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B cell activity in systemic lupus erythematosus: depressed *in vivo* humoral immune response to a primary antigen (haemocyanin) and increased *in vitro* spontaneous immunoglobulin synthesis

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SUMMARY

B lymphocyte studies, *in vivo* and *in vitro*, were performed in 28 patients with systemic lupus erythematosus (SLE). After immunization with the primary test antigen haemocyanin, a decreased antigen specific humoral immune response was observed for all three Ig classes, irrespective of disease activity or the use of corticosteroids. Levels of antibodies against (recall) viral and nuclear antigens were increased during active disease. The *in vitro* spontaneous production of IgM and IgG, determined in the supernatant of 8 days cultures by ELISA, was highly increased in patients with active disease (reversely correlated with levels of complement C3, $r = 0{\cdot}74$). Pokeweed mitogen-induced synthesis was decreased in all patients. The distribution of T cell subsets (OKT3, Leu 3a and OKT8 positive cells) was not different from controls, irrespective of disease activity. It is concluded that the primary humoral immune response is decreased in SLE, whereas a polyclonally activated B cell appears to be present. The normal T cell subset distribution does not support the primary role of the cell in the state of hyperactivity of the B cell.

Keywords B lymphocyte function primary immune response Ig production T cell subsets systemic lupus erythematosus

INTRODUCTION

In systemic lupus erythematosus (SLE) hyperactivity of the B lymphocyte system appears to be present: hypergammaglobulinaemia (Cass *et al.*, 1968), autoantibody production (Kunkel & Tan, 1964) and increased spontaneous activity of immunoglobulin secreting cells (Nies & Louis, 1978; Ginsburg, Finkelman & Ripsky, 1979; Blaese, Grayson & Steinberg, 1980; Wangel, Milton & Egan, 1982) have been demonstrated in patients with active SLE. Despite B cell hyperactivity, infection—often with common infectious agents (Carpenter & Sturgill, 1966)—is the major cause of morbidity and mortality in SLE (Ropes, 1964). Although infections are related to immunosuppressive drugs, untreated patients are also at risk (Staples *et al.*, 1974). To assess the primary humoral immune response in this regard, we immunized patients with SLE in various states of disease

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activity with the primary test immunogen α-*Helix pomatia* haemocyanin. We studied the kinetics of the *in vivo* class specific antibody response against this antigen. We related those findings to the state of *in vivo* activity of the B cell system, as reflected in the levels of immunoglobulins, antibodies against common viral antigens, and autoantibodies. At the same time, we assessed the *in vitro* activity of B cells from peripheral blood by determining their capacity to synthesize and secrete IgG and IgM *in vitro*, both spontaneously and after non-specific stimulation. In addition, we evaluated the numbers and the ratio of immunoregulatory T cell subsets ('helper' and 'suppressor' cells) as characterized by monoclonal antibodies (Kung *et al.*, 1979; Reinherz & Schlossman, 1980). We related the phenotype of the T cells to the state of activity of the B cells.

MATERIALS AND METHODS

Patients and controls. Twenty-eight patients (22 females and six males) were studied. The diagnosis of SLE was made according to the ARA criteria (Cohen *et al.*, 1971). Their clinical characteristics are given in Table 1. Mean age of the patients was 37·0 years (range 18–63). Different control groups consisting of healthy laboratory and clinical personnel and healthy blood donors, were used for the following tests: antibodies against α-*Helix pomatia* haemocyanin (HPH) (nine females and 14 males; mean age 48·8 years, range 26–69), antibodies against viral antigens (seven females and 12 males; mean age 43·0 years, range 26–72), and *in vitro* cellular studies (10 females and 10 males; mean age 29·3 years, range 22–50).

Patients and controls were immunized with 1 mg HPH subcutaneously in the deltoid region (De Gast, The & Snijder, 1973). Sera for antibody determination were collected before, and at 2 (only for controls), 3 and 6 weeks after immunization. Before immunization sera were drawn for determination of complement levels C3 and C4, levels of class specific immunoglobulins, and antibodies against viral antigens. At the second visit (3 weeks after immunization blood was drawn for cellular studies. In all patients written informed consent was obtained.

