Anti-cartilage antibody

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SUMMARY Antibody to cartilage has been demonstrated by indirect immunofluorescence on rat trachea in the serum of about 3% of 1126 patients with rheumatoid arthritis. Titres ranged from 1:20 to 1:640. The antibody was not found in 284 patients with primary or secondary osteoarthritis or in 1825 blood donors, nor, with the exception of two weak reactors, in 1314 paraplegic patients. In most cases the antibody appears to be specific for native type II collagen. Using this as an antigen in a haemagglutination test 94% of anti-cartilage sera were positive, whereas among 100 rheumatoid control sera there were only three weak positives. More than 80% of patients with antibody had some erosion of articular cartilage, but there was no correlation with age, sex, duration of disease, nor any recognisable clinical event or change.

Reports of anti-cartilage antibody in human sera are uncommon and have been limited to a few cases of relapsing polychondritis (Hughes *et al.*, 1972; Rogers *et al.*, 1973).

In the course of a survey of anti-tissue antibodies by immunofluorescence on rat tissues we found a serum which reacted strongly with a piece of tracheal cartilage accidentally included in one of the blocks. Since this occurrence in 1975, tracheal and costal cartilage have been included routinely in the tissue blocks, and we have found more than 40 patients with anti-cartilage antibody, most of whom had rheumatoid arthritis.

This paper describes the antibody, its probable identity with anti-type II collagen, and its incidence among 5000 patients and blood donors.

Material and methods

SOURCES OF SERA

1 Blood donors

1825 sera were kindly supplied by the Oxford BTS, who also provided details of the age and sex (but not the names) of donors.

2 Patients of the National Spinal Injuries Centre

Inpatients were tested once a month and outpatients at each six-monthly attendance. 1314 patients were tested on an average of 2.3 occasions.

3 Patients of the Oxford Regional Rheumatic Diseases Research Centre

Inpatients were tested once a month and outpatients usually at each attendance. 1785 patients were tested on an average of 3.0 occasions.

TEST FOR ANTI-TISSUE ANTIBODIES

Sera were tested by indirect immunofluorescence (IF) on cryostat sections of unfixed rat tissues. Rat tissues were assembled into two composite blocks: (i) kidney, liver, sciatic nerve, and stomach, (ii) costal cartilage, heart muscle, intercostal muscle, oesophagus, spinal cord, submaxillary gland, thyroid, and trachea.

Fluorescein-conjugated sheep anti-human sera: anti-immunoglobulin, anti-IgG, anti-IgM, anti-IgA and anti-C₃ were obtained from Wellcome Reagents Ltd. Sera to be tested were screened at a dilution of 1:10 using the anti-immunoglobulin. The immunoglobulin class of any antibody present was then determined and titrated with the specific immunoglobulin antisera.

SHEEP CELL AGGLUTINATION TEST (SCAT)

Sera to be tested were diluted in 2.5% sheep serum (Greenbury, 1960).

LATEX AGGLUTINATION

Slide tests were performed at a single serum dilution (1:20) using a suspension of latex particles (Bacto-Latex 0.81) mixed with human Cohn fraction II previously heated to 60°C for 2 minutes.

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HAEMAGGLUTINATION TEST (HA) FOR ANTIBODIES TO TYPE II COLLAGEN

Formolised sheep red blood cells were tanned with 1:40 000 tannic acid and then sensitised with human native type II collagen at the optimum dilution determined on the day of test. The collagen was derived from the nucleus pulposus of the intervertebral disc and was prepared and kindly supplied by Dr Helen Beard (Charles Salt Research Centre, Oswestry). Sera were absorbed with unsensitised cells before being titrated in microtitre trays.

Results

IMMUNOFLUORESCENCE

In rat tracheal cartilage the commonest pattern was fluorescence at the periphery of the cartilage only (Fig. 1b), although some of the strongest sera produced fluorescence throughout the matrix (Fig. 1a). If the sections were pre-treated with hyaluronidase all anti-cartilage sera produced a degree of generalised fluorescence. In costal cartilage fluorescence occurred throughout the matrix even with the weaker sera; pre-treatment with hyaluronidase enhanced the fluorescence but did not alter its distribution.

