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Reticuloendothelial Fc receptor function in SLE patients. I. Primary HLA linked defect or acquired dysfunction secondary to disease activity?

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SUMMARY

Reticuloendothelial system (RES) Fc receptor-mediated immune clearance was measured in 18 patients with systemic lupus erythematosus (SLE). Only two patients, with major disease activity, had a prolonged $T_{\frac{1}{2}}$ of the blood disappearance curve of injected IgG coated red cells in comparison to 22 healthy controls. Circulating immune complexes (CIC) were studied with three methods: PEG precipitation, C1q-ELISA and the indirect granulocyte phagocytosis test (IGFT). The $T_{\frac{1}{2}}$ of the blood disappearance curve related significantly to the IGFT ($r=0{\cdot}55$, $P<0{\cdot}05$) and not to the PEG and C1q-ELISA test. Although HLA-DR3 phenotype frequency was significantly increased in our SLE population ($P<0{\cdot}05$), it was not related to Fc receptor function. Similarly, HLA-DR2 phenotype was not related to RES Fc receptor function. These data do not support the concept that a genetic HLA linked defect in reticuloendothelial Fc receptor function is a primary cause of SLE, predisposing the inflicted individual to immune complex deposition. However, Fc receptor-mediated immune clearance seems to be related to disease activity itself and to levels of CIC

Keywords systemic lupus erythematosus Fc receptors reticuloendothelial system immune complexes HLA

INTRODUCTION

Defective *in vivo* reticuloendothelial system (RES) Fc receptor function in patients with active systemic lupus erythematosus (SLE) has been described by various groups (Frank *et al.*, 1979; Lockwood *et al.*, 1979; Hamburger *et al.*, 1981; Parris *et al.*, 1982). Most of these studies were done with IgG coated red cells. Erythrocytes with less than 2,000 IgG molecules on their cell surface are thought to be cleared from the blood by Fc receptors on macrophages, preferentially located in the spleen (Frank *et al.*, 1977). Although these original studies certainly revealed interesting and a perhaps unexpected series of associations (Frank *et al.*, 1983), a direct relationship between immune complex (IC) levels and RES function was not established. Nevertheless, it has been suggested that a direct blocking effect of circulating IC (CIC) on RES Fc receptor function can be reversed by plasmapheresis (Lockwood *et al.*, 1979; Hamburger *et al.*, 1981). The last author states, however, in a recent review article on the subject that CIC alone are not sufficient to cause blockage of reticuloendothelial function (Hamburger, 1983).

Defective RES Fc receptor function has also been described in normal volunteers and patients

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with dermatitis herpetiformis; in both groups there was no evidence for the presence of CIC (Lawley *et al.*, 1981). In this study the immune clearance defects were linked to HLA-B8 DR3 phenotype positivity. It was suggested that this linkage in controls and patients could be the explanation for the well documented predisposition of HLA-B8 DR3 positive persons towards immunologically-mediated diseases. Perhaps such individuals could have an HLA-B8 DR3 linked RES Fc receptor abnormality that could facilitate the tissue deposition of IgG containing IC.

A similar hypothesis has recently been put forward for the HLA-DR2 phenotype (Kimberley *et al.*, 1983). Although HLA-B8 DR3 and DR2 phenotype are significantly more frequent in SLE patients, a recent study (Parris *et al.*, 1982) did not show an association between HLA-B8 DR3 phenotype and RES Fc receptor function in SLE.

In this study we investigated Fc receptor function in a group of patients with SLE in relation to levels of CIC and HLA type. In addition, we looked at clinical symptoms in a prospective standardized protocol. We also performed standard serological studies that are currently used in our hospital to assess disease activity in SLE.

PATIENTS AND METHODS

Selection of patients. Twenty-three patients (18 women and five men, 18–63 years of age) with SLE satisfying four or more of the ARA preliminary criteria (Cohen *et al.*, 1971) were studied.

Their clinical characteristics are given in Table 1. ARA criteria with corresponding number are: (1) facial erythema; (2) discoid lupus; (3) Raynaud's phenomenon; (4) alopecia; (5) photosensitivity; (6) oral or nasopharyngeal ulceration; (7) arthritis without deformity; (8) LE cells or positive ANA test (titre) $\geq 1:100$; (9) chronic false positive Wassermann; (10) proteinuria > 3 g/day; (11) cellular casts; (12) pleuritis or pericarditis; (13) psychosis or convulsions and (14) leukopenia $< 4{,}000/mm^3$, thrombocytopenia $< 100{,}000/mm^3$ or haemolytic anaemia.

Major disease activity was defined as one or more of the following: severe proliferative glomerulonephritis, severe haemolytic anaemia (Hb < 5 g%) or thrombocytopenia ($< 50{,}000$ mm^3), pericarditis with tamponade, uveitis or retinitis, severe CNS involvement (convulsion, coma, transverse myelitis), myocarditis with arythmia and/or cardiac failure, severe myositis, systemic vasculitis with impending necrosis, pulmonary involvement with haemorrhage, fever e causa ignota ($> 38{\cdot}5$°C), serositis, haemolytic anaemia (Hb > 5 g%) and thrombocytopenia ($> 50{,}000/mm^3$) not responding to prednisone < 30 mg.

