

Antikeratin antibodies in serum and synovial fluid show specificity for rheumatoid arthritis in a study of connective tissue diseases

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SUMMARY Tests for antikeratin antibodies (AKA) were performed on 2152 disease-associated and control sera by indirect immunofluorescence (IF) on rat oesophagus substrate. The incidence of AKA was significantly raised in rheumatoid arthritis (37%) in comparison with systemic sclerosis (8%), psoriasis (7%), ankylosing spondylitis (6%), systemic lupus erythematosus (3%), and normal controls (2%). AKA were detected in synovial fluid obtained from patients with rheumatoid arthritis (RA) (48%) but not from patients with other conditions. Further experiments on AKA-positive sera showed reactivity with stratum corneum of rabbit prepuce and lips. A specific rabbit antihuman keratin antiserum was shown, by IF and inhibition studies, to have a different specificity from that of spontaneous human AKA.

AKA were associated with the presence of subcutaneous nodules in RA ($p=0.05$), but not with Raynaud's phenomenon, Sjögren's syndrome, or HLA-DR4 positivity. Rheumatoid factor (RF) was not associated with AKA either in RA or in RF-positive disease controls.

It has been suggested that defective immunoregulation may be central to the pathogenesis of rheumatoid arthritis.^{1,2} Several non-organ-specific autoantibodies have been described in RA and in other related connective tissue diseases.³ Certain autoantibodies may be more specific for RA,⁴ and we have previously described a possible relationship between rheumatoid factors and autoantibodies to keratin.⁵ Antikeratin antibodies were first described in RA⁶ but have since been reported in systemic lupus erythematosus (SLE),⁷ systemic sclerosis,^{6,8,9} and ankylosing spondylitis.⁹

In this study we have tested for any possible disease specificity of AKA, within a spectrum of connective tissue diseases and miscellaneous conditions reported to be associated with rheumatoid factor or AKA production. We included Sjögren's syndrome,¹⁰ psoriasis,¹¹ subacute bacterial endocarditis,¹² primary myxoedema,¹³ and silicosis.¹⁴ We also describe AKA in synovial fluid

and saliva in RA only and note an association between serum AKA and subcutaneous nodules in RA.

Materials and methods

A total of 2152 disease-associated and control sera were tested. These included 421 patients with classical or definite RA¹⁵ seen at the Brest University Hospital or The Middlesex Hospital, London. A particular note of the presence of Sjögren's syndrome, Raynaud's phenomenon and subcutaneous nodules was made in 144 cases of RA. Patients with other diseases were included as controls in the study (see Tables 1 and 2); all cases of systemic sclerosis, ankylosing spondylitis (AS), and primary Sjögren's syndrome fulfilled the proposed diagnostic criteria for the respective diseases.¹⁶⁻¹⁹ Normal controls included healthy clinical and laboratory staff, medical students, and residents of two homes for the elderly.

Synovial fluid (SF) was obtained from 58 patients with active RA, eight patients with other inflamma-

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Table 1 Incidence of antikeratin antibodies in serum, synovial fluid, and saliva of patients with rheumatoid arthritis, disease controls and normals

Diagnosis	No. positive*/No. tested (%) in:		
	Serum	Synovial fluid	Saliva
Rheumatoid arthritis	156/421 (37.1)	28/58 (48.3)	1/21† (3.2)
Systemic sclerosis	2/26 (7.7)	0/1	0/7
Ankylosing spondylitis	2/35 (5.7)	0/4	0/4
Systemic lupus erythematosus	1/30 (3.3)	0/3	0/6
Normal controls	6/247 (2.4)	0/20	0/30

*Serum antikeratin antibodies were screened at 1/10 dilution in PBS, while synovial fluid and saliva AKA were screened neat.

†Of these, 12 had a Sjögren's syndrome.

Table 2 Incidence of antikeratin antibodies in other connective tissue diseases and in other diseases associated with rheumatoid factor production

Diagnosis	No. tested	No. positive (%)
Primary myxoedema	34	4 (11.8)
Subacute endocarditis	10	1 (10.0)
Psoriasis	69	5 (7.2)
Alcoholic cirrhosis	35	2 (5.7)
Sjögren's syndrome	52	1 (1.9)
Silicosis	12	0
Polymyalgia rheumatica	25	0
Reiter's syndrome	22	0
Chronic active hepatitis	19	0
Pernicious anaemia	14	0
Primary biliary cirrhosis	11	0
Periarthritis nodosa	10	0

tory joint diseases, and 20 patients with meniscal tears or osteoarthritis which served as 'non-inflammatory' normal controls. Synovial fluid was collected in preservative-free heparin and immediately centrifuged at 400 g at 4°C for 20 min to remove cellular debris. The supernatant was then treated with hyaluronidase (150 U/ml, Sigma) for 15 min at 37°C and stored in aliquots at -70°C until tested.

Saliva from 21 RA patients, 17 disease controls and 30 normal controls was centrifuged, dialysed against borate-buffered saline 0.1 M pH 7.2, and stored at -70°C until tested.

