ANTIBODIES AGAINST NUCLEAR AND CYTOPLASMIC CELL CONSTITUENTS IN SYSTEMIC LUPUS ERYTHEMATOSUS AND OTHER DISEASES

G. L. ASHERSON*

From the Clinical Research Unit of the Walter and Eliza Hall Institute of Medical Research and the Royal Melbourne Hospital, Victoria, Australia

Received for publication November 17, 1958

GAJDUSEK (1957, 1958) showed that the sera of patients with systemic lupus erythematosus (SLE) and chronic hepatitis may contain complement fixing antibodies to human and animal tissues. Robbins, Holman, Deicher and Kunkel (1957) subsequently showed that sera of many patients with SLE reacted in the complement fixation test with nuclei and desoxyribose nucleic acid (DNA), of both human and animal origin.

This paper describes the results of complement fixation tests on sera from patients with SLE, chronic hepatitis, macroglobulinaemia and syphilis using human liver homogenates, rat liver nuclei and cytoplasm, calf thymus nuclei and calf thymus DNA as antigens.

MATERIALS AND METHODS

Antigens.—Human liver homogenate was prepared from autopsy material as 10 per cent suspension in 0·85 per cent saline (Gajdusek, 1958). Because of the difficulty in obtaining fresh human liver it was decided to make use of Gajdusek's observations that sera reacting against human tissue also reacted against animal tissue. For this reason rat liver was used in the study of the reactivity of the various cellular components.

Rat liver nuclei and calf thymus nuclei were prepared by the method of Mirsky and Pollister (1946). The rat liver was homogenized for 2 min. with a Teflon pestle with 9 vol. of cold 1 per cent citric acid. The calf thymus was homogenized in a Waring blendor with 19 vol. of cold 1 per cent citric acid. The citric acid lyses the mitochondria and inhibits the action of desoxyribonuclease. The nuclei were washed twice in 0·1 per cent citric acid and once in 0·25 M sucrose. Smears stained with methylene blue showed no unbroken cells or mitochondria.

Rat liver mitochondria and soluble cellular protein (supernatant) were prepared by a modification of the method of Schneider and Hogeboom (1950). The liver was homogenized with 9 vol. of cold 0·25 M sucrose with a Teflon pestle for 2 min. Nuclei and unbroken cells were removed by preliminary centrifugation at 500 *g* for 15 min. and the mitochondria isolated by centrifugation at 8000 *g* for 10 min. The mitochondria were washed in 0·25 M sucrose, kept overnight at −20° and rewashed twice in 0·25 M sucrose to remove soluble protein.

The microsomes were isolated at 40,000 *g* for 30 min. The residual fluid was then subjected to 2 centrifugations at 80,000 *g* for 40 min. to remove any remaining particulate matter. Care was taken to avoid contaminating the supernatant with the fatty layer that rose to the surface during high speed centrifugation. The final product remained clear on shaking and contained only soluble cellular protein.

Calf thymus DNA was prepared by the method of Signer and Schwander (1949).

Lipoid extract of whole rat liver was prepared by the methanol chloroform method of Rapport, Graf and Alonzo (1955).

* In receipt of a British Memorial Foundation Fellowship.

The cellular antigens were stored as a 20 per cent suspension in 0·25 M sucrose at −20°. The nuclear, mitochondrial and soluble cellular protein preparations were diluted with an equal volume of calcium magnesium saline (Gajdusek, 1958) before use. When the soluble cellular protein was highly anticomplementary it was used in a 5 per cent concentration. DNA was dissolved in physiological saline and used at a concentration of 100 μg./ml. The lipoid extract was used as a 10 per cent suspension.

Sera.—Sera from patients with SLE, lupoid hepatitis, primary biliary cirrhosis and macroglobulinaemia were obtained from the patients described by Mackay and Gajdusek (1958) and Mackay and Larkin (1958). Their system of code numbers has been followed in this paper; whenever possible the original sera employed by them were used. Two patients (R1 and R2) who presented with rheumatoid arthritis and a transiently positive LE cell test and 5 patients with syphilis and a positive Wassermann reaction were also studied. All the patients with SLE had given a positive LE cell test by the 2 hr. clot method of Magath and Winkle (1952) at some stage of their illness. Most of the patients were under treatment with cortisone and many were in remission at the time of bleeding.

Complement fixation test.—The sensitive method of Donnelley (1951) as modified by Gajdusek (1958) was employed. In order to overcome the anticomplementary activity of the antigens, complement was titrated in the presence of antigen and two 50 per cent haemolytic units of complement were used in the final titration. Antibody and antigen controls were included in each test.

