—I	1 r	59.3	40.7
8	72	5	60°II	4 r	64.7	35.3
9	60	5	60°II	4.5 l	49.9	50.1
10	54	6	60°II	4 l	60.0	40.0
11	55	6	90°III	4.5 l	46.6	53.4
12	56	7	80°II	4.5 l	61.9	38.1
13	31	8	30°I	3 r	52.4	47.6
14	52	8	30°I	4 l	41.4	58.6
15	59	8	80°II	5 l	41.1	58.9
16	62	10	90°III	4 l	87.6	12.4
17	56	10	45°II	4.5 l	52.3	47.7
18	43	10	90°III	5 l	50.9	49.1
19	64	11	—II	1 r	67.2	32.8
20	23	11	20°I	5 r	38.5	61.5
21	44	12	80°II	5 r	57.0	43.0
22	58	15	40°II	4.5 l	84.0	16.0
23	74	20	90°III	4 r	77.0	23.0
24	72	30	135°IV	5 r	40.3	59.7

*) Patients 14 and 19 are women

**) According to Hueston and Tubiana (1974)

dry tissue), 200 mg pepsin (Worthington Biochem. Corp., Freehold, N.J., USA) were added and solution was performed during two days at 4°C. The dissolved collagen was reprecipitated by adding sodium chloride to a concentration of 0.9 M/l. The precipitatate was dissolved in 0.5 M acetic acid and dialyzed against a large excess of Tris-HCl buffer pH 7.5 (0.05 M, containing 1.0 M/l NaCl). To the clear solution NaCl was added to a concentration of 1.6 M/l. This precipitated type III collagen. After 20 h at 4°C and centrifugation at 5000 g, the supernatant was brought to a NaCl-concentration of 2.4 M/l by addition of solid NaCl. The sediment (mainly

TABLE 2
RELATIVE CONTENT OF COLLAGENS TYPE I AND TYPE III IN NORMAL OR APPARANTLY NORMAL APONEUROSIS

Patient*	Age	Operated for	Localisation	Type I (%)	Type III (%)
25	16	Triphalangy	2 r	96.4	3.6
26	37	Injury, restitution of tendon	2 r	94.1	5.9
27	48	Carpal tunnel syndrome	2 r	93.5	6.5
28	49	Injury, restitution of tendon	4 r	97.6	2.4
29	58	Carpal tunnel syndrome	2,3 r	95.2	4.8
30	63	Carpal tunnel syndrome	2 r	97.4	2.6
4	35	apparently normal apon.	5 r (rad.)	88.8	11.2
4	35	apparently normal apon.	5 r (uln.)	86.4	13.6

* Patients 25 and 30 are women

type I collagen) was again harvested by centrifuge at 5000 g (Chung,1974). The amount of collagen of type I and type III was determined after hydrolysis in half concentrated hydrochloric acid using the method of Stegmann (1967). Since the hydroxy-proline content of type I collagen is different from that of type III collagen (Steven, 1967; Epstein, 1974), different factors were used in computing the amount of each collagen type. The identity of type I and type III collagen was confirmed by acrylamide gel electrophoresis before and after reduction with ß-mercaptoethanol (Bailey, 1975).

Immunofluorescence: The sera of all patients were investigated by immunofluorescence staining technique. The existence of the following antibodies in the sera of patients with Dupuytren's contracture was checked: Antinuclear antibodies, antimitochondrial antibodies, anti-basement membrane antibodies and antibodies against smooth and/or striated muscle. These investigations were performed on organs from rats (Ludwig, 1974). Human spleen was used for the detection of anticollagen antibodies. Rabbit anticollagen antibodies against human collagen type I were used as control in this investigation. All other tests were performed with the appropriate positive control sera. Moreover, sections of the palmar fascia from patients with Dupuytren's contracture were incubated with the autologous sera. The sections were obtained by cutting fresh snap-frozen tissue in 4 µ sections in a cryostat at —20°C. Staining was performed with FITC-coupled goat anti-human IgG, IgM and IgA (Behringwerke, Marburg, W.Germany) or, in the case of anticollagen antibodies derived from rabbits, with FITC-coupled goat anti-rabbit gamma globulin (Hyland, Div. Travenol Lab., Costa mesa, Calif.. USA).

Radioimmunoassay for anticollagen antibodies

Antibodies to collagen were determined using a radioimmunoassay (Menzel, 1977). As labelled antigen collagen type I from human dura mater was used in denaturated form.

TABLE 3
RESULTS OF RADIOIMMUNOASSAY FOR ANTICOLLAGEN ANTIBODIES

Patient	Age	Duration of disease	Radioactivity precipitated (dpm)**	Immunofluorescence
2	51	1	196	—
10	54	6	252	—
16	62	10	147	—
17	56	10	191	±
18*	43	10	187	—
19	64	11	173	—
24 24	72	30	158	—

All other patients were negative in radioimmunoassay (precipitated radioactivity in the normal range of 30 — 100 dpm) and in immunofluorescence.

