Clin. exp. Immunol. (1976) 25, 377–382.

Immunologic regulation of spontaneous antibodies to DNA and RNA

I. SIGNIFICANCE OF IgM AND IgG ANTIBODIES IN SLE PATIENTS AND ASYMPTOMATIC RELATIVES

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(*Received* 5 *February* 1976)

SUMMARY

Nine individuals from four families of patients with systemic lupus erythematosus (SLE) were studied by sucrose density gradient fractionation and filter radioimmunoassay for the presence of 19S IgM and 7S IgG antibodies to DNA, poly rA, and poly rA·poly rU. One individual in each family was totally asymptomatic, and at least one had active systemic lupus erythematosus (SLE).

The results indicate: (1) a correlation between 7S antibody to DNA and RNA and active SLE, and (2) the presence of 19S antibody to RNA in the asymptomatic relatives. These findings suggest that SLE may be a disorder of immunological regulation. The distribution of antibodies between IgM and IgG is closely related to disease severity. The asymptomatic relatives may have a partial regulatory abnormality resulting in the limited production of IgM antibodies to RNA. SLE patients may have a more complete failure of regulation permitting the additional synthesis of IgG antibodies to DNA and RNA.

INTRODUCTION

Antibodies to DNA and RNA are a characteristic feature of patients with systemic lupus erythematosus (SLE). They also occur in NZB/NZW F_1 mice, a strain genetically predisposed to a lupus-like syndrome (Talal, 1974; Burnet & Holmes, 1965; Koffler, Angello & Kunkel, 1974; Stollar *et al.*, 1962; Schur & Monroe, 1969). Interrelated genetic, immunological and viral factors play major roles in the pathogenesis of these disorders (Talal, 1970).

We have found an ordered sequential development of antibodies to DNA and RNA in NZB/NZW F_1 mice suggesting the presence of a preserved but defective regulatory mechanism (Papoian, Pillarisetty & Talal, submitted for publication; Talal, 1976). Both antibodies appeared initially in the 19S IgM fraction and then switched over to 7S IgG. The switch to 7S occurred earlier for DNA than for RNA, and earlier in female mice who develop a more severe, accelerated disease than do their male littermates.

In SLE, antibodies to DNA and RNA may belong to either the 19S or 7S immunoglobulin class (Arana & Seligmann, 1967; Rothfield & Stollar, 1967; Talal & Pillarisetty, 1975). Active lupus nephritis is associated in particular with serum 7S antibodies to DNA which deposit as immune complexes in the renal glomeruli (Koffler, Schur & Kunkel, 1967). At the other end of the spectrum, asymptomatic relatives of SLE patients have a significant increase of antibodies to RNA but not to DNA (DeHoratius *et al.*, 1975).

We now report studies on four families in which anti-nucleic antibodies were present both in the SLE

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probands and in asymptomatic family members. The results support the hypothesis that the autoimmune response to nucleic acids is regulated, and that 19S anti-RNA antibodies are associated with an asymptomatic 'carrier' state.

MATERIALS AND METHODS

Materials. Sera from nine individuals in four different families were stored frozen at −20°C until studied. Three Caucasian families lived in New Mexico and one Oriental family lived in San Francisco. At least one individual in each family fulfilled criteria for the diagnosis of SLE (Cohen, Reynolds & Franklin, 1971).

[^{3}H]poly rA (lot no. 57-7-303, 1 μCi/5·5 μg) and [^{3}H]poly rA·poly rU (lot no. 57-4-330, 1 μCi/11·6 μg) were purchased from Miles Laboratories, Elkhart, Indiana. [^{3}H]DNA (0·23 μCi/μg) from KB cells was purchased from Electro-Nucleonics Incorporated, Bethesda, Maryland. These nucleic acids are known to measure different antibody activities (Talal & Pillarisetty, 1975).