ANTIKERATIN ANTIBODY TEST

Serum antikeratin antibodies were screened at 1/10 dilution in phosphate-buffered saline 0.01 M pH 7.3 by indirect immunofluorescence, on a tissue substrate of the middle third of rat oesophagus as described by Young *et al.*⁶ Synovial fluid and saliva AKA were screened neat. Rabbit antihuman IgG,

isotype-specific (fluorescein isothiocyanate) conjugate was used (Kallestad).

TISSUE TYPING

Lymphocytes from 58 RA patients were tested for DR4 positivity by a microcytotoxicity test²⁰ with a panel of antisera from France-Transplant.

OTHER TECHNIQUES

A rabbit antihuman keratin antiserum was raised as described in detail elsewhere²¹ and used for blocking experiments. Sections of rat oesophagus were incubated with non-immune rabbit serum or rabbit antihuman keratin antiserum for 30 min, washed thoroughly, and then incubated with high titre AKA-positive RA sera.

Seven AKA-positive RA sera were tested by immunofluorescence on sections of rabbit prepuce and lips. A fluoresceinated sheep antirabbit Ig (Nordic) was used as conjugate for the rabbit antihuman keratin antiserum.

STATISTICS

The figures quoted below are percentages or geometric means. Data were analysed by the χ^2 test, with Yates's correction when required, or by the Mann-Whitney U test.

Results

Antikeratin antibodies of IgG class were frequently detected in RA sera (37.1%) and synovial fluid (48.3%) but only in 1/21 samples of RA saliva. Both normal and disease control groups showed a low incidence of AKA in serum (2.4-7.7%), and no positives were found in control SF or saliva samples (Table 1). Sera from another 12 diseases associated with spontaneous production of RF also showed a low incidence of AKA.

The titre of AKA in RA sera was significantly greater than disease controls ($p < 0.05$) and normals

($p < 0.04$) (Fig. 1). In a comparison of 58 paired samples of RA serum and SF (Fig. 2) we noted a consistently lower AKA titre in the SF samples.

Statistical analysis of the results from 144 patients with RA whose specific clinical details were available showed an association between AKA positivity and subcutaneous nodules ($p < 0.05$, Table 3) but no correlation with Sjögren's syndrome or Raynaud's phenomenon. Although there was slight increase in AKA positivity in the 36 DR4-positive cases of RA (44.4% positive) as against the 22 DR4-negative

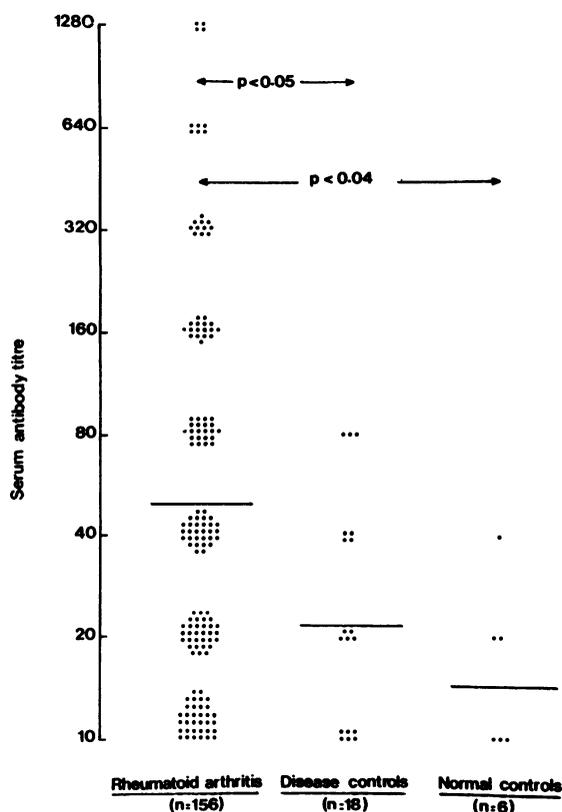


Fig. 1 Titres of serum antikeratin antibodies in rheumatoid arthritis patients, disease controls, and normals.

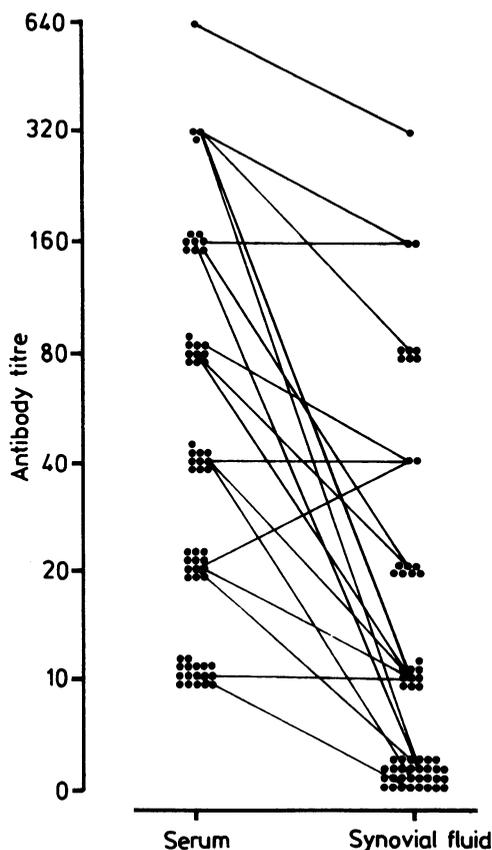


Fig. 2 Titres of antikeratin antibodies in 58 paired samples of rheumatoid arthritis serum and synovial fluid.

group (27.3% positive) this was not statistically significant.