Minor disease activity was defined as the presence of manifestations attributable to SLE, but not fulfilling the criteria for major disease activity (with the exception of Raynaud's phenomenon and photosensitivity). Five female patients (Nos 1, 3, 6, 15 and 21) with a theoretical chance of future pregnancy were excluded from the Fc receptor function study. Two physicians considered all clinical and laboratory findings in a formal system before studies of immune response and Fc receptor were performed. All patients gave written informed consent to participate in the study. This project was approved by the Hospital's Medical Ethical Committee. Normal values were obtained in studies of healthy laboratory and clinical personnel, from the department of Internal Medicine.

Clearance studies. Anti-rhesus antiserum was obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam and originated from a single donor who had previously been hyperimmunized and had been shown not to be a carrier of hepatitis B virus.

One donor unit of R2/R2 cells was incubated for 30 min at 37°C with a 1:2 dilution of the antiserum. After washing the cells thoroughly with saline, they were frozen, mixed with an equal volume of glycerol 38%, sorbitol 2·9% and NaCl 0·63%. A sample of these cells was checked for coating quantity on the cytofluorograph, showing that all cells were equally coated. The frozen cells were stored in 10 ml ampoules at -80°C. On the day of clearance study one ampoule at a time was thawed and centrifuged at 3,500 r/min for 5 min. Thereafter, cells were resuspended in sorbitol 17·5% and NaCl 0·8%. After recentrifugation at 2,500 r/min the erythrocytes were washed three times in saline. Thereafter, the cells were incubated with 18 Mbq (500 μCi) ^{99m}Tc for 5 min at room

temperature. A volume of freshly prepared 1% stannous chloride was added and the mixture allowed to stand for 5 min. The cells were then washed twice in saline, resuspended with an equal volume of saline and injected intravenously. Labelling efficiency was about 90%. Injection was done in 60 s, blood samples were taken at 0, 3, 8, 13, 18 and 23 min and the injected dose was determined by measuring the syringe before and after injection in a common dose calibrator. Over this period (up to 23 min) the blood clearance is monoexponential. The half-life ($T_{\frac{1}{2}}$) was calculated by regression analysis. The patients lay in a supine position on top of a large field of view gamma camera, so that the camera viewed the liver and spleen posteriorly. Digital images were recorded in 1 min frames during 60 min after injection. Curves using a region of interest technique over spleen and liver areas (corrected for physical decay of the radionuclide) were produced. Usually, an equilibrium between liver and spleen activity is reached in about 30 min. The 1 h liver and spleen uptake in counts per min (ct/min) per μCi administered were calculated. The quotient of the liver to spleen uptake was called the LS ratio.

IC tests. Blood samples were drawn on the day of the Fc receptor test. Blood was allowed to clot at room temperature and the serum was stored at $-20°C$. CIC were assayed by three different test: Polyethyleneglycol (PEG) precipitation, C1q-ELISA and the indirect granulocyte phagocytosis test (IGFT). PEG precipitation was done as follows: 50 μl serum was mixed with 150 μl 0·08 M $CaCl_2$ and 1 ml 5% PEG in 0·1 M borate buffer, pH 8·4. The mixture was left at 4°C overnight and centrifuged for 3 min at 3,500*g*. The clear supernatant was carefully removed and the pellet dissolved in 1 ml 0·1 N NaOH. Optical density (OD) was measured in a 0·5 cm cuvette at 280 nm. The test was performed in duplicate. The C1q-ELISA was carried out as described elsewhere (van der Giessen & The, 1980) with minor modifications. The wells of microtitre plates (Dynatech) were coated with 100 μl purified C1q (1–2 μg/ml) in 0·01 M Tris-HCl buffer, pH 8·0, containing 0·15 M NaCl at 4°C for at least 48 h.

After washing, the wells were filled with 100 μl of two-fold serum dilutions, starting with 1/20, in Tris-HCl buffer containing 0·15 M NaCl, 2% HSA (CLB), 0·1% gelatine and 0·05% Tween (Tris-HSA buffer). After incubation for 1h at 37°C, the wells were washed and filled with 100 μl HRPO-labelled anti-IgG (Dako) in Tris-HSA buffer, incubated for 1 h at 37°C, washed again, and filled with 100 μl substrate (0·2 mg/ml OPD [Kodak] in 50 mM phosphate buffer, pH 5·6, containing 0·0045% H_2O_2). After standing in the dark at room temperature for 45 min the reaction was stopped by adding 1 N H_2SO_4 and the colour was read automatically on a Titertek multiscan.

IC concentrations were determined by comparison with a standard curve of chromatographically purified IgG, which was heat aggregated for 30 min at 63°C (A-IgG), and results are expressed as μg equivalents A-IgG.