Serological studies. Anti-nuclear antibodies (ANA) and anti-double stranded DNA antibodies (a-dsDNA) were determined as described previously (Kallenberg *et al.*, 1982). Immunoglobulin levels were determined according to standard techniques. Complement levels C3 and C4 were assessed by radial immunodiffusion (Behring Werke). Antibodies against viral antigens (measles, rubella, cytomegalovirus, herpes simplex virus, varicella zoster virus) were determined by standard complement fixation. Sera with anti-complementary activity were excluded. Titres were expressed as titrestep of two-fold serum dilutions, 1:8 taken as 1. The titresteps of the five anti-viral antibodies were counted up, resulting in an 'anti-viral antibody score'.Class specific antibodies to HPH were determined using an indirect ELISA technique as described by Weits *et al.* (1978) with some modifications: briefly, flat bottom microtitre plates (Dynatech M 129 A) were coated with HPH in 0·1 M sodium carbonate pH 9·6 (5 μg HPH/ml) for at least 2 days at 4°C. Prior to the assay the plates were washed thoroughly with 10 mM Tris/HCl pH 8·0 containing 0·15 M NaCl and 0·05% Tween-20. Subsequently, the plates were incubated for 45 min at 37°C with 100 μl serum dilutions (1:100–1:1,600) in 0·01 M Tris/HCl pH 8·0 containing 0·05% Tween-20, 0·3 M NaCl and 4% bovine serum albumin (BSA). After a second wash step, the plates were incubated for another 45 min with 1:1,000 diluted heavy chain specific antisera conjugated to horse radish peroxidase (anti-γ, -μ and -α, Pasteur) in 10 mM Tris HCl pH 8·0 containing 0·3 M NaCl, 0·05% Tween-20 and 2% BSA for another 45 min, and washed again. Bound enzyme activity was measured by incubation with 100 μl 50 mM phosphate buffer pH 5·6 containing 0·2 mg/ml *o*-phenylene-diamine (OPD) and 0·0045% H_2O_2 for 30 min at room temperature. The reaction was stopped by the addition of 100 μl 1 N H_2SO_4. The optical density at 492 nm was read in a Titertek multiskan and the values were stored in a desk top computer for subsequent calculations. Each plate contained blank incubations and a reference sample, assayed in duplicate at six concentrations. The reference sample consisted of a pool of sera drawn from 20 high responders at 3 weeks after the primary HPH immunization. Anti-HPH concentrations were computed from the linearized titration curve obtained after log–logit transformation of the concentrations in the reference serum and the corresponding optical densities. Antibody concentrations were expressed as a percentage of the reference sample.

Table 1. Clinical characteristics of 28 patients with SLE

Patient No.	Age (years), sex (f/m)	ARA criteria* (in the past or at present)	Activity of disease at the time of the study†	Therapy at the time of the study‡
1	18, f	1,5,7,8	none	none
2	23, m	1,3,4,7,8,10,11,14	none	predn (50/0 mg)
3	25, f	1,4,6,7,8,10,11	none	none
4	25, f	1,3,4,7,8,10,11	none	indomethacin (75 mg) chloroquine (250 mg)
5	30, f	3,4,5,7,8,10,11,14	none	none
6	32, f	1,7,8,9,10,11,12,13,14	none	none
7	32, f	1,2,3,4,5,6,7,8	none	predn ($7\frac{1}{2}$/0 mg)
8	37, f	1,3,5,6,7,8,14	none	none
9	44, f	1,4,7,8	none	none
10	45, f	4,5,6,7,8,14	none	chloroquine (250 mg)
11	46, f	1,3,5,7,8	none	none
12	58, m	1,2,3,5,10,11	none	none
13	63, m	7,8,10,11	none	none
14	26, f	3,4,5,7,8	minor	none
15	31, f	1,2,3,4,5,6,8,10, 11,13,14	minor	predn (20/0 mg)
16	32, f	1,3,4,5,7,8,14	minor	chloroquine (250 mg)
17	33, f	1,4,7,8,12	minor	predn ($7\frac{1}{2}$/0 mg) indomethacin (0/100 mg)
18	37, m	5,7,8,11,14	minor	ibuprofen (1100 mg)
19	45, f	1,2,3,5,6,7,8	minor	ibuprofen (600 mg)
20	48, f	3,5,7,8	minor	none
21	49, f	1,2,3,4,5,6,7,8	minor	none
22	56, m	3,4,5,7,8,12	minor	predn (20/5 mg)
23	23, f	3,7,8,12	major	predn (60 mg)
24	24, f	1,5,6,7,8,12,14	major	predn (40 mg) azapropazon (600 mg)
25	28, f	1,7,8,9,10,11,14	major	predn (5 mg)
26	32, f	3,4,5,6,7,8,10,11, 12,13,14	major	predn ($7\frac{1}{2}$/0 mg) cyclophosphamide (50/0 mg)
27	40, m	1,3,5,7,8,10,11,13,14	major	none
28	53, f	1,3,4,5,6,7,8,10,11,14	major	predn (10/0 mg)

* ARA criteria with corresponding number (in brackets the percentage of patients with positive findings): (1) facial erythema (68%); (2) discoid lupus (18%); (3) Raynaud's phenomenon (64%); (4) alopecia (54%); (5) photosensitivity (68%); (6) oral or nasopharyngeal ulceration (36%); (7) arthritis without deformity (93%); (8) LE cells or positive ANA test (titre $\geq 1:100$) (96%); (9) chronic false positive Wassermann (7%); (10) proteinuria $>3{\cdot}5$ g/day (43%); (11) cellular casts (46%); (12) pleuritis or pericarditis (21%); (13) psychosis or convulsions (14%); (14) leukopenia $<4{,}000/mm^3$, thrombocytopenia $<100{,}000/mm^3$ or haemolytic anaemia (46%).

