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Respiratory involvement in systemic lupus erythematosus

A CLINICAL AND IMMUNOLOGICAL STUDY

S. T. HOLGATE, D. N. GLASS,* P. HASLAM, R. N. MAINI* & M. TURNER-WARWICK

*Cardiothoracic Institute, Brompton Hospital, London, and * Clinical Division, Kennedy Institute of Rheumatology and Charing Cross Hospital, London*

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SUMMARY

Thirty patients fulfilling conventional criteria for systemic lupus erythematosus and who presented with extensive pleural and pulmonary involvement were studied retrospectively. Four overlapping patterns of respiratory disease were identified and observations were made on their clinical presentation, radiographic abnormalities and response to treatment.

A low incidence of severe renal disease was found in this series of patients and this was in keeping with the general finding of low serum binding using native DNA in a globulin Farr-binding technique (greater than 20% binding in only 4/21 (19%) of the series) and normal or elevated serum complement (C3) levels.

Precipitating antibody detected by double diffusion and counter-current immunoelectrophoresis and probably reacting in most cases with single-stranded DNA was, however, detected in 66% of pretreatment serum samples tested. This evidence supports the idea that different types of anti-nuclear antibody may be associated with different clinical manifestations seen within a group of patients who broadly fulfil the criteria for SLE.

INTRODUCTION

The pathogenesis of systemic lupus erythematosus (SLE) remains conjectural. Experimental studies in man and animal models suggest that immune complex deposition may be important in initiating tissue damage (Cochrane & Koffler, 1973).

The lungs and pleura are among the many organs affected by the disease and although there have been numerous reports on the clinical, pathological and immunological aspects of the total syndrome, there have been only isolated studies on the pleuro-pulmonary features (Hoffbrand & Beck, 1965; Huang, Henniger & Lyons, 1965) or on the immunological aspects in cases with predominant intra-thoracic involvement (Turner-Warwick, 1974).

This report attempts to clarify the clinical aspects of pleuro-pulmonary SLE and draws attention to certain immunological features which appear to distinguish clinical sub-groups within the general definition of SLE.

Selection of patients

Thirty patients were selected with a diagnosis of SLE, all of whom presented with a predominant intra-thoracic disorder. Twenty-two patients fulfilled the criteria of the American Rheumatism Association for the diagnosis of SLE (Cohen *et al.*, 1971b) (Table 1). The remaining eight patients we considered had sufficient clinical features to be included in the broad definition of SLE (Table 2). All thirty patients met the Medical Research Council's criteria for SLE (MRC, 1961), and all had circulating anti-nuclear antibodies in a titre greater than 1/10 together with LE cells demonstrated on at least two occasions.

We have excluded from this report one patient with clinical features that overlapped with rheumatoid

Correspondence: Dr S. T. Holgate, The Medical Unit, Southampton General Hospital, Tremona Road, Southampton, Hants.

TABLE 1. The incidence of positive criteria in thirty patients with pleuro-pulmonary SLE based on fourteen manifestations suggested by the ARA

No.	Criteria	No. (%) in present series	Percentage in ARA preliminary criteria*
1	Facial erythema	16 (53·3)	63·7
2	Discoid lupus	3 (10·0)	17·1
3	Raynaud phenomenon	7 (23·3)	20·0
4	Alopecia	2 (6·7)	43·3
5	Photosensitivity	3 (10·0)	36·7
6	Oral or nasal ulceration	3 (6·7)	15·1
7	Arthralgia or arthritis	19 (63·3)	89·8
8	LE cells	30 (100·0)	91·8
9	Chronic false positive serological tests for syphilis	3 (10·0)	11·8
10	Profuse proteinuria	0 (0)	19·6
11	Cellular casts	2 (6·7)	47·8
12	Pleuritis	26 (86·7)	60·4
	Pericarditis	15 (50·0)	18·8
13	Psychosis or convulsions	5 (16·7)	19·2
14	Haemolytic anaemia	6 (20·0)	16·3
	Thrombocytopenia	9 (30·0)	11·4
	Leucopenia	12 (40·0)	39·6

The incidence of these manifestations in the present series were: six or more, seven patients; four or five, fifteen patients; three, five patients; two, three patients.

* Cohen *et al.* (1971b).

TABLE 2. The clinical features of eight patients that failed to meet the ARA criteria for SLE but had many additional clinical features that strongly suggested a diagnosis of SLE

No.	Sex	Age	Clinical features
1	F	61	Fibrosing alveolitis, Raynaud, myocarditis, endocarditis, haemolytic anaemia, hepatomegaly, conjunctivitis sicca, LE cells and ESR 110 mm in first hour
2	F	27	Pneumonitis, arthritis, thrombocytopenia, LE cells and ESR 87 mm in first hour
3	F	47	Pneumonitis, pleuritis, pericarditis, arthritis, LE cells and ESR 105 mm in first hour
4	F	45	Pneumonitis, pleuritis, pyrexia, facial erythema, sub-ungual infarcts, abdominal pain, LE cells and ESR 127 mm in first hour
5	F	50	Pleuritis, pyrexia, phlebitis, arthritis, rash, LE cells, ESR 70 mm in first hour
6	F	41	Fibrosing alveolitis, endocarditis, haemolytic anaemia, thrombocytopenia, mild proteinuria, hemiparesis, LE cells and ESR 58 mm in first hour
7	F	67	Pleurisy, folate-deficient anaemia, severe malaise, arthritis, LE cells and ESR 128 mm in first hour
8	F	65	Pneumonitis, haemolytic anaemia, leucopenia, LE cells and ESR 48 mm in first hour

arthritis and scleroderma who had an antibody which reacted with an RNase-sensitive antigen and one patient with a procaineamide-induced lupus syndrome.

