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TISSUE ANTIBODIES IN IDIOPATHIC AUTOIMMUNE HAEMOLYTIC ANAEMIA

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SUMMARY

Sera from patients with idiopathic autoimmune haemolytic anaemia (AIHA) were studied, by the immunofluorescent technique, for the presence of circulating organ-specific and non-organ-specific autoantibodies. In patients with warm AIHA there was an increased incidence in the non-organ-specific autoantibodies, compared to normal controls: In 15% of sera studied there were antinuclear antibodies detectable, and in 5% antimitochondrial antibodies were present. In cold AIHA, however, the frequency of these autoantibodies was not increased. The incidence of organ-specific autoantibodies was normal in both cold and warm AIHA. No correlation was found between the occurrence of these autoantibodies and pattern of serum immunoglobulin concentration, specific serological feature, or clinical manifestation. The significance of the increased incidence of tissue autoantibodies and their relation to autoimmune haemolytic anaemia remains to be elucidated, but in some way may be due to increased immunological reactivity.

INTRODUCTION

Circulating autoantibodies are present in many autoimmune diseases such as pernicious anaemia, Hashimoto's thyroiditis, myasthenia gravis, rheumatoid arthritis, systemic lupus erythematosus etc. In autoimmune haemolytic anaemia (AIHA) the circulating autoantibodies appear to be directed against intrinsic red-cell-surface antigens. These antibodies can usually be demonstrated on the red cell surface of such patients by means of the direct antiglobulin reaction and, in addition, red cell autoantibodies can usually be demonstrated in the sera of these patients.

Not infrequently, more than one manifestation of autoimmune disease occurs in the same patient. Thus the diagnosis of AIHA may be made, before, after, or at the time of diagnosis of the other autoimmune diseases (Dacie & Worlledge, 1969). In addition, all types of autoimmune diseases tend to occur in similar circumstances. For example, the same disease

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may be present in more than one member of a family, or families exist where the various members have different autoimmune diseases or serological abnormalities. Moreover they occur in association with hypogammaglobulinaemia, and in association with various lymphoreticular neoplasms (Pirofsky, 1968a; Pirofsky, 1968b; Blajchman *et al.*, 1969). These associations have given rise to speculation that the underlying pathogenic factor in all these disorders may reside in an abnormal immunological mechanism (Fudenberg, 1966; Pirofsky, 1968a). In AIHA the presence of low circulating levels of one or more classes of immunoglobulin in approximately 50% of patients, suggests that humoral immunity may be abnormal (Blajchman *et al.*, 1969). No apparent defect in cellular immunity, however, has been noted in AIHA or in the other autoimmune diseases. Lymphocyte response to phytohaemagglutinin, which may be a measure of lymphocyte function, has been reported as normal in systemic lupus erythematosus and rheumatoid arthritis (Patrucco, Rothfield & Hirshhorn, 1967; Kacaki, Bullock & Vaughan, 1969). The response of lymphocytes to phytohaemagglutinin in six untreated patients with idiopathic warm AIHA was normal (unpublished observations).

The association of AIHA with other disorders has been stressed by Pirofsky (1968a). His view is that the concept of idiopathic autoimmune haemolytic anaemia may be illusory and that it represents a partial diagnosis of a more generalized multisystem immunodeficient disorder. It was of interest, therefore, to investigate the incidence of the various tissue antibodies using an immunofluorescent technique in patients with autoimmune haemolytic anaemia, in an attempt to relate this disease to autoimmune disease in general.

MATERIALS AND METHODS

Patients

The sixty patients, thirty-eight female and twenty-two male, with warm autoimmune haemolytic anaemias were all adults who were investigated at the Royal Postgraduate Medical School between the years 1965–1970. All had overt haemolytic anaemia either before or at the time the serum was examined. The autoantibodies were considered to be of the warm type because they were as active at 37°C as at lower temperatures. The patients' diseases were classified as 'idiopathic' because no underlying or associated disease could be found, either before or during the period the patients were under observation. In addition there was no history of any drugs which could be incriminated in the production of autoimmune haemolytic anaemia.

