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AUTO-ANTIBODIES IN PATIENTS WITH CHRONIC PULMONARY TUBERCULOSIS

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ASHERSON AND ROSE (1963) found that rabbits infected with coccidia produced antibodies reacting with homologous organ homogenates. Sera from patients with *Schistosoma haematobium* infections may contain auto-antibodies that fix complement in the presence of normal human liver and lung homogenates (Shamma, Thewaini Ali and El-Shawi, 1965) and stain fresh-frozen human lung and liver sections when used in the fluorescent antibody technique (Shamma, Thewaini Ali and Rassam (1966).

Thewaini Ali and Oakley (1967) found that some rabbits infected with *Mycobacterium tuberculosis* or *Pasteurella pseudotuberculosis* or immunised with sterilised organ homogenates from infected animals develop auto-antibodies reacting with various homologous and heterologous tissue components. They did not provide evidence that auto-antibodies are produced in long-standing tuberculosis in man; the present paper attempts to supply it.

MATERIALS AND METHODS

Blood

Blood was collected from 80 patients suffering from chronic pulmonary tuberculosis with cavitation, but having no history of syphilis, bejel, schistosomiasis, acquired haemolytic anaemia, collagen diseases or infectious mononucleosis, at the Twaitha Tuberculosis Hospital, and from 20 healthy controls. The serum was separated and stored at -25°C until needed.

Auto-antibody tests

Complement-fixation tests. Sera were tested for complement-fixing antibody against human and rabbit liver, lung and kidney homogenates, before and after absorption with rabbit liver or kidney tissue, as described by Thewaini Ali and Oakley.

Anti-nuclear factor tests. The indirect method of Coons and Kaplan (1950) was used; unfixed smears of human buffy coat were treated with the serum under test for 30 min.; the slides were then washed with phosphate-buffered saline (PBS) at pH 7.2, and then treated for 20 min. with one drop of sheep anti-human-globulin conjugated with fluorescein isothiocyanate (Sylvana) absorbed with charcoal and with rat liver powder. The slides were washed again with PBS, drained, mounted in 90 per cent. phosphate-buffered glycerol pH 7.2 and examined for nuclear fluorescence with an ultraviolet microscope. Appropriate controls for the specificity of the fluorescent staining (Thewaini Ali and Oakley) were always included.

Rose-Waaler test. The modified Rose-Waaler test of Rose *et al.* (1948) was used, and the differential agglutination titre was obtained by dividing the reciprocal of the titre obtained

with sensitised sheep red cells by the reciprocal of the titre obtained with normal sheep red cells.

Other tests. Qualitative VDRL tests, and Wassermann tests with 3 and 5 MHD of complement were done on all sera.

RESULTS

Complement-fixation tests. Table I shows that of the sera from 80 patients with chronic tuberculosis, 12 contained antibodies fixing complement in the presence of human liver, lung and kidney homogenates; of these all but one reacted with comparable rabbit homogenates also, but usually to a lower titre. Only one of the 20 control sera gave a positive reaction.

Absorption of the 13 positive sera with rabbit liver or kidney powder removed all the complement-fixing activity against rabbit organ homogenates, and usually reduced the activity of the sera against human organ homogenates, and often abolished it completely. Two sera (no. 23 and 52) that still had a high titre after absorption had a higher titre against lung than against the other homogenates (table I).

Anti-nuclear factor tests. Four sera (all from tuberculous patients showing complement-fixing auto-antibodies) gave fluorescent nuclear staining in the indirect fluorescent antibody test. The titres were all low; three sera had to be used undiluted, the remaining one had a titre of 1 in 8. All positive sera were tested for non-specific staining; in all cases non-specificity could be excluded.

Rose-Waaler tests. Five of the 12 sera with complement-fixing auto-antibodies gave a differential agglutination titre of 1 in 16 or higher (usually considered positive), and another three gave titres of 1 in 8—i.e., doubtful or negative (table II).

Other tests. Nine sera gave false-positive reactions in the VDRL test, and 7 false-positive results in the Wassermann test (table II).

DISCUSSION

It is evident that the auto-antibodies demonstrated in about 15 per cent. of 80 patients with chronic pulmonary tuberculosis with cavitation are not organ-specific. They react with homogenates of at least three human organs, and also, to a less extent, with rabbit organ homogenates. There was, however, evidence that some of the sera contained antibody against one or more lung antigens. Five of the twelve sera had higher titres against lung than against liver or kidney homogenates (one noticeably higher), and absorption of the sera with rabbit liver or kidney powder left some activity against human lung homogenates in 7 out of 12 of them.