All positive sera reacted with human tracheal and articular cartilage. A small number of positive sera were tested with pig trachea, fowl trachea, and rat pinna; all reacted. Immunofluorescence titres varied from 1:20 to 1:640 with a mode of 1:40; the antibody

Table 1 Incidence of anti-cartilage antibody

Diagnosis	No. of individuals	Positive	
		No.	%
Rheumatoid arthritis	1126	37	3.3
Psoriatic arthropathy	67	1	1.5
Primary generalised osteoarthritis	97	0	—
Secondary osteoarthritis	187	0	—
Ankylosing spondylitis	183	0	—
Polymyalgia rheumatica	99	0	—
Gout	26	0	—
Paraplegia	1314	2	0.2
Blood donors	1825	0	—

was exclusively IgG and did not react with anti-IgM or IgA. There was no fixation of complement (C₃).

OCCURRENCE OF ANTI-CARTILAGE ANTIBODY

Anti-cartilage antibody was found in 40 patients, all but two of whom had rheumatoid arthritis or psoriatic arthropathy, an incidence of about 3% (see Table 1). The two non-rheumatoid patients with anti-cartilage antibody were from among 1314 paraplegics; they gave only weak but repeated positive reactions. In addition, there were 21 doubtful positives, that is, patients from whom a single serum, either one of a series or the only serum tested, gave a weak anti-cartilage reaction. At least 14 of these patients were suffering from rheumatoid arthritis or psoriatic arthropathy. No cartilage positive serum was found among blood donors or patients with locomotor disease other than rheumatoid arthritis, including 284 patients with osteoarthritis.

Fig. 1 Patterns of immunofluorescence in rat tracheal cartilage ($\times 100$): (a) generalised; (b) peripheral; (c) negative control.

The 40 patients with anti-cartilage antibody are detailed in Table 2. The only finding positively correlated with anti-cartilage antibody was the presence of articular erosions (x-ray grades 3 and 4), which were, or had been, present in 25 (78%) of the 32 patients on whom data were available. The actual incidence of erosions probably exceeded 78%, since the current practice is, after initial x-rays of hands and feet, to take no further radiographs except perhaps at three-year intervals, unless surgical intervention is contemplated.

The presence of anti-cartilage antibody was not related to age, sex, or duration of disease. The exact relationship with duration of disease could not be determined since 32 of the patients were positive on first testing, when in many cases the disease had been present for some years. The mean age of patients with anti-cartilage antibody was, however, the same as that of the whole population of rheumatoid patients attending the Rheumatic Research Centre. Antibodies to other tissues were often present with anti-cartilage, but none occurred with any greater

Table 2 Patients with anti-cartilage antibody

Patient	Sex	Age* (yr)	Diagnosis	Duration* of illness (yr)	x-ray grade	Rheumatoid factor†		Anti-cartilage antibody			Anti- collagen II antibody
						SCAT	LATEX	Period of testing (mth)	Antibody‡ change	IF titre	HA titre§
AA	M	71	RA	NA	NA	v	v	.	.	40	10
BE	M	75	RA	19	3	+	+	12		80	20
BH	F	56	RA	11	3	v	v	34	□	NT	NT
BI	F	72	RA	10	3	v	v	18	□	40	80
BK	F	55	RA	3	3	+	+	.	.	40	20
BW	M	63	RA	5	3	v	v	39	△	80	< 5
CB	M	43	RA	5	2	v	v	19	□	40	10
CL	F	55	RA	14	3	+	+	40	□	160	640
CV	F	64	RA	21	4	—	v	32	□	640	1600
DA	F	72	RA	19	2	—	—	29	□	80	80
ED	M	56	RA	8	3	v	v	16	□	320	> 1600
FA	F	54	PsA	14	3	v	v	23	.	40	640
FC	F	70	RA	1	NA	—	—	.	.	40	20
FE	F	70	RA	14	3	+	+	25	□	40	40
FH	M	69	RA	16	3	+	+	20	□	80	160
FJ	F	49	RA	15	NA	v	v	36	△	80	20
FL	F	67	RA	10	3	+	+	13	△	40	40
GI	F	52	RA	< 1	1	—	—	15	△	320	1600
HH	F	59	RA	4	3	v	v	20	△	NT	NT
HJ	F	36	RA	4	2	v	v	40	△	40	20
JM	F	65	RA	2	3	v	v	38	△	40	20
KI	F	51	RA	16	3	v	v	40	□	160	1600
KK	F	76	RA	17	NA	+	+	5	□	40	5
KM	F	62	RA	4	3	—	v	27	△	NT	NT
LW	M	77	RA	20	3	+	+	23	□	80	1600
MJ	M	45	RA	6	3	v	v	38	△	40	40
OH	M	61	RA	8	3	v	v	26	△	40	40
OT	M	57	RA	11	3	+	+	.	.	160	1600
PR	M	59	RA	NA	3	v	+	15	□	40	20
PS	F	68	RA	4	1	+	+	28	□	NT	NT
RE	M	71	RA	16	3	+	+	.	.	20	20
RM	F	44	RA	NA	3	+	+	18	□	NT	NT
SA	M	40	RA	NA	NA	v	+	8	□	20	< 5
SF	F	50	RA	2	2	—	—	10	△	160	160
SK	M	65	RA	14	NA	v	v	26	△	20	10
SM	F	56	RA	23	2	v	v	13	△	20	20
TC	M	75	RA	15	3	+	+	.	.	20	80
TI	F	66	RA	5	3	v	v	23	□	80	320
RI	F	59	Para	NA	NA	NT	NT	16	△	NT	NT
WS	M	18	Para	NA	NA	NT	NT	12	□	NT	NT