† Criteria for assessing disease activity: (1) Major disease activity: one or more of the following: (a) severe proliferative glomerulonephritis; (b) severe haemolytic anaemia (Hb <5 g %) or thrombocytopenia ($<50{,}000/mm^3$); (c) severe serositis (pericarditis with tamponade); (d) uveitis or retinitis; (e) severe CNS involvement (convulsions, coma, transverse myelitis); (f) myocarditis with arrhythmia and/or cardiac failure; (g) severe myositis; (h) systemic vasculitis with impending ischaemic necrosis; (i) pulmonary involvement with haemorrhage; (j) fever eci ($>38°C$), serositis, haemolytic anaemia (Hb >5 gr %) and thrombocytopenia ($>50{,}000/mm^3$), not reacting on prednisone <30 mg. (2) Minor disease activity: disease manifestations attributable to SLE, but not fulfilling the criteria for major disease activity (with the exception of Raynaud's phenomenon and photosensitivity).

‡ Data are given concerning immunosuppressive agents, antimalarial agents and non-steroidal anti-inflammatory agents (daily dosage in brackets). Predn = prednis(ol)on.

Antibody concentrations had to be covered by the titration curve. Therefore, three serum dilutions were measured in duplicate.

Cellular studies. Peripheral blood mononuclear cells (PBMNC) were obtained from fresh heparinized blood by Ficoll-Isopaque density gradient centrifugation. Cells were washed three times in RPMI 1640 with Hepes buffer (GIBCO-Biocult) supplemented with penicillin 100 u/ml and streptomycin 100 μg/ml.

(a) Culture of cells for Ig production

PBMNC were suspended at a density of 1×10^6 cells/ml in medium RPMI 1640 supplemented with 10% fetal calf serum (FCS), penicillin and streptomycin. A 1 ml aliquot was placed into a 12×75 mm culture tube (Falcon UD 57) and incubated at 37°C in an atmosphere of 5% CO_2 and 95% air for 8 days. To assess the passive transfer of Ig to the culture system, control cultures were included to which cycloheximide (50 μg/ml), a blocker of protein synthesis, was added. Cultures were performed in triplicate with and without pokeweed mitogen (PWM), 10 μl/ml. All cultures were done in triplicate. At the termination of the culture, the supernatants were removed and stored at −20°C until measurement of secreted Ig. We used FCS from a single lot with the least non-specific stimulative effect, and a single batch of PWM with optimal stimulative effect on Ig production throughout the study.

(b) ELISA for secreted IgG and IgM

IgG and IgM concentrations in the supernatants were assessed using an ELISA essentially the same as used for the measurement of anti-HPH antibodies. Flat bottom microtitre plates (Inotech) were used, coated for 1 h at 37°C with a 1:1,000 dilution of goat anti-human IgG or IgM (Tago). Supernatants were diluted at least 1:10 in the Tween-BSA buffer. For each assay blank incubations were included and a standard curve was prepared covering the range from 1·5–100 ng/ml for IgG and 3·5–480 ng/ml for IgM. IgG and IgM concentrations were calculated after log–logit transformation of the supernatant dilutions and optical densities, and were expressed as ng/ml IgG or IgM, using the standard curve. All determinations were performed in duplicate.

(c) Assessment of T cell subpopulations

The following mouse monoclonal antibodies were employed for lymphocyte phenotyping: OKT3 and OKT8 (Ortho Pharmaceuticals, Raritan, New Jersey, USA), and Leu-3a (Becton Dickinson and Company, Mountain View, California, USA). The preparations of these non-fluoresceinated monoclonal mouse hybridoma antisera were reconstituted by adding 1 ml of phosphate-buffered saline (PBS) supplemented with 1% BSA. Five hundredths of a millilitre of the reconstituted preparations further diluted 1:20 in PBS/1% BSA, were added to 2×10^6 PBMNC. The cells were resuspended and incubated for 30 min at 4°C. After centrifugation and washing twice with PBS/1% BSA at 4°C, the cells were incubated with fluorescein labelled goat anti-mouse IgG (Nordic) for 30 min at 4°C. After washing twice with PBS/1% BSA at 4°C, cells were resuspended in a drop of glycerol/PBS, mounted on a slide, covered with a coverslip, and examined in a Leitz Orthoplan fluorescence microscope. For each slide, 300 cells were counted by three different observers.

The cells that were used were either fresh or cryopreserved in liquid nitrogen. To ascertain that no changes in lymphocyte phenotype occurred due to cryopreservation, both fresh and preserved cells from a series of controls were assessed for the distribution of differentiation antigens as defined by the monoclonal antibodies described before. No differences were noted.