The sixteen patients with cold AIHA were similarly considered to have 'idiopathic' AIHA in that no underlying associated condition could be found. The diagnosis of cold AIHA was made on clinical and serological grounds. All patients had high titre cold autoantibodies in their serum.

Fluorescent antibody tests

The double layer technique of Weller & Coons (1954) was used to detect serum antibodies. Unfixed human thyrotoxic thyroid gland was mounted in a composite block with rat gastric mucosa and rat kidney. The tissues on this mounted block were sectioned together in a Pearse cryostat into 6 μ sections. These were then treated with serially diluted patients' sera for 30 min at room temperature, then washed three times in phosphate

buffered saline. The sections were then covered with a commercial preparation of rabbit antihuman immunoglobulin conjugated with fluorescein isothiocyanate (Hoechst Pharmaceuticals Ltd, London). Just prior to use, this antiserum was absorbed with guinea-pig liver powder (100 mg/ml). The slides were left for 30 min at room temperature, washed three times in phosphate buffered saline as before, and finally mounted in 50% glycerol in saline. The mounted sections were then examined under dark ground illumination using a Zeiss standard fluorescent microscope.

Haematological Studies were performed by standard techniques as described by Dacie & Lewis (1968), and the immunoglobulin measurements kindly performed by Professor J. R. Hobbs, using a modified Mancini technique.

RESULTS

Table 1 summarizes the incidence in the various tissue antibodies in the sera of patients suffering from warm and cold AIHA and compares them with 118 healthy controls.

Warm AIHA

There was an increased incidence in antinuclear (15% $P<0{\cdot}5$) and in antimitochondrial (5% $P<0{\cdot}1$) antibodies compared with normal controls. The age group and sex of the patients and controls and the positive tests in each are shown in Table 2. The tests show that the increased incidence of autoantibodies occurred only in women. Tables 3 and 4 summarize the various clinical and serological data in those patients with antibodies. Those

TABLE 1. Incidence of tissue antibodies in sixty patients with idiopathic warm AIHA and sixteen patients with idiopathic cold AIHA

Antibody	Warm AIHA (60)*		Cold AIHA (16)*		Healthy controls (118)*	
	No. positive	% positive	No. positive	% positive	No. positive	% positive
ANF	9†	15	0	0	5	4·2
Mitochondrial	3‡	5	0	0	0	0
Gastric parietal cell	2	3·3	2	12·5	2	1·7
Thyroid cytoplasmic	1	1·6	1	6·3	3	2·5
Smooth muscle	0	0	0	0	1	0·8

* Numbers in parentheses refer to the total number of patients examined.
† $P < 0{\cdot}05$.
‡ $P < 0{\cdot}1$.

patients who had antinuclear antibody did not appear to consist of a distinct group of patients. In all, the titre of ANA was low with none higher than 20. In four of the nine patients there was a low serum concentration of one or more class of immunoglobulin, a similar incidence to that in patients without ANA in their serum. Similarly the patients with mitochondrial antibodies did not appear to be a distinct group in any way. The liver

TABLE 2. Incidence of ANA and mitochondrial antibodies in patients with warm AIHA and controls

Age group	Sixty patients						118 controls					
	No. in age group		Number ANA+		Number M+		No. in age group		No. ANA+		No. M+	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
0–20	3	3	0	0	1	0	3	5	0	0	0	0
21–30	2	3	1	0	0	0	8	6	0	0	0	0
31–40	3	2	0	0	0	0	12	15	0	0	0	0
41–50	8	4	0	1	0	0	9	12	0	0	0	0
51–60	10	5	3	0	1	0	13	14	2	1	0	0
61–70	8	5	4	0	1	0	6	9	1	1	0	0
71–80	4	0	0	0	0	0	2	4	0	0	0	0
Total	38	22	8	1	3	0	53	65	3	2	0	0
Percentage			21·6	4·5	8·9	0			5·6	3·1	0	0