Assuming a stoichiometric behaviour of the anticomplementary activity of the serum, even when the serum was not anticomplementary, an antibody titre of 1 in 4 might have been due to the additive effects of the anticomplementary activity of the serum and antigen; a titre of 1 in 8 was the lowest titre which might be valid. When the serum was anticomplementary, the lowest titre which could be attributed to a specific complement fixation reaction was two dilutions higher than the anticomplementary titre of the serum.

RESULTS

Complement fixing titres of abnormal sera were expressed as the reciprocal of the highest serum dilution giving 50 per cent haemolysis. The results of tests against human liver homogenate, rat liver nuclei, calf thymus nuclei, calf thymus DNA, rat liver mitochondria and rat liver soluble cellular protein antigens are presented in Tables II and III. The titres against the microsomal preparations were variable and are not recorded.

Purity of antigens.—On both immunological and preparative grounds the nuclear, mitochondrial and soluble cellular protein antigens were regarded as distinct. The separate nature of the mitochondrial and soluble cellular protein antigens was established by serial antigen dilution studies (Table I). When using sera which failed to react to a given antigen, serial antigen dilution titration set an upper limit to the contamination of the non-reactive antigen by the reactive antigens.

Systemic Lupus Erythematosus

Of the sera of 25 patients with SLE 4 reacted with rat liver nuclei, calf thymus nuclei and calf thymus DNA, rat liver mitochondria and rat liver soluble cellular protein, 5 reacted with rat liver mitochondria and rat liver soluble cellular protein, 6 reacted with rat liver mitochondria, while 10 failed to react with any of the antigens. It should be noted that reactivity against nuclear preparations was always accompanied by reactivity against DNA. In general, the reactivity against human liver homogenate paralleled that against rat liver cytoplasmic proteins.

TABLE I.—*Antigen Titrations. Minimum Strength of Antigen Preparations Reacting with Sera*

Code number of patient	Serum dilution	Rat liver nuclei (per cent)	Rat liver mitochondria (per cent)	Rat liver supernatant (per cent)
L1	1/10	N.R.	<0·078	2·5
L2	1/15	„	0·078	0·625
L3	1/50	0·312	0·312	—
L17	1/10	N.R.	5	0·625
	1/15	„	N.R.	0·313
L22	1/20	—	0·625	2·5
L30	1/20	—	<0·078	N.R.
L36	1/10	—	<0·078	0·625
R1	1/20	—	0·312	N.R.
HL 1*a*	1/10	—	0·625	1·25
HL 1*b*	1/15	—	N.R.	1·25
HB 1	1/15	—	0·625	0·625

N.R. indicates that there was no reaction with the antigen at full strength (10 per cent).
— indicates that no estimation was available.

TABLE II.—*Titres of Sera of Patients with Systemic Lupus Erythematosus against Human and Animal Antigens*

Code number of patient	Concurrent response to LE cell test	Calf thymus nuclei	Rat liver nuclei	Calf thymus D.N.A.	Rat liver mitochondria	Rat liver supernatant	Human liver
Nuclear and Cytoplasmic Reactors							
L3 (Jan. 1958)	Positive	16	8	16	8	—	—
L3 (May 1958)	„	64	64	16	64	16	—
L3 (June 1958)	„	0	0	0	0	0	4
L27	„	4	8	16	8	8	8
L28	„	0	16	16	8	4	16
L37	„	32	64	128	64	32	64
Mitochondrial and Soluble Cellular Protein Reactors							
L2	Negative	0	0	0	32	64	64
L17	„	0	0	0	4	32	—
L19	—	0	0	0	128	64	256
L22	Negative	0	0	0	32	8	32
L36	Positive	0	0	0	>128	>128	>128
Mitochondrial Reactors							
L1	Negative	0	0	0	256	0	32
L4	Positive	0	0	0	8	0	8
L6	„	0	0	0	8	0	0
L25	„	0	0	0	64	0	8
L30	„	0	0	0	128	0	128
R1	Negative	0	0	0	128	0	0
Non-Reactors							
L5	Negative	0	0	0	0	0	0
L7	„	0	0	0	0	0	0
L8	Positive	0	0	0	0	0	0
L11	Negative	0	0	0	0	0	0
L15	Positive	0	0	0	0	0	0
L16	Negative	0	0	0	0	0	0
L18	„	0	0	0	0	0	0
L21	„	0	0	0	0	0	0
L31	Positive	0	0	0	0	0	0
R2	„	0	0	0	0	0	0

— indicates that no estimation is available.