RA = rheumatoid arthritis (4/5 ARA criteria); PsA = psoriatic arthropathy; Para = paraplegia.

*When cartilage antibody first detected.

NA = information not available.

†Rheumatoid serology for the entire period of surveillance:

+ always positive v variable
— always negative NT not tested

‡Direction of antibody change during period of testing:

▽ positive to negative □ always positive
△ negative to positive . patient tested once only

§The serum with the highest IF titre from each patient was used for the IF:HA comparison.

Anti-cartilage antibody

frequency than in the generality of patients with rheumatoid arthritis.

There is some suggestion of a correlation between anti-cartilage antibody and positive rheumatoid serology. Table 2 (cols 7 and 8) shows patients' serology for the whole period of their attendance at Stoke Mandeville Hospital, which in many cases extends well before 1975 when anti-cartilage testing was begun. Thirty-three (89%) of the 37 patients with rheumatoid arthritis in Table 2 were seropositive, that is, they have had positive tests for rheumatoid factor at some stage in their disease. Three of the four seronegative patients had rheumatoid arthritis of only short duration, and, statistically speaking, at least one of them is likely to become seropositive. This would result in an overall seropositivity rate of 92% for anti-cartilage positive patients, which contrasts with an overall rate of 82% for the 960 patients with rheumatoid arthritis whose serology we have followed for at least three years (unpublished data).

Most patients in Table 2 were tested for tissue antibodies on a number of occasions. In the majority, anti-cartilage antibody was present at the first test and persisted throughout the period of surveillance. In some the antibody appeared for the first time while the patient was under observation, and in others the antibody initially present disappeared; both groups of patients tended to be those whose disease was of shorter duration. In no case was it possible to link the appearance of the antibody with any clinical event.

SPECIFICITY OF ANTI-CARTILAGE ANTIBODY

Since no antibody to any other tissue present in the blocks regularly accompanied anti-cartilage antibody, it seemed probable that the latter owed its specificity to some constituent peculiar to cartilage—either proteoglycan or type II collagen (Miller, 1973). Absorption with proteoglycan derived from pig cartilage (kindly supplied by Dr Helen Muir, FRS, The Kennedy Institute of Rheumatology, London) failed to remove or to diminish the anti-cartilage titre in a serum shown to react with pig cartilage. Accordingly, sera from two patients were sent to Dr Helen Beard to be tested against type II collagen. Absorption on a column containing insolubilised type II collagen removed the antibody; and both HA and IF activity were subsequently recovered by elution.

After this demonstration in two sera of the close association, if not identity, of anti-cartilage with anti-type II collagen, we tested 33 IF positive sera by haemagglutination using cells coated with native type II collagen: 31 were positive in titres of 1:5 to 1:1600, average 1:80. Table 2 shows that there was an

obvious though not exact correlation between the HA and IF titres, although two of the IF positive sera were HA negative.