MATERIALS AND METHODS

Clinical methods. All patients had been investigated as in-patients at the Brompton Hospital between the years 1960 and 1973. A retrospective review of their clinical features and subsequent progress was made and as far as was possible the following data was tabulated systematically: presenting symptoms, evidence of respiratory, cardiac, visceral, skin and central nervous system involvement, chest radiographic appearances, laboratory data, including haemoglobin, white blood count, platelet count, erythrocyte sedimentation, Coombs test and evidence for renal and hepatic disease. Renal involvement was assessed by urinalysis, 24 hr urinary protein excretion, blood urea levels and creatinine clearances.

Respiratory function was recorded as the forced expiratory volume expressed in 1 sec (FEV_1), the forced vital capacity (FVC) both measured using a standard Vitalograph spirometer and the carbon dioxide diffusing capacity ($DLCO_2$) measured by the single breath technique (Ogilvie *et al.*, 1957).

Immunological methods. (1) *Antinuclear antibody* (*ANA*) was detected and titrated using a standard double layer immunofluorescent technique (Turner-Warwick & Parkes, 1970).

(2) *Rheumatoid* (*antiglobulin*) *factor* (*RF*) was measured as the differential agglutinating titre (DAT) (Rose *et al.*, 1948).

(3) *Total complement C3* (*BIC*, *BIA*) was measured by a radial immunodiffusion technique using the standard Immuno-Plate® (Hyland) (Mancini, Carbonara & Heremans, 1965).

(4) *Serum globulin–DNA-binding capacity* was measured by a modified Farr technique (Farr, 1958; Glass *et al.*, 1973). ^{125}I-labelled double-stranded (Ds) HeLa cell DNA was used as the antigen and DNA binding activity was expressed as the percentage radioactivity in the globulin fraction precipitated by the addition of anti-human immunoglobulin.

(5) *DNA precipitins* were detected by two methods in uninactivated sera and after heat inactivation at 56°C for 30 min. (a) Ouchterlony double-diffusion in 0·4% agarose gel was performed using a modification based on the method of Tan & Kunkel (1966). Commercial calf thymus DNA (Miles Seravac Laboratories) was used as the test antigen. It was prepared as a freeze-dried double-stranded material. Biochemical data indicated a DNA content of 83·3%, RNA less than 1%, protein 0·23% and moisture 9·4%. Viscosity measurements on material reconstituted with minimal stirring to avoid strand sheer and possible further dissociation into single strands suggested that there was nevertheless some damage to the double-stranded structure. For this reason we have described it as 'Ds'-calf thymus DNA. It was used as the antigen at a concentration of 1 mg/ml, buffered with 0·01 M K_2HPO_4-KH_2PO_4, pH 7·2. Wells were cut using a standard template, DNA placed in the central well and undiluted sera in the peripheral wells. The plates were incubated at 4°C and examined for lines of immunoprecipitation after 48 hr using indirect illumination (Fig. 1a). (b) Two-stage (counter-current) electro-immunodiffusion in 1·2% agarose gel was performed using the modification of Johnson, Edmonds & Holborow (1973). Calf thymus

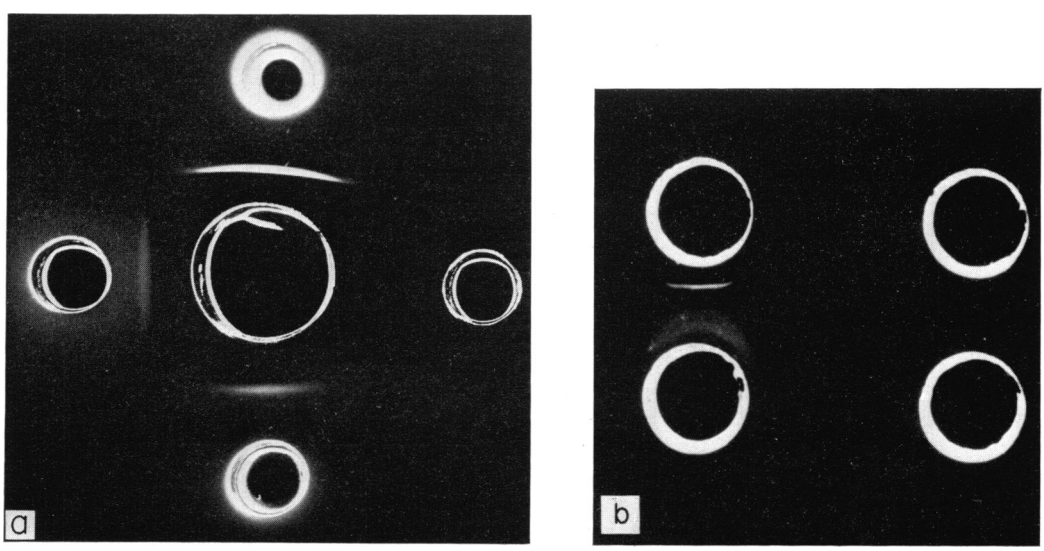

FIG. 1. Techniques used to detect precipitating antibodies to commercial DNA: (a) Double diffusion in agar gel. Antigen is placed in the centre well and test serum samples in the peripheral wells. (b) Electro-immunodiffusion. Antigen is placed in the upper wells and test serum samples in the lower wells. Precipitins are seen as a line nearest and concave towards the antigen cathodal well. The haze adjacent to the serum wells is non-specific.