Statistics. For statistical analysis of difference between groups of patients and controls Wilcoxon's rank sum test was used. For correlation studies Spearman's rank correlation was used. Only P values less than 0·05 were considered significant.

RESULTS

In vivo *humoral immune response to the primary test immunogen HPH*

Twenty-two patients with SLE and 23 healthy controls were immunized with HPH. No complications, especially no flare-up of the disease, was observed after immunization. In none of the

patients and controls antibodies to HPH of IgG and IgA class were detectable before immunization; some controls, however, had low levels of IgM class antibodies reacting with HPH before immunization. At 3 and 6 weeks after immunization, class specific antibodies to HPH were determined. Both at 3 and at 6 weeks, levels of IgG, IgM and IgA class antibodies were lower in the patient group than in the controls ($P < 0{\cdot}01$, Fig. 1). Similar differences were noted when comparing the controls with the group of patients with inactive disease only ($P < 0{\cdot}02$, Fig. 1). No differences were observed in class specific antibody response to HPH between patients with active and those with inactive disease. Also, the dosage of prednisone was not related to the height of the antibody response. Levels of HPH antibodies and total levels of immunoglobulins did not correlate in the patients. Concerning the kinetics of the response, the decline in levels of IgM and IgA class antibodies at 6 weeks after immunization was greater in patients than in controls. The difference between patients and controls was, however, only significant for IgA class antibodies ($P < 0{\cdot}05$).

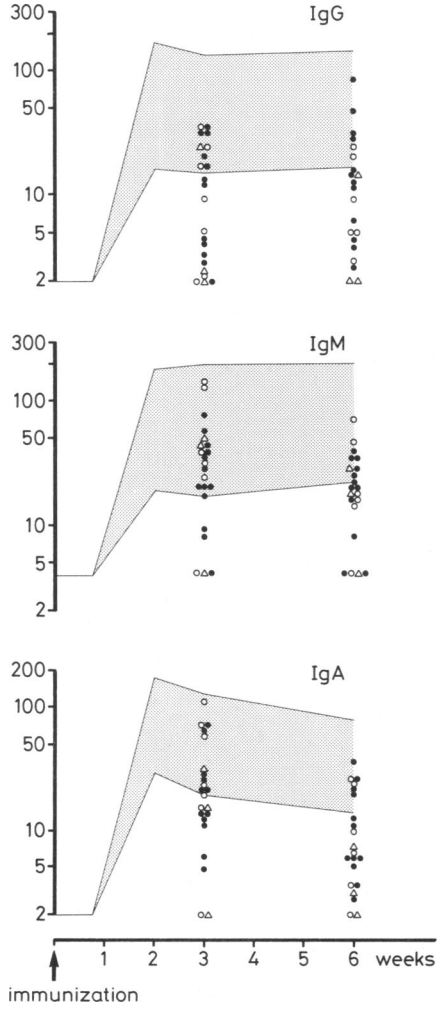

Fig. 1. Class specific antibody response to HPH at 3 and 6 weeks after immunization in 22 patients with SLE. The antibody levels are expressed as a percentage of a positive standard. The shaded area represents the normal range (±s.d.). ● = patient without activity of the disease; ○ = patient with minor activity of the disease; △ = patient with major activity of the disease.