Unfortunately the serum samples obtainable were too small and their titres were too low to allow of extensive antibody analysis of the kind carried out by Thewaini Ali and Oakley (1967).

Davis (1944) found that sera from patients with trypanosomiasis contain auto-antibodies and give false-positive tests for syphilis; some of the sera from Iraqi tuberculous patients behaved similarly, and some gave positive Rose-Waaler tests. Antibodies to anti-nuclear factor were present in four patients, but the titre was always low.

Presumably the destruction of tissues in chronic pulmonary tuberculosis in man alters some tissues in such a way as to make them auto-antigenic; the tubercle bacilli present may also fulfil the same "completing" role as they do in Freund's complete adjuvant in provoking the production of auto-antibody (Hunter, Hackel and Heyman, 1960; Jones and Roitt, 1961; Sclaire and Taylor, 1961; Åström and Waksman, 1962; Wight, 1968).

Antibodies to lung antigen can be produced in experimental animals (Burrell, 1963; Thewaini Ali and Oakley), and are also found in the sera of patients with primary atypical pneumonia (Thomas, 1964).

SUMMARY

Fifteen per cent. of single serum samples from patients with chronic pulmonary tuberculosis with cavitation contained antibodies reacting with human and rabbit liver, lung and

TABLE I
Complement-fixing auto-antibodies in tuberculous patients and in a normal control

Source of serum	No. of persons	Person no.	Complement-fixation titre of serum									
			before absorption*, against homogenates of						after absorption†, against homogenates of			
			human			rabbit			human			
			liver	lung	kidney	liver	lung	kidney	liver	lung	kidney	
Tuberculous patients	68		—	—	—	—	—	—	—	—	—	—
	4	22	16	32	16	16	16	16	8	8	8	—
		37	16	32	16	16	16	16	—	—	—	—
		43	16	32	16	16	16	16	—	—	—	—
		70	16	32	16	16	16	16	—	—	—	—
	3	8	32	32	32	8	8	8	—	—	—	—
		67	32	32	32	8	8	8	8	16	8	—
		68	32	32	32	8	8	8	—	—	—	—
	1	52	32	32	8	—	—	—	16	32	8	8
	3	13	16	16	16	4	8	8	8	8	8	8
		21	16	16	16	4	8	8	4	8	8	4
		45	16	16	16	4	8	8	8	8	8	4
	1	23	32	128	32	16	16	16	4	32	4	4
Normal controls	19		—	—	—	—	—	—	—	—	—	—
	1	6	16	16	16	8	8	8	—	—	—	—

* Absorbing material rabbit liver or kidney powder.

† Absorption with rabbit liver or kidney powder removed all complement-fixing activity against rabbit homogenates.

— = No complement-fixing activity with serum diluted 1 in 4.

TABLE II
Other evidence for auto-antibody production in chronic tuberculosis

Source of serum	Number of samples	Person no.	Complement-fixation titre against homogenates of human			Rose-Waaler test (DAT)	False-positive		Nuclear fluorescent staining
			liver	lung	kidney		Wassermann reaction	VDRL test	
Tuberculous patients	68		—	—	—	—	—	—	—
	4	22	16	32	16	16	+	+	—
		37	16	32	16	16	+	+	—
		43	16	32	16	16	+	+	—
		70	16	32	16	16	+	+	—
	3	6	32	32	32	8	—	—	+
		67	32	32	32	8	—	—	+
		78	32	32	32	8	—	—	+
	1	52	32	64	16	—	+	+	+
	3	13	16	16	16	—	—	+	—
		21	16	16	16	—	—	+	—
		45	16	16	16	—	—	+	—
	1	23	32	128	32	32	+	+	—
Normal controls	19		—	—	—	—	—	—	—
	1	6	16	16	16	—	—	—	—

— = Titre <4 in complement-fixation tests; < 8 in Rose-Waaler tests; negative in Wassermann and VDRL tests, and in nuclear fluorescent staining tests by sandwich method.

kidney homogenates. Some of the positive sera also gave false-positive reactions in Rose-Waaler tests and in tests for syphilis; a few contained weak anti-nuclear antibodies.

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