DNA was used as the antigen at a concentration of 20 μg/ml and the test buffered with 0·05 M sodium barbitone–HCl, pH 8·2. Electrophoresis was carried out for 15 min in the first stage and 45 min in the second stage and plates examined immediately by indirect illumination (Fig. 1b).

(6) *Heat-denatured DNA (single-stranded, ss-DNA)* was prepared from commercial calf thymus DNA as described by Cohen *et al.* (1971b).

(7) *Enzymatically denatured DNA* was prepared by treating commercial calf thymus DNA with beef pancreas DNase 1 (Miles Seravac Laboratories) using the method described by Sharp *et al.* (1971).

RESULTS

Clinical results

The age of onset of symptoms ranged from 13 to 71 years with a mean of 43·9 years. Females outnumbered males by 4:1 and all the male patients presented over the age of 50 years (Fig. 2).

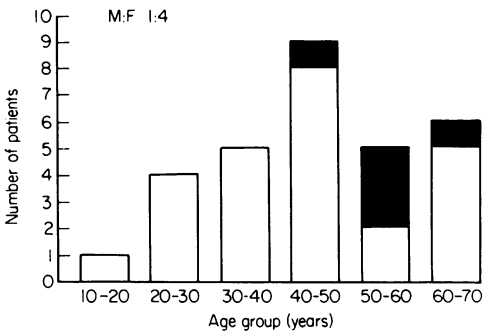

FIG. 2. Age and sex distribution of thirty patients with respiratory systemic lupus erythematosus. (■) Male patients: (□) female patients (r 1:4).

The incidence of systemic features and laboratory abnormalities are summarized in Figs 3 and 4. Only ten patients had any detectable renal abnormality (Table 3): proteinuria was present in nine patients but never exceeded 350 mg in 24 hr and only the two patients with urinary cellular casts in addition to proteinuria fulfilled the ARA criteria for lupus nephritis. Impaired glomerular filtration

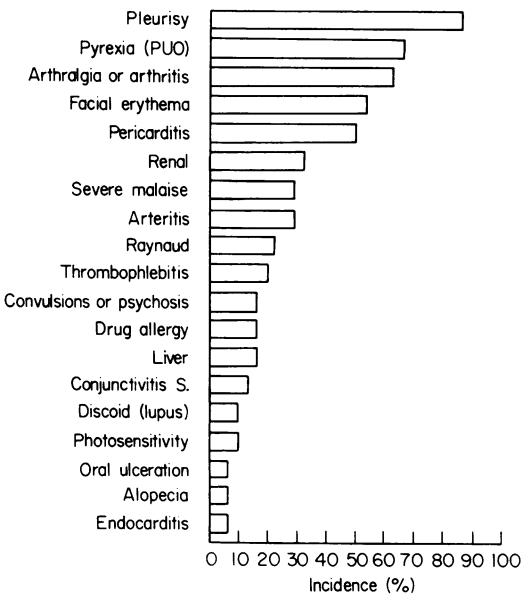

FIG. 3. Incidence of the clinical features in thirty patients with respiratory systemic lupus erythematosus.

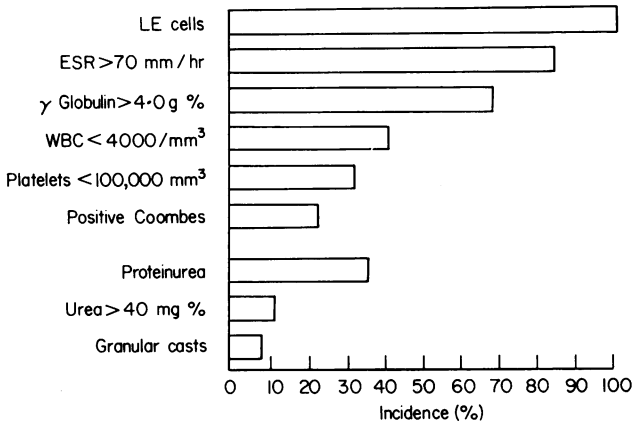

FIG. 4. Incidence of the laboratory abnormalities in thirty patients with respiratory systemic lupus erythematosus.

detected by a reduced creatinine clearance (<70 ml/min) was present in four patients during their period of observation. Some of the relatively minor renal functional abnormalities recorded in this series may have related to other features of these patients' clinical illness. For instance, one patient with impaired renal function had severe general debility associated with carcinoma of the pancreas (patient 6), and in two other patients severe congestive cardiac failure (patient 7) and a large pericardial effusion (patient 1) were considered to be important contributory factors.