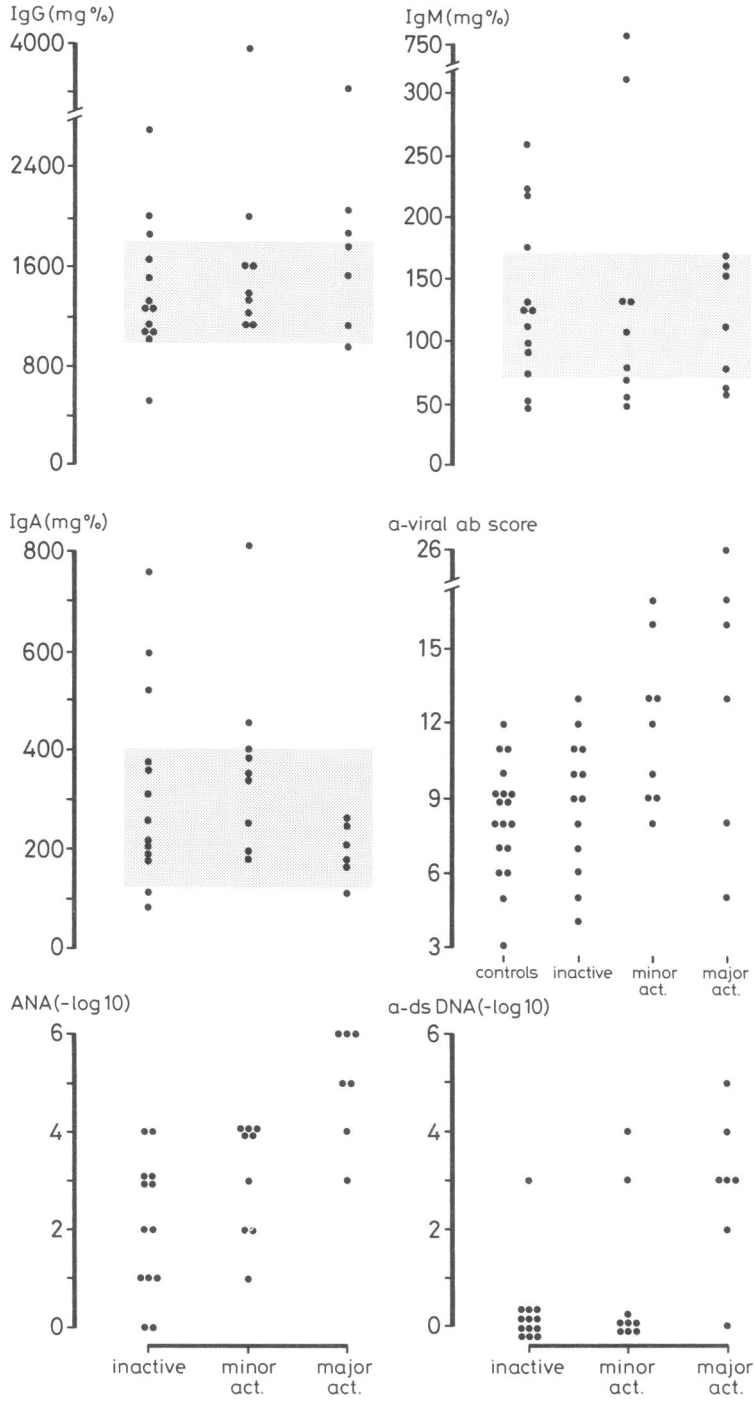

Fig. 2. Levels of immunoglobulins, antiviral antibody score and titres of ANA and a-dsDNA in controls, patients with SLE without disease activity, patients with minor activity, and patients with major activity of the disease. The shaded area represents the normal range (± 2 s.d.) in our laboratory.

In vivo *antibodies against viral antigens and autoantigens*

To evaluate the *in vivo* activity of the B cell system, we studied levels of immunoglobulins, titres of antibodies against common viral antigens (patients and controls had no signs of viral infections), and titres of ANA and a-dsDNA. Elevated levels of immunoglobulins were present in a minority of the patients; IgG in 29% of the patients, IgM in 21% and IgA in 21%. Except for the presence of lower levels of IgA in patients with major disease activity than in those with minor activity ($P < 0{\cdot}05$), no differences were noted in levels of immunoglobulins between patients with inactive, slightly active and very active disease (Fig. 2). On the contrary, titres of anti-viral antibodies, expressed as the anti-viral antibody score, were higher in patients with active disease than in those without disease activity ($P < 0{\cdot}05$, Fig. 2). The anti-viral antibody score of these latter patients did not differ from that of the controls. Titres of ANA and a-dsDNA, also, were higher in patients with active than in those with inactive disease ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively). Major disease activity was associated with higher titres than minor activity of disease ($P \leqslant 0{\cdot}05$, Fig. 2). No correlation was observed between the anti-viral antibody score and titres of ANA and a-dsDNA.

In vitro *B cell activity*

Peripheral blood mononuclear cells (PBMNC) were isolated both from patients and controls, and cultured for 8 days with and without pokeweed mitogen (PWM). For each test 1×10^6 cells were cultured in tubes with 1 ml culture medium containing 10% FCS. At the end of the culture period supernatants were analysed for the amount of secreted IgG and IgM using an ELISA technique. The spontaneous activity of the B cells and their capacity to secrete immunoglobulin on non-specific stimulation were evaluated. In healthy controls spontaneous secretion of IgG never exceeded 250 ng/ml (median value 85 ng/ml, range < 15–230). On stimulation with PWM, values of secreted IgG were measured up to 4,020 ng/ml (median value 975 ng/ml, range 390–4,020; one control did not have any stimulation at all), Fig. 3. The median ratio of PWM stimulated to spontaneous cultures in the controls was 15·3 (range 1·0–35·3), Fig. 5. Similar results were obtained for the *in vitro* secretion of IgM, although both spontaneous secretion (median value 150 ng/ml, range < 35–820) and PWM stimulated secretion (median value 2,280 ng/ml, range 450–7,910) were higher for IgM than for IgG (Fig. 3). In patients with SLE different results were obtained. PBMNC from patients without activity of their disease secreted spontaneously an amount of IgG comparable to that of controls

