We thank Dr P C N Clarke, Dr C W Jones, Dr C Engler, and Mrs S Reid for their help.

References

- ¹ Prahl, P, Wilken-Jensen, K, and Mygind, N, *Archives of Disease in Childhood*, 1975, **50**, 875.
- ² Shore, S C, and Weinberg, E G, *Archives of Disease in Childhood*, 1977, **52**, 486.
- ³ Turkeltaub, P D, Norman, P S, and Crepea, S, *Journal of Allergy and Clinical Immunology*, 1976, **58**, 597.

- ⁴ Bloom, F L, *et al*, *Annals of Allergy*, 1977, **38**, 408.
- ⁵ Schulz, J I, Johnson, J D, and Freeman, S O, *Clinical Allergy*, 1978, **8**, 313.
- ⁶ Sahay, J N, Chatterjee, S S, and Engler, C, *Clinical Allergy*, 1979, **9**, 17.
- ⁷ Strem, E L, Austrian, S, and Geller, G, *Journal of Allergy and Clinical Immunology*, 1978, **61**, 150.
- ⁸ Siegal, S, *et al*, *Journal of Allergy and Clinical Immunology*, 1978, **61**, 152.
- ⁹ Wood, B, *A Paediatric Vade-Mecum*, p 129. London, Lloyd-Luke, 1974.
- ¹⁰ Morrison Smith, J, *et al*, *British Medical Journal*, 1975, **2**, 255.
- ¹¹ Tarlo, S M, *et al*, *Journal of Allergy and Clinical Immunology*, 1977, **59**, 232.
- ¹² Norman, P S, *et al*, *Journal of Allergy and Clinical Immunology*, 1967, **40**, 57.

(Accepted 26 May 1979)

Anti-keratin antibodies in rheumatoid arthritis

B J J YOUNG, R K MALLYA, R D G LESLIE, C J M CLARK, T J HAMBLIN

British Medical Journal, 1979, **2**, 97-99

Summary and conclusions

A naturally occurring antibody that reacts with the keratinised tissue of animal oesophagus was found in the serum of 75 out of 129 patients (58%) with classical or definite rheumatoid arthritis (RA) but not in sera from 105 healthy people.

Detection of the antibody, which is unrelated to rheumatoid factor, is more specific for RA than the reaction in the sheep-cell agglutination test but less sensitive.

Introduction

Naturally occurring anti-keratin antibodies (AKA) in human sera were first noted in this laboratory by R S Lloyd in 1970 (unpublished data) using rat oesophagus tissue and fluorescent antibody techniques. The keratins are a family of intracellular, fibrous, and highly complex insoluble proteins exhibiting pronounced heterogeneity. Their structure and chemistry has been extensively studied and reviewed.¹ Their antigenicity was first shown experimentally by Pillemer *et al*.² Later studies disclosed considerable cross-reactivity between keratins derived from both human³⁻⁵ and animal⁶ tissues. These differences were partially resolved by Lee *et al*,⁷ who isolated four polypeptides from the precursor protein prekeratin. These are designated A, A¹, B, and B¹ and form two immunologically distinct families of A and B chains in mixed proportions. Furthermore, antisera raised to A and B chains react with the major structural protein of the epidermis. Although these findings confirm the work of Bauer,³ Kemp and Rogers⁶ detected no such antigenic relation with keratins derived from differentiating cortical cells.

Departments of Immunology and Medicine, Royal Victoria Hospital, Bournemouth, Dorset BH1 4JG

B J J YOUNG, MSC, FIMLS, senior chief medical and laboratory scientific officer

R K MALLYA, MB, MRCP, medical registrar (present address: Department of Medicine, Bristol Royal Infirmary, Bristol BS2 8HW)

R D G LESLIE, MB, MRCP, medical registrar (present address: King's College Hospital, London SE5 9RS)

C J M CLARK, MD, FRCP, consultant physician

T J HAMBLIN, MRCP, MRCPATH, consultant haematologist

We now think that the antigenic specificity of the keratin molecule may be modified by reorganisation of the A and B chains during the keratinisation process itself; this is discussed by B J J Young (paper in preparation). Preliminary testing for AKA was carried out on 10 589 sera from patients being investigated with standard autoantibody tests for a wide variety of medical diseases and on whom adequate clinical data were not available. AKA were found in 552. The finding that 380 of these sera also produced a positive reaction in the sheep-cell agglutination test (SCAT) (table I) led to the present investigation.