TABLE 3. Laboratory data on the ten patients with a detected renal abnormality

Patient no.*	Age	Sex	Urinary cellular casts	Pyuria	Proteinuria (mg/24 hr) (maximum)	Blood urea (mg/100 ml) (maximum)	Creatinine clearance (ml/min) (minimum)
1	31	F	++	0	50	75	30
3	57	M	0	0	300	19	70
20	21	F	++	0	130	22	104
17	45	F	0	0	++	33	—
21	34	F	0	0	80	80	25
19	45	F	0	0	200	28	90
0†	31	F	0	0	350	19	124
0†	22	F	0	0	0	53	40
7	21	F	0	0	120	28	58
9	60	M	0	0	200	40	73

* Patient numbers correspond to Table 5.
† No serum available and thus not included in Table 5.

Respiratory symptoms were a major feature in all the patients studied: dyspnoea in 80%, pleuritic pain in 87%, dry cough in 33% and small haemoptyses in 17%. They were the presenting feature of the disease in fourteen patients (47%) and the only symptomatic manifestation in seven patients (24%). From the clinical, radiological and physiological data, four overlapping types of respiratory disease in SLE were identified (Table 4).

Pleurisy was the most frequent symptom encountered (87%). It occurred either in isolation (Type 1, 30%) or complicated the other forms of respiratory involvement. Small pleural effusions (bilateral in 33%) and pericarditis (55%) were associated findings and although the lung fields could be radiologically clear, a restrictive defect of lung function and a reduction in the gas transfer suggested the possibility

TABLE 4. The incidence, clinical and radiological features of the four types of pleuropulmonary involvement seen in systemic lupus erythematosus

Type	Clinical features	Chest X-ray features	Response to steroids	No. of patients
1	Episodic pleuropericarditis	Pleural thickening, effusions and normal lung fields	Good	9
2	Pleuropericarditis with moderate dyspnoea, cough or haemoptyses	(a) Pleural changes, basal line shadows, thickened and elevated hemi-diaphragm	Good	7
		(b) Segmental or lobar infiltrates or collapse ('changing shadows')	Good	6
3	Frequent episodes of Type 1 or 2 but with dyspnoea that persists and progresses after each attack	Bilateral thickened and elevated diaphragms with or without pleural changes and line shadows ('shrinking lungs')	Poor	4
4	Progressive dyspnoea	Diffuse basal reticulo-nodular shadowing (fibrosing alveolitis)	Poor	4

of coexistent lung disease. The symptoms and physiological disturbance usually responded well to corticosteroids.

Type 2 disease (43%) had, in addition to pleural changes, radiographic evidence of pulmonary involvement (lupus pneumonitis). Clinically and radiologically two broad sub-groups could be identified. In the first (Type 2a, 23%) patients presented with pleural pain, moderate dyspnoea and small haemoptyses and had the radiological findings of unilateral diaphragmatic elevation, short line shadows 1–2 cm long in the basal lung fields and, less commonly, long bizarre line shadows 2–5 cm long extending across the

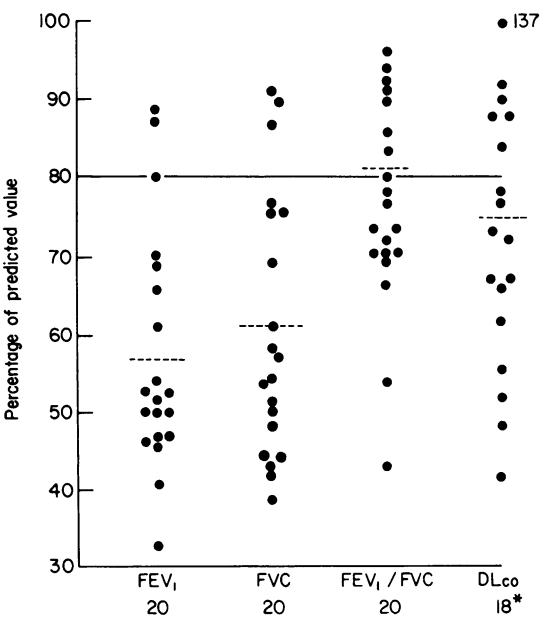

FIG. 5. Spirometric and gas transfer values in twenty patients with respiratory systemic lupus erythematosus. The results are expressed as the percentage of the predicted value for age, sex and body surface area. (- - -) Mean value; (———) lower limit of normal. * Two patients were too dyspnoeic for the $DLCO_2$ to be obtained by the single breath method.

lung fields to communicate with a pleural surface. In the second (Type 2b, 20%) severe dyspnoea and cough were the presenting features, and radiologically transient changing areas of lobar and segmental infiltrates or patches of collapse ('changing shadows') were characteristic. Both groups responded well to steroids.

Patients with Type 3 disease (13%) initially had the clinical and radiological features of Types 1 or 2, but rapidly progressed with persistent dyspnoea that was only partially responsive to steroids. The radiological features of bilateral elevation of thickened diaphragms, pleural changes and lower zone line shadows have led to the term 'shrinking lungs' to describe this syndrome.