TABLE 3. Patients with idiopathic warm AIHA and antinuclear antibodies

						Red cell antibody titres			Immunoglobulins* (mg/100 ml)		
							Papainized cells				
Patient	Sex	Age	ANA titre	LE test	DAGT†	IAT	Agg	Lysin	IgG	IgA	IgM
A.K.	M	43	10	negative	C′	0	128	8	840	135	160
L.F.	F	68	10	negative	IgG+C′	8	8	0	600	85	20
M.W.	F	57	20	negative	IgG+C′	4	32	4	1100	140	120
G.J.	F	63	20	negative	IgG	0	512	0	1260	50	480
N.P.	F	66	20	negative	IgG	0	2	0	550	150	124
A.F.	F	56	10	negative	IgG	0	8	0	440	280	90
E.M.	F	25	20	negative	IgG+C′	0	8	0	1600	290	120
F.B.	F	53	20	negative	IgG+C′	0	32	0	1500	170	120
E.B.	F	68	10	negative	IgG	0	8	0	300	110	90

* Normal range ±2 SD. IgG, 500–1600 mg/100 ml; IgA, 125–425 mg/100 ml; IgM, 45–170 mg/100 ml.
† DAGT = Direct antiglobulin test.
Underlined values represent those values outside the normal range.

TABLE 4. Patients with idiopathic warm AIHA and mitochondrial antibodies

Patient	Sex	Age	Mitochondrial antibody titre	DAGT	LFT*	Red cell antibody titres			Immunoglobulins (mg/100 ml)		
						IAT	Papainized cells		IgG	IgA	IgM
							Agg	Lysin			
M.M.	F	54	10	IgG	Normal	16	512	0	1200	460	63
B.W.	F	68	20	IgG+C′	Normal	1	128	1024	1350	265	1080
D.E.	F	13	20	IgG+C′	Normal	0	0	8	850	190	128

* LFT = Liver function tests

function tests in all three were normal and in one patient a liver biopsy obtained at the time of splenectomy showed no evidence of autoimmune hepatitis.

Cold AIHA

The incidence of tissue antibodies in this group of patients was similar to that in the control group when compared for age and sex.

DISCUSSION

The increased incidence of non-organ-specific autoantibodies in our patients with idiopathic warm AIHA confirms the reports of other authors (van Loghem, 1965; Gerbal *et al.*, 1968; Tan & Chaplin, 1968) and is similar to that found in other autoimmune diseases. Thus the incidence of antinuclear antibodies has also been reported to be increased in rheumatoid arthritis, myasthenia gravis and Hashimoto's thyroiditis (Seligmann, Cannat & Hamard, 1965). Similarly there is an increased incidence of antimitochondrial antibodies in various autoimmune diseases (Editorial, 1970). Conversely, the incidence of red cell antibodies as manifested by a positive direct antiglobulin test, is increased in various autoimmune diseases. The pathogenic mechanism giving rise to the red cell autoantibodies and the other antibodies, however, remains speculative. Possibly, patients with autoimmune diseases have an underlying immunological hyper-reactivity. Several analogous increases in tissue antibodies occur in experimental situations. For example, rabbits made hyperimmune by non-specific bacterial antigens produce anti-DNA antibodies (Christian & Abruzzo, 1965). Similarly the NZB/BL strain of mice have been noted to be immunologically hyper-responsive to stimuli by various foreign antigens, nonspecific stimuli, and show a resistance to the development of immunological tolerance (Morton, Olson & Siegel, 1967; Staples & Talal, 1969; Morton & Siegel, 1970). Patients with chronic infective illnesses similarly have an increased incidence of ANA and rheumatoid factors (Seligmann, Cannat & Hamard, 1965; Lindgvist, Coleman & Osterland, 1970), presumably because of continued immunological stimulation.

That this immunological hyper-reactivity gives rise to multisystem immunological disease is adequately demonstrated by the repeatedly reported incidence of more than one type of autoimmune disease in individual patients. The underlying aetiologic mechanism, however, remains to be elucidated. That immunodeficiency occurs in some patients with autoimmune disease has been extensively reviewed and documented. However, the concept that this is the underlying defect in autoimmune disease, while acceptable theoretically, remains unproven.

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