We record here accumulated evidence of the prevalence of AKA in human serum, and more specifically the serum of patients with rheumatoid arthritis (RA).

TABLE I—Prevalence of anti-keratin antibodies (AKA) and rheumatoid factor (positive sheep-cell agglutination test (SCAT) result) in 10 589 serum samples examined over two years

	AKA-positive	SCAT-positive	AKA/SCAT-positive	AKA-positive/SCAT-negative	Total
No (%) of sera examined	552 (5.2)	1054 (10.0)	380 (3.6)	170 (1.6)	10 589 (100.0)

Patients and methods

Three groups of sera were examined for antibodies to rat oesophageal keratin and rheumatoid factor.

Group 1 comprised sera from 129 patients with classical or definite RA; 84 were women and 45 men, and their ages ranged from 18 to 80 years (mean 57 years). The duration of disease ranged from six months to 35 years (mean 7.5 years).

Group 2 comprised sera from 52 patients with non-rheumatoid diagnoses—namely, ankylosing spondylitis (8), psoriatic arthropathy (9), scleroderma (7), keratoconjunctivitis sicca (10), osteoarthritis (4), systemic lupus erythematosus (10), and polymyalgia rheumatica (4). Their ages ranged from 31 to 72 years (mean 59 years).

Group 3 comprised sera from 105 healthy blood donors. These sera served as controls.

Twenty AKA-positive samples from group 1 were also tested against mouse, monkey, and human oesophageal tissue.

Sera were tested for autoantibodies routinely at a 1/10 dilution.⁸ Freshly prepared cryostat sections were examined by the indirect fluorescent antibody technique⁹ with a fluorescein-labelled anti-human immunoglobulin G (IgG) (Wellcome Reagents Ltd). Unfixed cryostat sections of human skin and hair follicles were similarly treated with selected sera. All sera were screened with RA latex reagent (Hyland Laboratories) and positive sera titrated for rheumatoid factor

using the SCAT described by Roitt and Doniach.¹⁰ An agglutination of 1/20 or more was taken as positive.

Aggregated human IgG was freshly prepared with Cohn fraction II (Sigma), as described.¹¹ Dilutions of AKA-positive pooled human sera were absorbed at 37°C for one hour and then kept at room temperature overnight. Next day the samples were centrifuged and assayed by immunofluorescence for AKA activity. The immunoglobulin fraction was purified from pooled AKA-positive sera by sodium sulphate precipitation (18% w/v) and dialysed against 0.02M TRIS (trometamol)/HCl/NaCl buffer pH 8.2. The reconstituted proteins were then passed down a column containing Ultragel (ACA 34 LKB) equilibrated with the same buffer and precalibrated with blue dextran (Pharmacia) containing purified human IgG. The purity of the eluted fractions was tested by radial immunodiffusion¹² with commercially available plates (ICL Scientific). The AKA activity of each undiluted fraction was determined as described above.

Results

Seventy-five of the sera in group 1 (58%) were AKA-positive, including eight from 24 patients who did not have rheumatoid factor (table II). Thirty-eight SCAT-positive sera were AKA-negative. Twelve out of 16 patients with RA and clinical features of Sjögren's syndrome and all four patients with Felty's syndrome had AKA. All group 2 sera were AKA-negative except one from a patient with scleroderma. None of the control sera were AKA-positive, although two were SCAT-positive. Most of the patients with RA (group 1) had had treatment of varying duration with various antirheumatoid agents, including penicillamine, gold, and corticosteroids.