Patients with late onset dyspnoea and persisting diffuse or small irregular shadowing, predominantly in the lower zones and resembling fibrosing alveolitis (Type 4, 13%). This was confirmed by open lung biopsy in two patients who demonstrated gross alveolar wall fibrosis, organizing intra-alveolar exudate and a variable peri-alveolar chronic inflammatory cell infiltrate. The disease was progressive and the response to steroids was poor.

Twenty patients had had lung function studies performed (Fig. 5). These showed a restrictive defect in eighteen (with a reduced gas transfer in fourteen) and irreversible airway obstruction in two. Patients with Types 3 and 4 disease had the most severe physiological abnormalities which failed to reverse with steroids, while patients with Types 1 or 2 disease had abnormalities of varying severity which could neither be correlated with the clinical or the radiological extent of involvement, but usually improved on steroid treatment.

TABLE 5. The immunological features found in twenty-one sera from untreated patients with respiratory systemic lupus erythematosus

No.	Sex	Age	No. of ARA criteria	ANA titre†	DAT titre	Native DNA binding ($n = < 20\%$)‡	Double diffusion (0 to +++)§	Immuno-electrophoresis (0 to +)	Total C3 complement (mg/100 ml) ($n = 62$–212)
1*	F	31	6	1/800	1/64	11	++	+	215
2	F	21	7	1/800	1/16	7	+++	+	175
3*	M	57	4	1/80	0	12	0	0	—
4	F	50	4	1/160	1/32	8	++	±	—
5	F	49	5	1/10	1/16	8	++	+	181
6	F	67	3	1/200	1/256	49	++	+	—
7*	F	21	4	(m) 1/160	1/8	10	0	0	187
8	F	67	4	1/40	1/8	8	+++	+	—
9*	M	60	4	(nu) 1/400	0	8	+	+	230
10	F	37	5	1/800	0	13	+++	+	—
11	F	27	4	1/10	0	6	0	0	—
12	F	67	5	1/320	1/4	12	0	0	—
13	M	42	4	1/640	0	17	+	0	250
14	F	36	2	1/400	1/64	7	+++	+	170
15	F	44	7	(s) 1/160	0	11	+++	+	—
16	F	61	3	1/40	1/16	9	+++	+	180
17*	F	45	8	(m) 1/800	0	13	±	0	—
18	M	53	4	1/40	0	8	0	0	230
19*	F	45	6	1/320	0	30	+++	+	—
20*	F	21	7	1/800	0	39	0	0	—
21*	F	34	3	1/640	0	47	+++	+	—

n = Normal range.

* Patients with renal abnormality (see Table 3).

† All ANAs were of the diffuse type except where prefixed by s (speckled), m (membranous) or nu (nucleoli).

‡ N-DNA binding activity is expressed as percentage of radioactivity in the precipitate brought down by addition of the second antibody (Glass *et al.*, 1973).

§ DNA precipitins by diffusion were graded from 0 to +++ according to the intensity of the precipitin line produced.

The mean follow-up period for the thirty patients was 9·8 years. Six patients died; two from incidental causes (carcinoma of the pancreas and extensive burns) and four from respiratory complications (two infective and two from respiratory failure). The mean follow-up period for the twenty-four surviving patients was 9·5 years ranging from 1 to 29 years. No patient in this series died from renal failure.

Immunological results

Serum samples from twenty-one patients obtained before steroid treatment were available for analysis and the results are summarized in Table 5.

Anti-nuclear antibody was present in all sera in titres varying between 1/10 and 1/800 (titrations were not continued beyond 1/800). The pattern of nuclear fluorescence was diffuse in seventeen (80%), peripheral in only two (10%), speckled in one (5%), and mixed nucleolar and diffuse in one (5%).

Rheumatoid factor in a titre of greater than 1/16 was detected in seven sera (33%).

Serum complement C3 levels in nine sera tested were within the normal range in five and elevated in four.

Globulin-DNA binding capacity using ^{125}I-labelled HeLa cell Ds-DNA was surprisingly low, seventeen out of twenty-one (81%) sera tested showing insignificant binding of less than 20%; four sera only (19%) showed increased DNA binding at levels of 39%, 48%, 49% and 30%. Three of the four positive sera were from patients with a detectable renal abnormality (patients 19, 20 and 21). The remaining patient with positive DNA binding was a woman aged 67 with fibrosing alveolitis but no evidence of nephropathy (patient 6).

Precipitating antibodies to calf thymus ds-DNA were detected in fourteen sera (67%) by Ouchterlony immunodiffusion and in twelve sera (57%) by two-stage electro-immunodiffusion. There was very close agreement between the results with the two methods ($P < 0{\cdot}01$ using Fisher's Exact Test). There were only two sera positive in the Ouchterlony system not detected by electro-immuno-diffusion.

Of the seven patients with evidence of renal disease, four had DNA precipitins using the Ouchterlony technique and three of these were positive using counter-current electrophoresis. Three of the four sera showing significant DNA binding also showed DNA precipitins by both techniques.

In three studies to identify an immunoglobulin component to the precipitin lines, immuno-electrophoresis combined with autoradiography using ^{131}I-labelled anti-human immunoglobulin, confirmed the presence of IgG. The precipitin lines were also removed from all these three sera by prior absorption with anti-human immunoglobulin.