As virtually all our anti-cartilage sera were from cases of rheumatoid arthritis, 100 random anti-cartilage negative RA sera were tested by HA as controls. Among these 100 sera there were only five weak positives with titres from 1:2 to 1:10. These were retested by immunofluorescence for anti-cartilage antibody; the two strongest (HA titre 1:10) were found to be IF positive at a titre of 1:5 (that is, below the dilution at which sera were originally screened).

Discussion

Our findings seem to be of some interest because they concern an autoantibody to a tissue directly involved in a disease generally thought to be of auto-immune origin. The antibody hitherto regarded as rare is certainly very limited in its distribution and is practically confined to patients with rheumatoid arthritis.

The evidence points strongly to the anti-cartilage antibody here described being closely related to, if not identical with, antibody to native type II collagen, the distribution of which in the body is almost entirely confined to cartilage and intervertebral disc (Eyre and Muir, 1975, 1976; Miller, 1973). However, the two antibodies are not identical in every case, since two of the patients with anti-cartilage antibody had negative HA tests for anti-collagen type II; moreover, the titres of anti-cartilage correlate only roughly with those of anti-collagen type II. There is thus some heterogeneity within the anti-cartilage sera, possibly in the specificity for different antigenic sites on the collagen molecule; there are also variations in patterns of fluorescence with tracheal cartilage. These heterogeneities and the more detailed specificities of the antibodies are being further investigated.

There have been a number of reports of anti-collagen antibodies in rheumatoid arthritis. These mostly concern native or denatured type I collagen: in serum (Adam *et al.*, 1977; Michaeli and Fudenberg, 1974); in synovial fluids (Cracchiolo *et al.*, 1975; Menzel *et al.*, 1976); or antigen-antibody complexes in synovial fluid cells (Steffen *et al.*, 1974). One group of workers, however, have found antibody to type II collagen in synovial fluids (Mestecky and Miller, 1975) and in serum in both the IgG and IgM fractions (Andriopoulos *et al.*, 1976a, b, c). It is only with the last group that we can compare our findings, and we find great difficulty in reconciling our results with theirs. These authors found a high incidence (in the range 60-75%) of antibodies to both

native and denatured collagens of types I, II, and III compared with 3-4% of anti-cartilage and anti-type II collagen in our series. It is unlikely that this wide discrepancy is due to a simple difference in sensitivity of the methods used, since the two tests described here gave results in close agreement, yet differed widely in both substrate and method. A more probable explanation could lie in differences in the antigen used, or perhaps in some fibronectin- or rheumatoid factor-like substance present in rheumatoid arthritis which has been responsible for haemagglutination in the Birmingham, Alabama, series.

Collagen, especially type II, would fit so admirably the part of antigen in a self-perpetuating autoimmune arthritis that it is not surprising that those who have found anti-collagen antibodies should attribute to them a causative role in the aetiology of rheumatoid arthritis (Steffen, 1970). Indeed, it has been shown experimentally that immunisation with type II collagen can produce arthritis in rats (Trentham *et al.*, 1977). We can offer no evidence on this topic, but the low incidence of anti-cartilage antibody suggests that it is unlikely to be a prime aetiological factor. No doubt anti-cartilage antibody is present at some time in the disease more often than we have found it here, since we have seen it sometimes to be only fleetingly present, or to disappear after being well established; even so, our results suggest that it is unlikely to occur at all in more than a small minority of patients. This argument against a causative role for anti-cartilage antibody would have less force if the serum antibody were, as very well may be the case, only an 'overflow' of that produced in the synovial tissues. We have not found anti-cartilage antibody in the few rheumatoid synovial fluids examined, but the fluids were not pre-treated with collagenase or hyaluronidase. The high correlation of anti-cartilage antibody with erosions is no better evidence for it being a cause rather than a consequence of cartilage damage. It is nonetheless interesting to speculate why antibody to cartilage is not found in osteoarthritis which involves extensive damage to cartilage.

The figures are too small for any statistical comment on psoriatic arthropathy, but it is worth noting that the single patient with anti-cartilage antibody and psoriasis was seropositive and therefore may more properly belong in the group in which rheumatoid arthritis and psoriasis are coincidental.

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