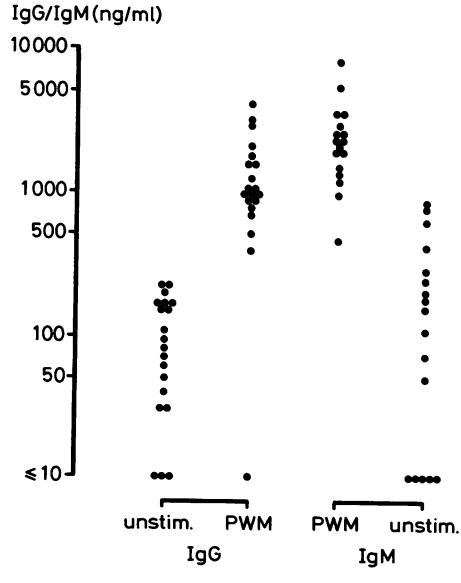

Fig. 3. Spontaneous and pokeweed mitogen stimulated secretion of IgG and IgM in 8 days cultures of mononuclear cells from healthy controls. Data are plot on a logarithmic scale as IgG (ng/ml) or IgM (ng/ml).

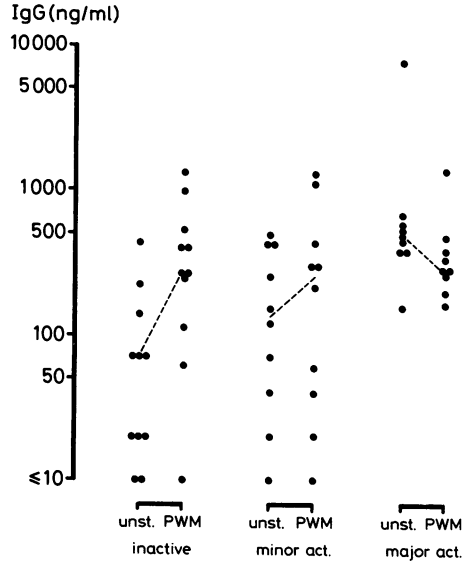

Fig. 4. Spontaneous and pokeweed mitogen stimulated secretion of IgG in cultures of mononuclear cells from patients with SLE without disease activity, with minor, and with major disease activity. The dotted lines connect the median values of unstimulated and stimulated cultures.

(median value 70 ng/ml). However, their capacity to secrete IgG on stimulation with PWM (median value 260 ng/ml) was decreased compared to controls ($P < 0{\cdot}01$), Figs 4 & 5. In patients with minor disease activity, PWM stimulated secretion of IgG was also lower than in the controls ($P < 0{\cdot}01$). Spontaneous *in vitro* secretion was somewhat higher in this group compared to controls and to patients with inactive disease (not reaching statistical significance). Consequently, the ratio of PWM stimulated and spontaneous IgG secretion in patients with minor disease activity was lower than that in patients with inactive disease ($P < 0{\cdot}05$, Fig. 5). When major activity of SLE was

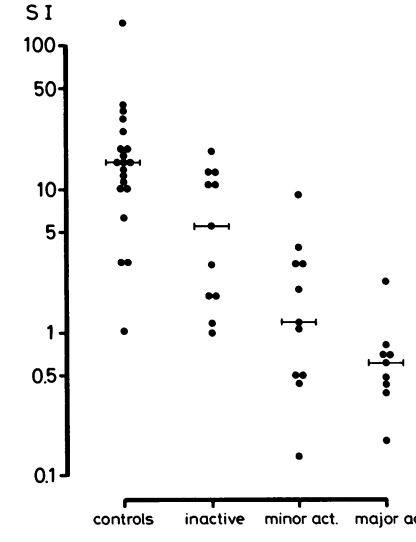

Fig. 5. Ratio (SI) of IgG secretion in cultures with and without pokeweed mitogen. SI values are given for controls and for patients with various degrees of activity of SLE. Horizontal bars indicate median values.

present, spontaneous *in vitro* secretion of IgG was increased compared to the other groups of patients and the controls ($P < 0{\cdot}01$). The median value of spontaneous IgG secretion was 480 ng/ml, the range 150–7,480 ng/ml, Fig. 4. In contrast, PWM stimulated secretion was low (median value 280 ng/ml, range 160–1,370). These values were lower than those of unstimulated cultures in all but one patient. Consequently, the ratio of PWM stimulated and spontaneous IgG secretion was less than one (Figs 4 & 5). To evaluate more precisely the relationship between spontaneous *in vitro* IgG secretion and the activity of the disease, we related the values of spontaneous IgG secretion to the levels of complement C3 in all patients. A highly significant reversed correlation was observed ($P < 0{\cdot}001$, $r = 0{\cdot}735$).

In general, similar findings were present in all the studies as for the *in vitro* secretion of IgM (data not shown).