In six other experiments to study antibody specificity the precipitin lines were removed not only by prior absorption of sera with calf thymus DNA, but also with heat-denatured (single-stranded) calf thymus DNA and with enzymatically denatured calf thymus DNA. The lines were not removed by prior absorption of sera with RNA.

DISCUSSION

A significant number of patients with SLE have lung involvements although this may not be clinically evident and demonstrated only after functional or pathological investigation (Huang *et al.*, 1965; Laitinen *et al.*, 1973).

Four overlapping clinical and radiological types of respiratory involvement have been recognized and correspond well with earlier descriptions (Bulgrin, Dubois & Jacobson, 1960; Hoffbrand & Beck, 1965; Gold & Jennings, 1966; Gross *et al.*, 1972; Olsen & Lever, 1972).

In the series that we report, pleuropulmonary (and/or pericardial) involvement was deliberately selected as the predominating feature of the patients studied and to this extent this series may contain differences in clinical emphasis from those selected by other specialists such as rheumatologists, nephrologists, neurologists and dermatologists. The incidence of non-respiratory features in the present series of SLE patients is, however, comparable to that found in other series with the exception of alopecia, skin involvement and severe nephritis (Table 1) (Dubois & Tuffanelli, 1964; Cohen *et al.*, 1971b; Estes &

Christian, 1971). It is possible that part of the explanation for the low incidence of skin involvement and alopecia may reflect the limitations of a retrospective survey, but this is unlikely to be the whole explanation because of the closely similar prevalence of many other subjective and objectively observed clinical features.

Up to 50% of patients in several large reported series of SLE developed renal functional impairment which, in a significant proportion, is severe, and becomes one of the major factors adversely influencing prognosis (Estes & Christian, 1971). In contrast, patients in our series had a much lower overall incidence of renal functional impairment. In only ten patients (33%) could any renal abnormality be detected, in each case this was mild and in three instances it could be accounted for by associated disease. Only two of our thirty patients fulfilled criteria for lupus nephritis (Cohen *et al.*, 1971b) and in all cases it appeared to have little or no influence over the disease course.

Immunologically, the onset of active lupus nephritis is frequently reflected by a measurable decrease in one or more serum complement components (Schur & Sanderson, 1968), and by a marked increase in the serum globulin–DNA Farr-binding capacity (Pincus *et al.*, 1969; Hughes, 1971). It has been suggested that low affinity antibodies to DNA may be particularly important in the initiation of the renal lesions, soluble immune complexes forming *in vivo* which can gain access to the kidney and become deposited on the glomerular basement membrane (Johnson *et al.*, 1973). Recent direct evidence in support of the importance of low affinity antibody has been provided by Steward *et al.* (1974) who demonstrated that the avidity of DNA in *in vitro* immune complexes using the sera of patients with renal disease is lower than from SLE patients without renal disease. Additional indirect evidence was provided by Johnson, Edmonds & Holborow (1973, 1974) who, using counter-current electrophoresis found that precipitating antibodies to DNA were often absent from the serum of patients with active renal disease but readily demonstrable in many patients without renal disease. These workers suggested that low affinity antibodies tending to form soluble non-precipitating immune complexes would not be readily detectable in gel immunoprecipitation systems.

The normal or high serum C3 levels in ten of the patients studied from the present series, and the small number with globulin-DNA binding, are both also in keeping with the low prevalence of renal involvement observed. There was perhaps also indirect evidence of low affinity antibody to DNA in the serum of one patient where DNA antibodies were not detected using immunoprecipitation techniques but were detected using Farr-binding and it was interesting that this patient had abnormal renal function with proteinuria (130 mg/24 hr) and cellular casts and fulfilled the criteria for lupus nephritis (patient 20, Table 5).

These immunological findings lend support to the validity of the clinical observation that patients with SLE, with predominant pleuropulmonary involvement, fall into a clinical sub-group with an unusually low incidence of active nephritis.

Our immunological investigations have also demonstrated an interesting anomaly of antibodies to DNA which are demonstrable by immunoprecipitation but which were very rarely detected using the Farr globulin-DNA binding assay. The discrepancy is not unique to our series and has been noted in other studies on SLE sera (Johnson *et al.*, 1973; Davis & Winfield, 1974; Dorsch & Barnett, 1974). However, the degree of the discrepancy is particularly striking in our series. Theoretically one might predict that all antibodies including precipitating and non-precipitating ones would be identified using the Farr-binding test. The inconsistent findings between Farr-binding and precipitation reported here have been studied in greater detail and reported elsewhere (Haslam *et al.*, 1976).

From the observations reported here, the possibility of false positive reactions between highly cationic DNA and highly anionic substances such as C1q or non-immunoglobulin serum proteins has been largely excluded as an explanation for the high incidence of precipitins, by using the counter-current method of electrophoresis to separate non-specific and immunologically specific precipitin lines. Further we have demonstrated in a small number of experiments an IgG immunoglobulin component to the precipitin arc.

Another possibility which was considered was that anti-DNA antibodies in our patients sera were already complexed with autologous DNA and therefore gave a negative DNA binding in the Farr assay,

but under the conditions of precipitation tests in agar they became dissociated and allowed a reaction to occur between anti-DNA antibody and calf thymus DNA.

It is more probable that differences in the antigenic characteristics and the quantities of DNA used in the two test systems may be of fundamental importance.