Sucrose density gradient ultracentrifugation. 0·3 ml of serum was layered on top of a 13·5 ml sucrose gradient ranging from 10–35% (w/v) sucrose in borate-buffered saline, 0·15 M, pH 8·0. The tubes were centrifuged in a Beckman Model L2-65 centrifuge at 4°C for 20 hr at 39,000 rev/min. Approximately forty fractions were collected by piercing the bottom of the tube. Fifty microlitres of each fraction were analysed for nucleic acid binding by radioimmunoassay. The distribution of immunoglobulins in the 19S and 7S regions of the density gradient was established on representative sera by either the Ouchterlony technique (adopted to microscale) or by radial immunodiffusion. This technique has been described in detail (Talal & Pillarisetty, 1975). Monospecific anti-human immunoglobulin sera of goat origin (obtained from Burroughs-Wellcome Laboratories, Research Triangle Park, North Carolina) or 'immunoplates' (obtained from Hyland Laboratories, Costa Mesa, California) were used for determining immunoglobulin concentrations by quantitative radial immunodiffusion.

Cellulose ester filter radioimmunoassay. The radioimmunoassay for detection of antibodies to poly rA, poly rA·poly rU, or DNA was performed as follows: 10 μl of whole serum or 50 μl of sucrose gradient fractions were made up to 90 μl with borate-buffered saline (0·15 M, pH 8·0). After heating at 56°C for 30 min, 10 μl of radioactive nucleic acid (750 ct/min/7·74 ng [^{3}H]poly rA; 750 ct/min/95·5 ng [^{3}H]DNA; 700 ct/min/91·64 ng [^{3}H]poly rA·poly rU) was added. The reaction mixtures were incubated at 37°C for 30 min and then in the cold at 2°C for 18–20 hr. The contents of the tubes were then passed through cellulose ester filters (Millipore HAWP, 0·45 μm/25 mm). The filters were processed and radioactivity determined in a liquid scintillation counter as previously described (Talal & Pillarisetty, 1975).

Calculation of 7S : 19S ratios for nucleic acid binding. The radioactive binding profiles showed clear distribution into 7S and 19S peaks of activity after sucrose density gradient fractionation. The graphed 7S and 19S peaks were cut out and weighed. The weight in grams of the area under each peak was used to determine the 7S:19S binding ratios.

TABLE 1. Antibodies to DNA, poly rA, poly rA·poly rU in SLE probands and family members

	Nucleic acid (μg) retained on filter/ml serum			Ratio of 7S:19S binding		
	DNA	poly rA	poly rA·poly rU	DNA	poly rA	poly rA·poly rU
Family One						
D.F. Proband	9·42	0·53	3·74	2·96	2·4	0·36
L.P. Sister	2·98	0·42	4·08	0·44	0·8	0·14
M.P. Mother	n.s.	0·09	3·65	n.s.	0*	0·11
Family Two						
V.K. Proband	3·71	0·67	4·75	ω†	ω	12·8
M.K. Daughter	n.s.	0·11	2·11	n.s.	0	0
Family Three						
F.W. Proband	2·48	0·32	1·28	0·54	0	0
S.W. Daughter	n.s.	n.s.	2·88	n.s.	n.s.	0
Family Four						
Do. C. Proband	2·76	0·40	2·49	0·29	0·8	0
De. C. Sister	1·95	0·16	n.s.	0·35	0	n.s.
Normal values (mean ± S.D.)	0·52 ± 0·24	0·02 ± 0·02	0·69 ± 0·49			

n.s. = Not significant.

* No 7S peak present in the sucrose gradient.

† Presence of a 7S peak without detectable 19S binding.