TABLE II—Prevalence of anti-keratin antibodies (AKA) and rheumatoid factor (SCAT) in rheumatoid arthritis. Figures are numbers of sera (percentages in parentheses)

Group	No patients	AKA-positive	AKA-negative	SCAT-positive	SCAT-negative	AKA/SCAT-positive
1	129	75 (58.1)	54 (41.9)	105 (81.4)	24 (18.6)	67 (51.9)
2	52	1 (1.9)	51 (98.1)	0	0	0
3	105	0	105 (100.0)	2 (1.9)	0	0

A few of the 20 AKA-positive sera tested reacted with the keratinised layer of monkey oesophagus, but none with human oesophagus, which is not keratinised. Only six of these sera, however, reacted with mouse oesophageal keratin, which is less differentiated than that of rats.¹³

The keratin antibody is easily recognised in both rat oesophageal tissue (fig 1) and the keratogenous zone of human hair follicles (fig 2). Figs 3 and 4 show negative reactions with normal human sera.

FIG 1—Positive immunofluorescence of keratin fibres in rat oesophageal mucosa. This AKA-positive serum also contains antibodies to nuclear constituents. $\times 200$ (original magnification).

FIG 2—Cross-section of human hair follicle showing positive immunofluorescence of keratogenous zone with AKA-positive serum. $\times 400$ (original magnification).

FIG 3—Negative immunofluorescence of rat oesophageal mucosa with normal human serum. $\times 200$ (original magnification).

FIG 4—Serial section of human hair follicle in fig 2 showing negative immunofluorescence with normal human serum. $\times 400$ (original magnification).

Absorption of AKA-positive sera with aggregated human IgG did not remove the antibodies. The antibody activity was shown by column chromatography to correspond to the IgG peak, and contamination with IgM could not be detected with a sensitive radial immunodiffusion method.

Discussion

The anti-keratin antibody described here gives a highly specific staining pattern with rat, mouse, and monkey oesophageal keratin and, to a less extent, with human skin. The antibody also reacts with the cortical cells of both human and non-human hair follicles. In human tissue the reaction appears to be both stronger and more specific for the keratogenous zone (fig 2). Experimentally produced antibodies to these proteins cross-react with low-sulphur keratins in the cortical cells of the differentiating hair follicles of guinea-pigs but do not react with the keratogenous zone.⁶ This suggests that our reaction is different and more specific for fully differentiated keratins. Our results with the less differentiated mouse oesophageal keratin¹³ and comparisons with the morphological reactions obtained in the cortical cells of mice (B J J Young; in preparation) support this interpretation.

Absorption experiments with heat-aggregated human IgG and chromatography showed that the keratin antibody is an IgG autoantibody, which is unlikely to be related to rheumatoid factor.

Thus compared with the SCAT detection of AKA appears to be highly specific for RA; only one patient with scleroderma was positive in the non-RA group. The SCAT is sensitive but not specific for RA. Kellgren *et al*¹⁴ reported positive reactions

in 41% of patients with systemic lupus erythematosus, 41% with systemic sclerosis, 13% with dermatomyositis, and 20% with polyarteritis nodosa, whereas specificity for RA was shown by Valkenburg *et al*¹⁵ to increase only as the SCAT titre rises. Moreover, in random sampling in the north of England only 26% of people with a positive SCAT result had clinical or radiological evidence of RA.¹⁶

AKA should have a clinical value in being more specific though less sensitive than the SCAT reaction for RA.

References

- Bradbury, J H, *Advances in Protein Chemistry*, 1973, **27**, 111.
- Pillemer, L, Ecker, E E, and Wells, J R, *Journal of Experimental Medicine*, 1939, **69**, 191.
- Bauer, F W, *Dermatologica*, 1972, **144**, 301.
- Frater, R, *Australian Journal of Biological Sciences*, 1968, **21**, 815.
- Carruthers, C, *Journal of the Society of Cosmetic Chemists*, 1970, **21**, 169.
- Kemp, D J, and Rogers, G E, *Journal of Cell Science*, 1970, **7**, 273.
- Lee, L D, *et al*, *Biochimica et Biophysica Acta*, 1975, **412**, 82.
- Young, B J J, *Medical Laboratory Technology*, 1975, **32**, 133.
- Coons, A H, and Kaplan, M H, *Journal of Experimental Medicine*, 1950, **91**, 1.
- Roitt, I M, and Doniach, D, *World Health Organisation Manual of Auto-immune Serology*. Geneva, WHO, 1969.
- Dickler, H B, and Kunkel, H G, *Journal of Experimental Medicine*, 1972, **136**, 191.
- Mancini, G, Carbonara, A O, and Heremans, J F, *Immunochemistry*, 1965, **2**, 235.
- Ham, A R, *Histology*, 7th ed. Philadelphia and Toronto, Lippincott, 1974.
- Kellgren, J H, Ball, J, and Bier, F, *British Medical Journal*, 1959, **1**, 523.
- Valkenburg, H A, *et al*, *Annals of Rheumatic Diseases*, 1966, **25**, 497.
- Lawrence, J S, *Rheumatism in Populations*, p 179. London, Heinemann, 1977.