The material used in the Farr-binding assay was double-stranded Hela cell DNA, freshly extracted from Hela cells with every precaution to preserve the double-stranded helix. On the other hand, the material used in the immunoprecipitation tests was commercial calf thymus double-stranded DNA which had been freeze dried and reconstituted. Evidence of some damage to the double-stranded helix has been found using viscosity measurements (Haslam *et al.*, 1976). The difficulty in stabilizing ds-DNA and of establishing its exact physical form at the moment of use has been emphasized by Tan & Natali (1970). The possibility therefore arises that using Farr-binding with Hela-cell DNA we may be demonstrating the few sera containing antibodies reacting with double-stranded DNA, but that using the immunoprecipitation methods with calf thymus ds-DNA, we (and many others using this material), are demonstrating an additional majority containing antibodies reacting with antigens presenting on single-stranded DNA. The fact that in a few studies the precipitin lines were usually removed by absorption with single-stranded DNA as well as with ds-DNA and enzymatically denatured DNA suggests that the antibodies in these sera were not specific to double-stranded material. The heterogenous nature of antibodies to DNA in SLE is clearly established (Koffler *et al.*, 1969, 1971; Cohen *et al.*, 1971b) and the significance of antibodies reacting with ss-DNA have been said to differ from those reacting with ds-DNA in that their levels do not reflect disease activity (Koffler *et al.*, 1971). They also frequently occur in the serum of patients with other connective tissue disease, e.g. rheumatoid arthritis, chronic active hepatitis and Sjögren's syndrome, as well as in the lupus-like syndrome occasionally seen after procaineamide ingestion (Blomgren, Condemi & Vaughan, 1972; Davis & Winfield, 1974).

One other possibility explored in the present study was that the precipitin methods might in many instances have demonstrated immunological reactions to contaminants such as RNA or protein histones (Reichlin & Mattioli, 1972) which may be present in differing amounts in the calf thymus and Hela cell DNA. However, the calf thymus DNA used in the present study contained only trace amounts of RNA and protein, and in the few sera tested the precipitin lines were not removed by serum absorption with RNA, nor were any lines observed when RNA was used directly as the antigen.

We feel that the balance between ds and ss structure in the DNA materials used is the most likely explanation for the disparity between binding and precipitating systems in our series. Whether other material related factors, such as quantitative or molecular weight differences or species origin which may affect the numbers of available antibody-combining sites, can also influence the test results remains to be resolved.

The similarity between the patients with predominant respiratory SLE in our series, and the procaine-amide-induced lupus syndrome is striking, especially with regard to the high incidence of pleuropulmonary involvement, the low incidence of renal impairment, the presence of normal or elevated serum complement levels, and the finding of a high incidence of antibodies more probably reacting with ss-DNA, but a strikingly low incidence of Farr-binding antibodies (Dubois, 1969; Blomgren *et al.*, 1972; Auerbach, Snyder & Bragg, 1973). We were, however, unable to incriminate any particular drug as an initiating agent in our patients.

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REFERENCES

Auerbach, R.C., Snyder, N.E. & Bragg, D.G. (1973) Chest roentgenographic manifestations of Pronestyl induced lupus erythematosus. *Radiology*, **109**, 287.

Blomgren, S.E., Condemi, J.J. & Vaughan, J.H. (1972) Procainamide induced lupus erythematosus—clinical and laboratory observations. *Amer. J. Med.* **52**, 338.

Bulgrin, J.G., Dubois, E.L. & Jacobson, J. (1960) Chest roentgenographic changes in systemic lupus erythematosus. *Radiology*, **74**, 42.

Cochrane, G.C. & Koffler, D. (1973) Immune complex disease in experimental animals and man. *Advanc. Immunol.* **16**, 186.

COHEN, S.A., HUGHES, G.R.V., NOEL, G.L. & CHRISTIAN, C.L. (1971a) Character of anti-DNA antibodies in systemic lupus erythematosus. *Clin. exp. Immunol.* **8**, 551.

COHEN, S.A., REYNOLDS, W.E., FRANKLIN, E.C., KULKA, J.P., ROPES, M.W., SHULMAN, L.E. & WALLACE, S.L. (1971b) Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. rheum. Dis.* **21**, 643.

DAVIS, J.J. & WINFIELD, J.B. (1974) Serum antibodies to DNA by counterimmunoelectrophoresis (CIE). *Clin. Immunol. Immunopath.* **2**, 510.

DORSCH, C. & BARNETT, E.V. (1974) The occurrence and nature of precipitating antibodies in anti-DNA sera. *Clin. Immunol. Immunopath.* **2**, 310.

DUBOIS, E.L. (1969) Procainamide induction of a systemic lupus-like syndrome. *Medicine (Balt.)*, **48**, 217.

DUBOIS, E.L. & TUFFANELLI, D.L. (1964) Clinical manifestations of systemic lupus erythematosus: computer analysis of 520 cases. *J. Amer. med. Ass.* **190**, 104.

ESTES, D. & CHRISTIAN, C.L. (1971) The natural history of systemic lupus erythematosus by prospective analysis. *Medicine (Balt.)*, **50**, 85.

FARR, R.S. (1958) A quantitative immunochemical measure of the primary interaction between 1* BSA and antibody. *J. infect. Dis.* **103**, 239.