T cell subsets as defined by monoclonal antibodies

In 19 patients and 12 controls, PBMNC were assessed for T cell subsets using monoclonal antibodies. The following antibodies were used: OKT3, which reacts with all peripheral T cells; Leu-3a, which reacts with the inducer or helper cell subset of peripheral T cells; OKT8, which reacts with the suppressor or cytotoxic cell subset of peripheral T cells (Kung *et al.*, 1979; Reinherz & Schlossman, 1980; Ledbetter *et al.*, 1981; Howard *et al.*, 1981). The data are shown in Table 2. Generally, a wider range was observed in the percentages of T cell subsets in the patients than in the controls. However, no significant correlation was present between the percentage of a specific T cell subset and the activity of the disease. In order to adjust for differences in the number of total T cells between individual subjects, we calculated the ratio of the percentage of Leu-3a cells (helper cells) to the percentage of OKT8 cells (suppressor cells). Again, no consistent change in the ratio was

Table 2. T cell subsets in 19 patients with SLE (percentage of reactive peripheral blood lymphocytes)

Patient No. (see Table 1)	OKT3	Leu-3a	OKT8	Ratio Leu-3a/OKT8	Disease activity
25	70	37	34	1·09	
24		55	37	1·49	major
26		38	31	1·23	
27	85	45	43	1·04	
19	72	49	25	1·96	
18	79	40	38	1·05	
20	85	39	57	0·68	minor
21	67	63	31	2·03	
14	63	45	29	1·55	
17	80	53	39	1·36	
1	72	55	30	1·83	
3	81	56	36	1·56	
4	80	58	38	1·53	
6	83	58	36	1·61	
8	79	41	52	0·79	none
10	63	48	40	1·20	
11	58	44	32	1·38	
12	68	54	24	2·25	
13	57	42	28	1·50	
Mean ± s.d. in the patients	73·1 ±9·3	48·4 ±7·6	35·7 ±9·3	1·43 ±0·41	
Mean ± s.d. in the controls	75·8 ±5·5	47·3 ±6·0	33·9 ±6·9	1·45 ±0·34	

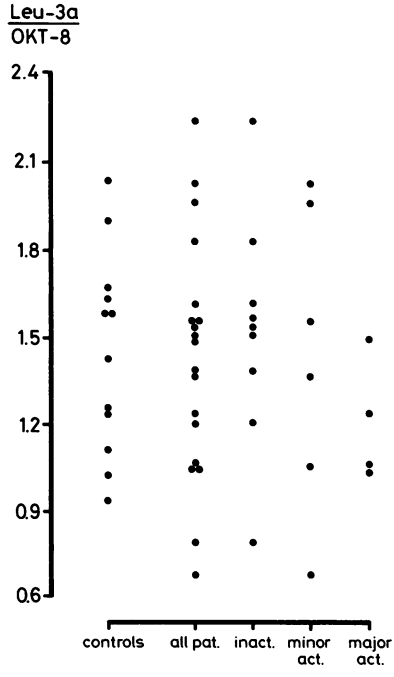

Fig. 6. Helper–suppressor ratio (Leu-3a/OKT8) of peripheral blood lymphocytes as determined by monoclonal antibodies in controls and patients with various degrees of activity of SLE.

observed in the patients compared to the controls (Fig. 6). Also, the ratio did not correlate with the activity of the disease.

Finally, we evaluated whether spontaneous or PWM stimulated *in vitro* IgG secretion was related to the percentage of Leu-3a or OKT8 T cell subsets. No correlation was noted.

DISCUSSION

We studied immune responsiveness in patients with SLE. Since SLE is characterized by B cell hyperactivity, we paid attention to the humoral immune response. After immunization with the primary test immunogen HPH, a decreased humoral response was observed in all three immunoglobulin classes, but especially in the IgG class. In addition, levels of IgA class antibodies declined faster in the patients than in the controls. Thus, despite the supposed B cell hyperactivity, the humoral immune response to newly encountered antigens is blunted in SLE. This, apparently, is an intrinsic defect in SLE, since the severity of the defect in the humoral immune response was not related to the activity of the disease nor to the use of (low doses of) corticosteroids. The latter is in accordance with other data demonstrating no influence (Butler, 1975) or even enhancement (Tuchinda, Newcomb & DeVald, 1972) of the primary humoral immune response by (low doses of) corticosteroids. An impaired humoral immune response to immunization in patients with SLE was also recorded in other studies (Baum & Ziff, 1969; Williams *et al.*,1978; Jarrett *et al.*, 1980), although a normal response was also described (Brodman *et al.*, 1978; Louie *et al.*, 1978). However, in all these studies viral and bacterial antigens were used. These antigens probably do not represent primary immunogens in most patients. It should be stated that the impairment of the humoral response in our patients with SLE does not necessarily represent a defect in B lymphocyte function, since antibody formation after exposition to a complex antigen like HPH is a multifactorial process, requiring adequate handling of antigen by macrophages, and T–T and T–B cell co-operation. Nevertheless, B cell hyperactivity in SLE apparently does not apply to the immune response to