TABLE III.—*Titres of Sera of Patients with Lupoid Hepatitis, Primary Biliary Cirrhosis, Macroglobulinaemia and Syphilis against Human and Anima Antigens*

Code number of patient	Calf thymus nuclei	Rat liver nuclei	Rat liver mito-chondria	Rat liver super-natant	Human liver
		Lupoid Hepatitis			
HL1*a*	0	0	256	2,048	256
HL1*b*	—	—	16	1,024	—
HL3	0	0	0	0	—
HL5	0	0	0	0	—
HL6	0	0	0	16	0
		Primary Biliary Cirrhosis			
HB1	0	0	1,024	1,024	128
		Macroglobulinaemia			
MG3	0	0	>16,000	0	4,000
		Syphilis			
S1	—	—	8	0	—
S2	—	—	64	64	—
S3	—	—	>128	>128	—
S4	—	—	64	0	—
S5	—	—	8	0	—

— indicates that no estimation is available.

Liver Disease

Of the 4 patients with lupoid hepatitis, one reacted with both soluble cellular protein and mitochondria, one reacted with soluble cellular protein only, while 2 failed to react with any of the antigens. However, none of these 4 patients had a concurrently positive LE cell test. The one patient with primary biliary cirrhosis (see Mackay, 1958) reacted with both mitochondria and soluble cellular protein in very high titres.

Macroglobulinaemia

One case of macroglobulinaemia reacted in extremely high titre to mitochondria but failed to react with the nuclei or soluble cellular protein.

Syphilis

All the 5 sera from syphilitic patients with a positive Wassermann reaction reacted with mitochondria and with lipoid derived from mitochondria. This is in keeping with the findings of Furth and Kabat (1941). Two sera also reacted with soluble cellular protein. Eleven of the high titre sera were tested against lipoid extract of rat liver and Eagle's heart antigen (Eagle, 1937). Only one of the patients with SLE (L30) reacted with these antigens.

Clinical Correlations in Patients with SLE

The 4 patients with positive reactions to nuclei and DNA also had a concurrently positive LE cell test. The converse was not true, for, of 9 other patients

with a concurrently positive LE cell test, 5 reacted only to human liver and rat cytoplasmic antigens, while 4 failed to react to any of the antigens.

The severity of the initial illness and the subsequent clinical course were graded by another observer (Mackay and Larkin, 1958). No definite correlation could be made between the presence and degree of renal, hepatic or haematological involvement and the clinical severity of the disease in individual patients on the one hand, and the corresponding titres and cell fraction specificities of the serum antibodies on the other.

DISCUSSION

These results demonstrate the occurrence in SLE of circulating complement fixing antibodies against both nuclear and cytoplasmic antigens. Circulating complement fixing antibodies against cytoplasmic antigens were also found in lupoid hepatitis, macroglobulinaemia, primary biliary cirrhosis and syphilis. The occurrence of the LE phenomenon in lupoid hepatitis suggests that antinuclear antibodies might have been found had more patients been studied.

All patients in the present series with positive nuclear and DNA reactions also showed a concurrently positive LE cell test. However, of 9 other patients with a concurrently positive LE cell test 5 reacted with cytoplasmic antigens only and 4 failed to react with any antigen. Thus the presence of the LE factor in the serum did not mean that circulating complement fixing antinuclear antibody would be detected.

The finding of complement fixing antibody against nuclei and DNA is in agreement with the work of Robbins, Holman, Deicher and Kunkel (1957). Friou (1958) and Friou, Finch and Detre (1958) used a fluorescent antibody technique capable of detecting complete and incomplete antibody, and demonstrated circulating antibody against nuclei in the sera of 27 out of 28 patients with active and quiescent SLE. Complement fixing antinuclear antibodies were detected in 4 out of 23 patients in the present series. As a sensitive method of complement fixation was used it is probable that the failure to demonstrate antibody against nuclei in many of the patients was due to an inability of the antinuclear antibody to bind complement.

The poor correlation between the serological and clinical findings in this series of patients with SLE may have been due either to the unimportance of circulating complement fixing antibodies or to certain special factors. *In vivo* absorption of antibody was a factor which could not be assessed. Only one specimen of serum was available from most patients and in many cases the natural history of the disease had been altered by treatment with cortisone. Nevertheless the poor correlation favours the view that the various complement fixing antibodies, particularly those against cytoplasmic components, are not themselves pathogenic in SLE but represent only one facet of altered immunological function.

Circulating complement fixing antibodies also seem unimportant in experimental auto-immune disease and in hypersensitivity of the delayed type. In experimental allergic encephalomyelitis there is no correlation between tissue damage and the titre of complement fixing antibodies (Lumsden, Kabat, Wolf and Bezer, 1950); Freund, Thompson and Lipton (1955) found no constant association between these factors in experimental allergic testicular damage. Similar observations have been made in experimental thyroiditis (Witebsky,

Rose, Terplan, Paine and Egan, 1957). In both experimental encephalomyelitis (Lipton and Freund, 1953) and in tuberculosis, skin hypersensitivity could be transferred passively by cells but not by serum.