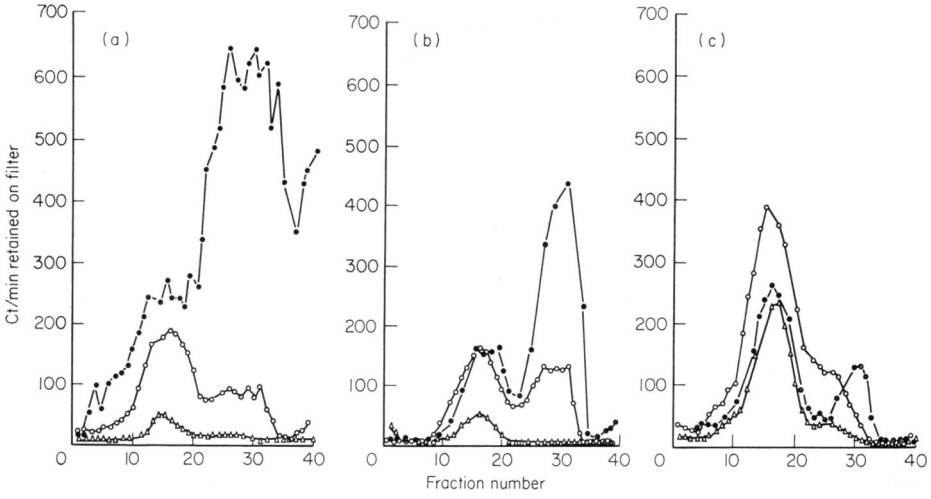

FIG. 1. Separation of serum from members of Family One by sucrose density gradient ultracentrifugation. Individual fractions were assyed for antibody activity to DNA (a), poly rA (b), and poly rA·poly rU (c). Heavier fractions are to the left. (●) D.F. (proband); active SLE; (○) L.P. (sister) mild SLE; (△) M.P. (mother), no symptoms.

RESULTS

Family One

The proband (D.F.) is a 22-year-old female with active SLE characterized by glomerulonephritis, severe myositis, rash, anaemia, leucopenia and marked hypocomplementaemia. Her 21-year-old sister (L.P.) has milder SLE manifest as Raynaud's phenomenon, alopecia, photosensitivity, non-deforming arthritis, thrombocytopaenia and mild hypocomplementaemia. Both have positive antinuclear factor and LE cells. The proband requires prednisone and azathioprine for control, whereas the sister is controlled on prednisone only. The mother (M.P.) is asymptomatic.

The binding activity of whole serum for DNA, poly rA, and poly rA·poly rU is shown in Table 1. The two sisters with SLE have significant concentrations of all three antibodies, whereas the asymptomatic mother shows significant binding of poly rA and poly rA·poly rU only.

Sera were fractionated by sucrose density gradient ultracentrifugation and assayed for antibodies to DNA, poly rA and poly rA·poly rU (Fig. 1). The 7S:19S ratios were calculated for each nucleic acid antigen. D.F. (with active SLE) had a large 7S peak of anti-DNA and a smaller 19S shoulder. L.P. (with milder SLE) had a large 19S peak of anti-DNA with a smaller 7S region of activity. M.P. (asymptomatic) showed only a small peak of DNA binding in the 19S region.

A similar pattern was observed for antibodies to poly rA. D.F. and L.P. both had 7S and 19S peaks of activity, with the former being more prominent in the active patient and the latter more prominent in her less ill sister (Fig. 1b). The asymptomatic mother again had some 19S binding activity.

All three individuals had significant binding of poly rA·poly rU in the 19S region (Fig. 1c). The greatest activity was present in the patient with mild SLE (L.P.). D.F., with active SLE, had in addition a small 7S peak of activity. Thus, for all three nucleic acids, active disease was associated with a higher 7S:19S ratio (Table 1).

Family Two

The proband (V.K.) is a 38-year-old female with active SLE characterized by arthritis, rash, psychosis, leucopenia, anaemia and hypocomplementaemia. She requires treatment with prednisone and azathioprine. Her daughter (M.K.) is asymptomatic.