(Accepted 5 June 1979)

SHORT REPORTS

Field trial of a heat-stable measles vaccine in Cameroon

Heat-labile measles vaccines are rapidly inactivated at room temperature and require a rigorously controlled cold chain for conservation without loss of potency. Recently the development of more-heat-stable measles vaccines has been reported by manufacturers. Laboratory studies of Rimevax, one such vaccine, were carried out by the manufacturer and showed that the minimum required titre (10^3 median tissue culture infective dose/dose) was maintained after 14 days' storage at 37°C and after 21 days at 20°C–25°C.¹ In order to assess this vaccine for use under field conditions in the United Republic of Cameroon we undertook the following study in Yaoundé, the capital.

Methods and results

Rimevax was transferred from storage at –20°C to a covered, dark container and maintained unfrozen at room temperature for from 24 to 192 hours before its reconstitution and use. A continuous temperature monitor kept with the vaccine recorded temperatures ranging from 23–1°C to 25–4°C. At 24-hour intervals eight phials of the unfrozen vaccine were removed from storage. Four phials were refrozen at –20°C for later titration and four were immediately transported, at ambient temperatures and in a dark container, to the vaccination site, where they were reconstituted with diluent at the same temperature as the vaccine. With informed consent of the parents 0.5 cm³ of the vaccine was administered subcutaneously by needle and syringe to children aged 9–24 months who had no history of measles. Vaccine was administered within 30 minutes of reconstitution; before and 30 days after immunisation capillary blood was collected on filter-paper discs from finger puncture as described by Mathews *et al*.² The discs were dried for 24 hours and stored sealed with a desiccant at –20°C. Measles hemagglutination inhibition antibody determination was performed on this blood according to the techniques developed by Hierholzer and Suggs³ and Norrby.⁴ Vaccine virus titration was performed on the Rimevax using the method described by Peetermans.⁵

None of the children had a history of measles during the period of the study. Seroconversion was defined as a rise in titre from seronegative (<1:10) to ≥1:20; all children who failed to seroconvert were revaccinated. Out of 55 children aged 13–24 months, 53 seroconverted (96% seroconversion rate), while out of 77 children aged 9–12 months, 69 seroconverted (rate of 90%) (table). The titre of Rimevax remained essentially stable during the 192 hours of the study at a level above 3.5 log₁₀ per dose.

Seroconversion to Rimevax in children aged 9–24 months in Cameroon during 1977

Duration of vaccine storage at 23°–25°C (hours)	Age (months)			
	9–12		13–24	
	No of susceptible children vaccinated with 30-day follow-up	No (%) successfully seroconverted	No of susceptible children vaccinated with 30-day follow-up	No (%) successfully seroconverted
24–48	4	3 (75)	5	5 (100)
48–72	13	13 (100)	8	8 (100)
72–96	14	13 (93)	11	10 (91)
96–120	12	11 (92)	4	4 (100)
120–144	8	7 (88)	14	14 (100)
144–168	10	8 (80)	6	6 (100)
168–192	16	14 (88)	7	6 (86)
Overall	77	69 (90)	55	53 (96)

Comment

Under field conditions in Cameroon Rimevax showed a negligible loss of titre during exposure to temperatures between 23°C and 25°C for seven days and induced seroconversion in over 90% of the children vaccinated. Though this does not eliminate the need for a well-controlled cold chain of vaccine conservation, such stability will