When immunological damage cannot be attributed to a classical circulating antibody, then presumably either an incomplete circulating antibody is present, as seems likely in the case of the antinuclear and anti-DNA antibodies in SLE, or tissue damage is produced by immunologically competent cells without the mediation of soluble antibody. These possibilities are not mutually exclusive.

The lymphocyte and plasma cell infiltration seen in various organs including the liver (Mackay, Taft and Cowling, 1958) in SLE and related diseases may be due to a non-specific attraction of these cells to the sites of tissue damage and to the sites of antibody-antigen reactions. Alternatively a specific accumulation of immunologically competent cells may occur when antigens are present with which they can react. This hypothesis would explain the aggregation of mesenchymal cells and the occurrence of cellular damage in the absence of circulating antibody.

The auto-immune nature of the haematological changes in SLE rests on firm evidence (Dameshek, 1958). The present finding of complement fixing antibodies against both nuclear and cytoplasmic components of tissue cells in a proportion of cases of SLE supports the concept that a deep seated anomaly of immunological response is related to the pathogenesis of the disease. It also indicates that the presence of circulating antibody demonstrable by complement fixation is inconstant and does not seem to be an essential part of the disease picture.

SUMMARY

Previous observations of Robbins, Holman, Deicher and Kunkel (1957), on the occurrence of complement fixing antibodies against nuclei and desoxyribose nucleic acid in the sera of patients with systemic lupus erythematosus have been confirmed.

The sera of certain patients with systemic lupus erythematosus also reacted in the complement fixation test with soluble and insoluble cytoplasmic protein of rat liver.

Some patients with lupoid hepatitis, primary biliary cirrhosis, macroglobulinaema and syphilis reacted with cytoplasmic antigens, but none reacted with nuclei.

These findings support the auto-immune concept of the pathogenesis of SLE.

I am deeply grateful to Sir Macfarlane Burnet, Dr. Ian Wood and Dr. Ian Mackay for their encouragement and advice. I should also like to thank Miss Lois Larkin for detailed technical advice, and Mr. G. L. Ada for the preparation of calf thymus DNA.

REFERENCES

DAMESHEK, W.—(1958) *Ann. intern. Med.*, **48**, 707.

DONNELLEY, M.—(1951) *Aust. J. exp. Biol. med. Sci.*, **29**, 137.

EAGLE, H.—(1937) ' The Laboratory Diagnosis of Syphilis '. St. Louis (C. V. Mosby), p. 144.

FREUND, J., THOMPSON, G. E. AND LIPTON, M. M.—(1955) *J. exp. Med.*, **101**, 591.
FRIOU, G. J.—(1958) *J. Immunol.*, **80**, 476.
Idem, FINCH, S. C. AND DETRE, K. D.—(1958) *Ibid.*, **80**, 324.
FURTH, J. AND KABAT, E. A.—(1941) *Science*, **94**, 46.
GAJDUSEK, D. C.—(1957) *Nature*, **179**, 666.—(1958) *Arch. intern. Med.*, **101**, 9.
LIPTON, M. M. AND FREUND, J.—(1953) *J. Immunol.*, **71**, 380.
LUMSDEN, C. E., KABAT, E. A., WOLF, A. AND BEZER, A. E.—(1950) *J. exp. Med.*, **92**, 253.
MACKAY, I. R.—(1958) *New Engl. J. Med.*, **258**, 185.
Idem AND GAJDUSEK, D. C.—(1958) *Arch. intern. Med.*, **101**, 30.
Idem AND LARKIN, L.—(1958) *Australasian Ann. Med.*, **7**, 251.
Idem, TAFT, L. I. AND COWLING, D. C.—(1959) *Lancet*, i, 65.
MAGATH, T. B. AND WINKLE, V.—(1952) *Amer. J. clin. Path.*, **22**, 585.
MIRSKY, A. E. AND POLLISTER, A. W.—(1946) *J. gen. Physiol.*, **30**, 117.
RAPPORT, M. M., GRAF, L. AND ALONZO, N.—(1955) *Cancer*, **8**, 546.
ROBBINS, W. C., HOLMAN, H. R., DEICHER, H. AND KUNKEL, H. G.—(1957) *Proc. Soc. exp. Biol. N.Y.*, **96**, 575.
SCHNEIDER, W. C. AND HOGEBOOM, G. H.—(1950) *J. biol. Chem.*, **183**, 123.
SIGNER, R. AND SCHWANDER, H.—(1949) *Helv. Chim. Acta*, **32**, 853.
WITEBSKY, E., ROSE, N. R., TERPLAN, K., PAINE, J. R. AND EGAN, R. W.—(1957) *J. Amer. med. Ass.*, **164**, 1439.