* This patient suffered from rheumatoid arthritis.
**Disintegration per minute

RESULTS

Contractures of 24 patients with Dupuytren's disease, six tissue specimens from six normal adult persons and two specimens from normal regions of the aponeurosis of patient 4 (Table 1) were investigated as to the relative amounts of type I and type III collagens. The results are shown in Table 1 (patients with Dupuytren's disease) and Table 2 (controls). We found a significant increase ($p<0.001$) in the relative content of type III collagen in the contractures of the patients with Dupuytren's disease as compared to the controls (average content of type III collagen in Dupuytren's contractures: 40.55%, S.D. = 13.95; controls: 4.32%, S.D. = 1.71).

In preliminary experiments using artificial mixtures of ^{3}H-labelled type I collagen with type III collagen we found that 2-5% type I collagen are coprecipitated in salt fractionation at 1.6 M/l NaCl concentration with type III collagen. The content of type III collagen may therefore be overestimated, if this separation technique is used. This is true, however, both for Dupuytren contractures and controls. In acrylamide gel electrophoresis the characteristic pattern of type I and type III collagen was obtained, if the precipitates formed at 2.4 molar or 1.6 molar NaCl concentration, respectively, were examined.

In radioimmunoassay using denatured type I collagen as labelled antigen, we found in 7 out of 24 patients low concentrations of anticollagen antibodies (Table 3). In the immunofluorescence studies only patient 17 (Table 1) showed a weak positive anticollagen fluorescence. Antinuclear fluorescence was negative in all patients with the exception of patient 18, who was weakly positive. All other immunofluorescence tests were negative in all cases.

DISCUSSION

The investigation of contractures from patients with Dupuytren's disease as to the distribution of type I and type III collagens revealed the presence of significant amounts of type III collagen. In contrast to this, the aponeurosis of normal subjects

contained only low amounts of collagen precipitating at 1.6 molar NaCl concentration. This finding accords well with the results of Bazin (1977), who used a different collagen extraction method. It also correlates well with amino-acid analyses of Dupuytren's contractures published by Hunter (1975), which indicated a clearcut increase in the hydroxyproline content as compared to control aponeurosis. It is well known that type III collagen contains much more hydroxyproline than type I collagen (Epstein, 1974; Steven and Jackson, 1967). As far as the type distribution of collagen is concerned, the fibrous tissue of Dupuytren's contracture is therefore similar to scar tissue, which it also resembles histologically.

TABLE 4
CHARACTERISTICS OF COLLAGEN I AND III

	Type I	Type III +
Occurrence	Skin, bone, tendon, aorta	Skin, aorta
Hydroxyproline content (residues per molecule)	290	375
Proline: Hydroxyproline	1.0	1.0
Contains Cysteinyl residues	no	yes
Precipitates from neutral solution at a NaCl-concentration of	2.2-2.4 M	1.5-1.6 M

+) Increased proportions of type III collagen typical for embryonic tissue or granulation tissue as formed during wound healing or in acute or chronic inflammation

Some features of Dupuytren's disease suggest a possible involvement of immunologic mechanisms in the pathogenesis of the disease (Gay, 1972; Noeva, 1977). In 1959 Millesi proposed an autoimmunological mechanism accounting for the cell proliferation in Dupuytren's disease (Millesi, 1959. Gay, 1972) discussed the hypothesis of collagen autoimmunity (Steffen, 1970) in connection with the disease. One important piece of evidence for such hypothesis would be the demonstration of circulating anticollagen antibodies. To this aim, we used two sensitive methods: Immunofluorescence and radioimmunoassay.

In the latter technique we employed type I collagen as labelled antigen, since this collagen type can be extracted from human tissues, such as dura mater, using buffers without the addition of pepsin. Therefore, this collagen still contains the non-triplehelical telopeptide regions, which may be of immunologic importance.

Our results do not bolster the hypothesis of collagen as the auto-antigen, supporting self-perpetuation of Dupuytren's disease. In 7 out of 24 patients only very low concentrations of antibodies to denatured type I collagen were present. One of the positive patients (Patient 18, table 1) suffered from rheumatoid arthritis, which is known to cause the appearance of anticollagen antibodies in the patients' sera. As compared to other diseases which are characterised by high concentrations

of anticollagen antibodies, such as rheumatoid arthritis (Steffen, 1973; Menzel, 1977), the low amounts of anticollagen antibodies found by us in the sera of patients with Dupuytren's disease seem to be of minor relevance. It cannot be excluded, however, that a large part of anticollagen antibodies is tissue-fixed and therefore not circulating. In this context a paper of Gay (1972) is of interest. The authors found in Dupuytren's aponeuroses, that had been surgically removed, large amounts of IgG, IgA and IgM. Elution studies must show, whether these antibodies have anticollagen specificity.

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