The binding activity of whole serum and 7S:19S ratios are presented in Table 1. V.K. (active SLE) had all three antibodies, whereas M.K. (asymptomatic) bound only poly rA and poly rA·poly rU. In

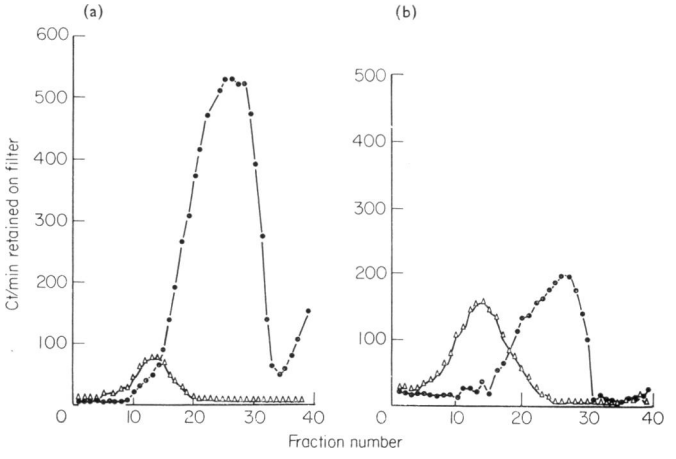

FIG. 2. Separation of serum from members of Family Two by sucrose density gradient ultracentrifugation. Individual fractions were assayed for antibody activity to poly rA (a), and poly rA·poly rU (b). (●) V.K. (proband), active SLE; (△) M.K. (mother), no symptoms.

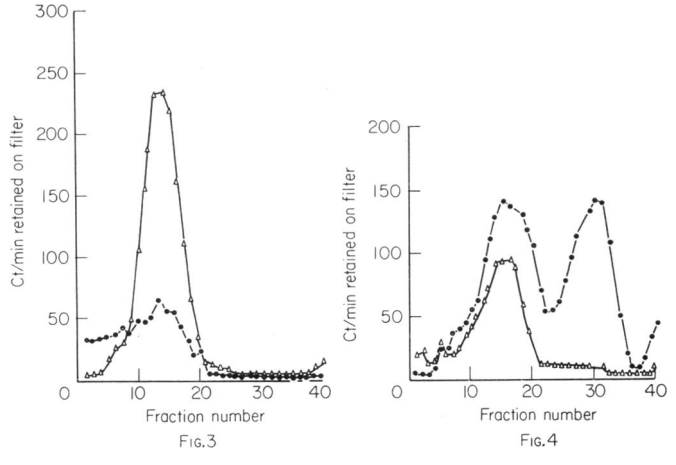

FIG.3 FIG.4

FIG. 3. Separation of serum from members of Family Three by sucrose density gradient ultracentrifugation. Individual fractions were assayed for antibody activity to poly rA·poly rU. (●) F.W. (proband), active SLE; (△) S.W. (daughter), no symptoms.

FIG. 4. Separation of serum from members of Family Four by sucrose density gradient ultracentrifugation. Individual fractions were assayed for antibody activity to poly rA. (●) Do. C. (proband), active SLE; (△) De. C. (sister), no symptoms.

V.K., large peaks of activity for DNA and poly rA, and a smaller peak of poly rA·poly rU, were maximal in the 7S region (Fig. 2). M.K., like the asymptomatic relative in Family One, had prominent 19S binding of poly rA·poly rU and minimal activity for DNA and poly rA.

Family Three

The proband (F.W.) is a 45-year-old Chinese female with SLE characterized by glomerulonephritis, pulmonary hypertension, sicca symptoms and hypocomplementaemia. Her daughter (S.W.) is clinically normal.

The binding activity of whole serum and 7S:19S ratios are shown in Table 1. The patient (F.W.) had antibodies to DNA and poly rA; the daughter bound only poly rA·poly rU. F.W. (active SLE) had large 19S and 7S peaks of anti-DNA, whereas no significant DNA binding was seen with S.W. S.W. had

a large 19S peak of antibodies to poly rA·poly rU. Much less binding of poly rA·poly rU was seen in the patient (Fig. 3).

Family Four

The proband (Do.C.) is a 17-year-old female with active SLE characterized by membranous glomerulonephritis, arthritis, facial rash, leucopenia, anaemia and arteritis demonstrated on muscle biopsy. She is managed on prednisone only. Her sister (De.C.) is asymptomatic.