Further tests on seven AKA positive RA sera showed binding to stratum corneum of rabbit prepuce and lips, but we also found four sera which bound to the upper cell layer and four which bound to the glandular orifices (Thivolet *et al.*, manuscript in preparation).

When a rabbit antiserum to human keratin was used to define the specificity of naturally occurring

Table 3 Clinical associations of antikeratin antibodies in a study of 144 patients with rheumatoid arthritis

Antikeratin antibodies	Raynaud's phenomenon		Sjögren's syndrome		Subcutaneous nodules	
	Yes	No	Yes	No	Yes	No
Positive (No.)	9	47	14	41	15*	48
Negative (No.)	15	73	31	58	9	72

* $p < 0.05$ between patients with and without subcutaneous nodules.

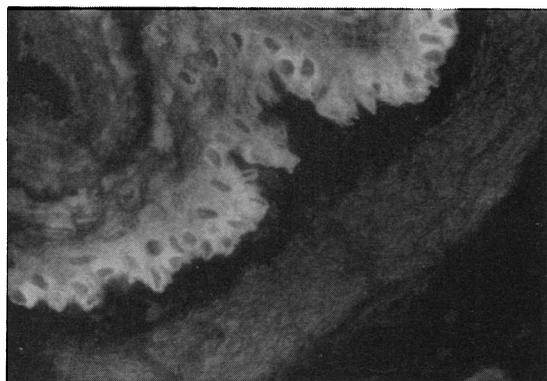


Fig. 3a

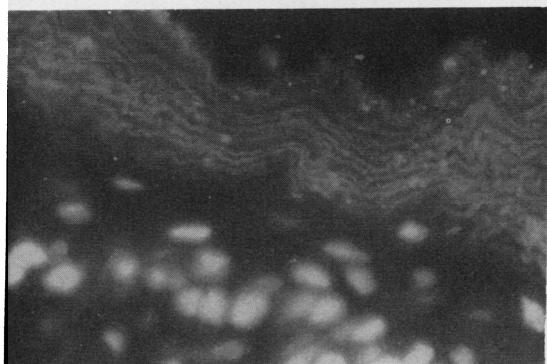


Fig. 3b

Fig. 3 Immunofluorescence pattern obtained on rat oesophagus stained with: (a) rabbit antihuman keratin antiserum; (b) high titre antikeratin antibody positive human RA serum.

support comes from our finding that AKA are not associated with DR4 positivity, unlike the reported association of DR4 with RF-positive RA.²² Additional clarification of this question will however be required as Mallya *et al.*²³ have recently reported a close association between AKA abundance and RF and anti-ss-DNA antibodies, in a study of 98 patients. They also found an association with Sjögren's syndrome and subcutaneous nodules. This latter finding is confirmed in our survey (Table 3).

High titres of AKA were mainly restricted to RA patients and we propose that a titre of 1:40 or higher may be helpful in the diagnosis of RA (Fig. 1). In addition we could only detect AKA in synovial fluid from RA patients, and although we did not correct for total Ig content when comparing AKA titres of paired serum and SF samples, our results confirm the report of Quismorio *et al.*⁹ that AKA are not concentrated in SF. Despite the absence of IgG anti-AKA in our study of RA saliva, it is possible that IgA anti-AKA could be present; IgA rheumatoid factors have been detected in RA and Sjögren's saliva.¹⁰

Antibodies to rabbit stratum corneum have not previously been described in association with AKA positivity, but further analysis of AKA specificity on a rat substrate showed restriction of (spontaneous) AKA binding (in RA sera) to the more superficial layers, whereas a specific rabbit antihuman keratin antiserum bound only to the basal cell layer (Figs 3a and b). These two patterns of binding may represent differences in epitopic specificity between the two types of antibody tested, and further studies are in progress.

In conclusion we have found antikeratin antibodies in titres of 1/40 or above to be relatively specific for rheumatoid arthritis in a study of serum and synovial fluid. The significance and the epitopic specificity of these antibodies require further elucidation.

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human AKA, we found it did not block human AKA activity and instead showed binding to the basal cell layer (Fig. 3a) in contrast with the human sera which found more superficial layers of keratin in the rat oesophagus preparation (Fig. 3b).

Discussion

In this extensive long-term study we have found serum AKA to be relatively specific for RA. Although it is of low sensitivity at a prevalence of 37% in RA, we detected AKA in only 3-8% of other connective tissue diseases. This appears to be lower than some previous reports of AKA in Sjögren's syndrome, 50%;⁸ AS, 25%;⁹ and SLE, 18%.⁷ We included other RF-positive syndromes as disease controls, which also showed a low incidence of AKA, supporting the earlier demonstration that AKA do not have RF activity.⁶ Further indirect

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