primary antigens. On the contrary, increased titres of antibodies against viral recall antigens were observed in patients with active disease, but not in patients in remission. Similar observations were made in longitudinal studies in some patients (data not shown). Elevated levels of antibodies against viral antigens in patients with SLE were also recorded by others (Hollinger *et al.*, 1971; Hurd *et al.*, 1972; Phillips & Christian, 1973). In one study a relation with the activity of the disease was observed (Phillips & Christian, 1973). None of our patients demonstrated clinical signs of viral infection during activity of the disease. Thus, clinically, the increase in anti-viral antibody titres cannot be explained by antigenic stimulation, and suggests autonomous B cell activity. However, most of the antibodies studied were directed against viruses that may persist in the host. Active virus replication without clinical signs, cannot be excluded as a cause of the observed increase in antiviral antibody titres. Reactivation of persistent viral infections may even have resulted in polyclonal activation of the B cell system.

As could be expected—and has been reported already in many studies—levels of autoantibodies (ANA and a-dsDNA) correlated also with disease activity. These findings fit in the concept of autonomous, polyclonal activation of B cells in active SLE. Despite polyclonal B cell activation, levels of total immunoglobulins, although elevated in some of our patients, did not parallel the activity of the disease. This can be explained by increased immunoglobulin turnover (Levy *et al.*, 1970), or by a more selective, although still polyclonal, activation of the B cells.

In *in vitro* studies we indeed observed an activated B cell system in patients with active SLE as reflected in the high rate of spontaneous Ig synthesis. The same observations were made by others (Nies & Louie, 1978; Ginsburg *et al.*, 1979; Blaese *et al.*, 1980; Wangel *et al.*, 1982) using different assays. Interestingly, a highly significant correlation was present between the rate of spontaneous Ig synthesis and the decrease in levels of complement factor C3. The latter is regarded as the most sensitive indicator of disease activity in SLE (Lloyd & Schur, 1981). Beside an increased *in vitro* spontaneous Ig synthesis, we observed a decreased capacity of our patients' PBMNC to synthesize IgG or IgM *in vitro* on stimulation with PWM. Although this finding was noted even in patients without disease activity, it was much more outspoken in patients with active disease. In the latter patients, lower values were recorded for PWM stimulated Ig synthesis than for unstimulated synthesis. The significance of this observation, also made by others (Nies & Louie, 1978; Ginsburg *et al.*, 1979; Bobrove & Miller, 1977; Beale *et al.*, 1982), is not clear. B cells, already activated, might have lost their capacity to react on stimulatory factors. Activated B cells unresponsive to signals from helper T cells may still react on signals from suppressor T cells. Otherwise, since PWM-induced Ig synthesis is a T cell-dependent process, abnormalities in the number or function of T cell subsets may underly the defective stimulation by PWM. Results of re-constitution experiments, as reported in the literature, using normal and SLE B and T cells in various combinations, are unconclusive; in some studies defective B cell function in SLE could be (partially) restored by normal T cells (Beale *et al.*, 1982; Tan, Pang & Wilson, 1981), in other studies the defective response was considered to reside only in the B cells (Nies *et al.*, 1980; Pelton & Denman, 1982).

To evaluate changes in T cell subsets, including helper and suppressor cells, we studied the percentages of those subsets in patients with various degrees of activity of SLE, using well defined monoclonal antibodies. Although the total number of peripheral blood lymphocytes is known to be severely depressed in active SLE, we could not demonstrate a relative deficiency of one or more subsets (total number of T cells, helper cells and suppressor cells, respectively) in our patients. However, both in patients and controls the sum of cells staining with anti-Leu-3a and anti-OKT8 separately, frequently was greater than the number of cells staining with anti-OKT3. This overlap may (partly) be explained by the existence of a small subset of cells bearing both the Leu-3a and OKT8 antigens, as also reported by others (Smollen *et al.*, 1982). In addition, no consistent change in the ratio of helper to suppressor cells was noted. These findings are in contrast to those of Morimoto *et al.* (1980, 1982) who described the loss of OKT8 suppressor cells in active SLE. However, in a recent paper from the same group, different results were reported (Smollen *et al.*, 1982): no consistent relation was found between disease activity and the distribution of T cell subsets in a group of SLE patients. However, a low helper : suppressor ratio was observed in patients with renal disease and thrombocytopenia, whereas patients with a high ratio had multisystem

disease without renal involvement. We could not reproduce these findings in our group of patients. Two other remarks have to be made. First, the phenotype of T cells as determined by monoclonal antibodies, does not necessarily correspond with the functional state of the cells (Damle & Gupta, 1982). Secondly, in these *in vitro* experiments we are only studying the peripheral blood compartment, whereas important abnormalities in the immune system may occur in spleen, bone marrow and lymphnodes and remain undetected.

Whatever the role of the T lymphocytes, exacerbations of SLE seem characterized by the presence of polyclonally activated B lymphocytes. Why and how the B cell becomes activated is not clear at the moment.

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