The binding activity of whole serum and 7S:19S ratios are shown in Table 1. The patient (Do.C.) had all three antibodies; her sister (De.C.) had antibodies to DNA and poly rA. Antibodies to DNA were predominantly 19S, and antibodies to poly rA·poly rU were exclusively 19S, in both individuals. The only significant difference was seen with poly rA (Fig. 4). The patient (Do.C.) had prominent 19S and 7S peaks of poly rA binding, whereas De.C. had a smaller 19S peak and no 7S activity. Even though De.C. remains asymptomatic, the pattern of nucleic acid binding is suggestive of SLE.

DISCUSSION

There are two major findings in this report: (1) the correlation of 7S antibody to nucleic acids, both DNA and RNA, with active SLE; and (2) the presence of 19S antibody to RNA in asymptomatic relatives of SLE patients.

Family One contains two sisters who differ in the severity of their SLE. The one with more active disease had more 7S than 19S antibody. This correlation between disease activity and 7S antibody to DNA and poly rA was supported by results in Family Two where the patient (V.K.) had large 7S peaks for DNA and poly rA. In Family Three, the patient (F.W.) had both 19S and 7S antibodies to DNA. In Family Four, the patient (Do.C.) was distinguished from her asymptomatic sister by having 7S antibodies to poly rA.

IgM antibodies appear before IgG both in phylogeny and in ontogeny. The switch from IgM to IgG production often involves the action of T cells (Grumet, 1972; Mitchell, 1974). There is a premature loss of T-suppressor cell function in NZB/NZW F_1 mice (Talal, 1974; Chused, Steinberg & Parker, 1973; Barthold, Kysela & Steinberg, 1974). We have found an abnormality of T-cell regulation to be associated with the ordered, sequential development of IgM, and later IgG, antibodies to DNA and RNA in B/W mice (Talal, 1976).

The asymptomatic relatives in the first two families present an identical picture characterized by significant 19S binding of poly rA·poly rU. The asymptomatic relative in Family Three also had a large 19S peak for poly rA·poly rU as well as for poly rA. In an earlier study, we reported the presence of antibodies to poly rA and poly rA·poly rU in significant numbers of asymptomatic relatives of SLE patients correlating with lymphocytotoxic antibody (DeHoratius *et al.*, 1975). The present study demonstrates that these antibodies are exclusively 19S, indicative of a limited immune response. Such a limited 19S IgM immune response occurs normally in mice immunized with 'thymic-independent antigens' (Baker *et al.*, 1974; Anderson & Blomgren, 1971; Gershon, 1974). The antibody response to classical T-independent antigens is regulated by T-suppressor cells, as has been demonstrated for pneumococcal polysaccharide (Baker *et al.*, 1974) and polyvinyl pyrrolidone (Anderson & Blomgren, 1971).

Small numbers of B cells with receptors for DNA (Bankhurst & Williams, 1975) and thyroglobulin (Bankhurst, Torrigiani & Allison, 1973) have been reported in normal individuals. Proliferation of these cells leading to clonal expansion and large antibody titres may normally be prevented by T-suppressor cells. A small number of antibodies to DNA and RNA may be present in normal individuals and represent the limited expression of these small clones of B cells.

SLE may be a disorder of immunological regulation in which the mechanism for switching from IgM to IgG bears an intimate relationship to disease severity. Certain asymptomatic relatives of SLE patients appear to show a limited abnormality of T-cell regulation permitting the synthesis of IgM antibodies to RNA. These individuals may represent a 'carrier state' for the lupus diathesis. SLE patients may have a more complete abnormality of T-cell regulation.

Although the few families studied show a heterogeneity from one individual to another, the results taken together support a general conclusion that 7S antibodies to DNA and poly rA are associated with a more active disease picture. This is reflected in a higher 7S:19S ratio in patients with active SLE. The heterogeneity could reflect individual selection factors related either to the immunogenic stimulus or to the regulatory failure. Genetic and virologic factors might also contribute to this heterogeneity.

Supported by research grants from the Veterans Administration and USPHS grants AM16140 and AM 13789.

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