GLASS, D.N., CAFFIN, J., MAINI, R.N. & SCOTT, J.T. (1973) Measurement of DNA antibodies by double antibody precipitation. *Ann. rheum. Dis.* **32**, 342.

GOLD, W.M. & JENNINGS, D.B. (1966) Pulmonary function in patients with systemic lupus erythematosus. *Amer. Rev. resp. Dis.* **93**, 556–56.

GROSS, M., ESTERLY, J.R. & EARLE, R.H. (1972) Pulmonary alterations in systemic lupus erythematosus. *Amer. Rev. resp. Dis.* **105**, 572.

HASLAM, P., Griffiths, I.E., MAINI, R.N. (1976) Antibodies to deoxyribonucleic acid in systemic lupus erythematosus. (In press.)

HOFFBRAND, B. & BECK, E.R. (1965) 'Unexplained' dyspnoea and shrinking lungs in systemic lupus erythematosus. *Brit. med. J.* **i**, 1273.

HUANG, C.T., HENNIGAR, C.R. & LYONS, H.A. (1965) Pulmonary dysfunction in systemic lupus erythematosus. *New Engl. J. Med.* **272**, 288.

HUGHES, G.R.V. (1971) Significance of anti-DNA antibodies in systemic lupus erythematosus. *Lancet*, **ii**, 861.

JOHNSON, G.D., EDMONDS, J.P. & HOLBOROW, E.J. (1973) Precipitating antibody to DNA detected by two stage electro-immunodiffusion: study in SLE and rheumatoid arthritis. *Lancet*, **ii**, 883.

JOHNSON, G.D., EDMONDS, J.P. & HOLBOROW, E.J. (1974) Precipitating antibodies in systemic lupus. *Lancet*, **i**, 750.

KOFFLER, D., CARR, R.I., AGNELLO, V., FEIZI, T. & KUNKEL, H.G. (1969a) Antibodies to polynucleotides: distribution in human sera. *Science*, **166**, 1648.

KOFFLER, D., CARR, R.I., AGNELLO, V., THOBURN, R. & KUNKEL, H.G. (1971) Antibodies to polynucleotides in human sera: antigenic specificity and relation to disease. *J. exp. Med.* **134**, 294.

LAITINEN, O., SALORINNE, Y. & POPPIUS, H. (1973) Respiratory function in systemic lupus erythematosus, scleroderma and rheumatoid arthritis. *Ann. Rheum. Dis.* **32**, 531.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.

MEDICAL RESEARCH COUNCIL COLLAGEN DISEASES AND HYPERSENSITIVITY PANEL (1961). Treatment of systemic lupus erythematosus with steroids. *Brit. med. J.* **ii**, 915.

OGILVIE, C.M., FORSTER, R.E., BLAKEMORE, W.S. & MORTON, J.W. (1957) A standardised breath-holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J. clin. Invest.* **36**, 1.

OLSEN, E.G.V. & LEVER, J.V. (1972) Pulmonary changes in systemic lupus erythematosus. *Brit. J. Dis. Chest*, **66**, 71.

PINCUS, T., SCHUR, P.H., ROSE, J.A., DECKER, J.L. & TALAL, N. (1969) Measurement of serum DNA-binding in systemic lupus erythematosus. *New Engl. J. Med.* **281**, 701.

REICHLIN, M. & MATTIOLI, M. (1972) Correlation of a precipitin reaction to an RNA protein antigen and a low prevalence of nephritis in patients with systemic lupus erythematosus. *New Engl. J. Med.* **286**, 908.

ROSE, H.M., RAGAN, C., PEARCE, E. & LIPMAN, M.O. (1948) Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proc. Soc. exp. Biol. (N.Y.)*, **68**, 1.

SCHUR, P.H. & SANDERSON, J. (1968) Immunological factors and clinical activity in systemic lupus erythematosus. *New Engl. J. Med.* **278**, 533.

SHARP, G.C., IRVIN, W.S., LA ROQUE, R.L., VELEZ, C., DALY, V., KAISER, A.D. & HOLMAN, R.H. (1971) Different nuclear antigens and clinical patterns of rheumatic disease and responsiveness to therapy. *J. clin. Invest.* **50**, 350.

STEWARD, M.W., GLASS, D.N., MAINI, R.N. & SCOTT, J.T. (1974) Role of low avidity antibody to native DNA in human and murine lupus syndromes. Extracts from VI Pan-American Conference on Rheumatic Diseases. Toronto, June 1974 Abstract 75.

TAN, E.M. & KUNKEL, H.G. (1966) Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J. Immunol.* **96**, 464.

TAN, E.M. & NATALI, P.G. (1970) Comparative study of antibodies to native and denatured DNA. *J. Immunol.* **104**, 902.

TURNER-WARWICK, M. (1974) Immunological aspects of systemic diseases of the lungs. The Philip Ellman Lecture delivered to the Royal Society of Medicine, 1973. *Proc. Roy. Soc. Med.* **67**, (Symp. No. 14), 541.

TURNER-WARWICK, M. & PARKES, W.R. (1970) Circulating rheumatoid and antinuclear factors in asbestos workers. *Brit. med. J.* **iii**, 492.