The IGFT (Van Wingerden *et al.*, 1978) was done as follows: normal donor granulocytes containing $< 1\%$ mononuclear cells, were obtained by dextran sedimentation (NH_4Cl for removal of erythrocytes) and Isopaque-Ficoll gradient centrifugation from freshly drawn heparinized blood. 100 μl of a cell suspension of 20×10^6/ml were incubated with 100 μl patient serum and 25 μl fresh human complement at 37°C for 1 h, with occasional mixing. After washing the cells, cytocentrifuge preparations were made. The slides were air dried and fixed with water free acetone. Phagocytosed IC were detected by indirect immunofluorescence using specific rabbit antisera against IgG (CLB) and as a second layer FITC conjugated sheep anti-rabbit Ig. Cells containing at least six fluorescent granules were regarded as positive. A total of 1,000 granulocytes in fields chosen at random were screened by two observers independently.

Serological studies. ANA were detected with an indirect immunofluorescence technique using human fibroblast layers as a substrate (Kallenberg *et al.*, 1983). Antibodies to double stranded DNA (a-dsDNA) were detected using an indirect immunofluorescence technique with the kinetoplast of *Crithidia luciliae* as a substrate (Aarden, de Groot & Feltkamp, 1975).

C3 and C4 levels were assayed by radial immunodiffusion with monospecific antisera (Behringwerke). Rheumatoid factor was measured with a Waaler–Rose and Latex test. The detection of anti-red cell and anti-lymphocyte antibodies was performed according to standard methods. HLA-A, -B and -C typing for 32 antigens was done by the NIH lymphocyte microcytotoxicity technique. HLA-DR typing was performed with the two colour fluorescence technique (van Rood, van Leeuwen & Ploem, 1976).

Statistical analysis. Wilcoxon's rank sum test was used for differences between groups of

patients and controls. For correlation studies Spearman's rank correlation test was used. For HLA typing, the observed differences between populations were tested for significance with the chi-square test. Only P values less than 0·05 were considered significant.

RESULTS

Fc receptor-mediated immune clearance

Two patients with major disease activity had a marked defect in clearance of ^{99m}Tc labelled IgG sensitized erythrocytes (Table 1). The half life values in these patients were 61 and 70 min, clearly above the values obtained in the 22 controls (Fig. 1).

Both patients had rapid declining kidney function at the time of investigation, due to a biopsy proven diffuse proliferative glomerulonephritis. Patient No. 22 (Table 1) also had a Coombs positive haemolytic anaemia. $T_{\frac{1}{2}}$ values in controls and patients were not related to ABO or rhesus group. Although only five patients (Nos 2, 14, 20, 22 and 23) had received steroids at the time of the RES Fc receptor test, there was no relation between $T_{\frac{1}{2}}$ and steroid medication. LS ratio was always below 0·20, confirming the major role of the spleen in the clearance of these cells. Spleen and liver size were normal in all subjects studied.

IC tests

Patients without disease activity had low levels of CIC in the PEG test (mean = 109, range 46–275). Levels were higher in patients with minor (mean = 122, range 117–180) and major (mean = 191, range 119–294) disease activity. Comparing the patients with minor and major disease activity against the non-active group, the difference was statistically significant ($P < 0·05$, Wilcoxon's rank sum test). There was no significant relation with the $T_{\frac{1}{2}}$ of the IgG coated red cells ($r = 0·28$). Three patients (Nos 11, 15 and 23, Table 1), one in each group of disease activity, had a positive C1q-ELISA test. The IGFT for IgG containing IC gave the following results: no disease activity—mean 120, range 20–240; minor disease activity—mean 218, range 30–920; major disease activity—all three patients had a score of 1,000.

Again, comparing the patients with minor and major disease activity with the non-active group, the difference was statistically significant ($P < 0·05$). There was a significant relationship with the $T_{\frac{1}{2}}$ of the Fc receptor test ($r = +0·55$, $P < 0·05$), see Fig. 2. However, there appears to be no relationship if the two patients with major disease activity are excluded. There was no significant difference between the patients with (Nos 4, 5, 7, 8, 9, 10, 13 and 15) or without anti-lymphocyte antibodies.

HLA typing

The six controls with the longest $T_{\frac{1}{2}}$ (Fig. 1) of the injected IgG coated red cells were negative for both B8 DR3 and DR2 phenotype. Nine patients were HLA-B8 positive, and nine patients were DR3 positive (Table 1). Eight out of 23 were B8 DR3 phenotype positive. In a random blood donor population in the Netherlands a B8 frequency of 0·29 ($n = 198$) and a B8 DR3 frequency of 0·16 ($n = 168$) was found. So B8 and DR3 frequency separately were not significantly increased. However, B8 DR3 frequency was significantly increased ($P < 0·05$). Fc receptor-mediated immune clearance was not related to B8 DR3 positivity: mean $T_{\frac{1}{2}}$ of B8 DR3 positive subjects was 28·1 (range 15–70 min), mean $T_{\frac{1}{2}}$ of other SLE patients was 34·0 (range 16–61 min). DR2 positivity was present in seven of the 23 patients and in a random blood donor population in the Netherlands this was the case in 54 of the 198 studied controls. DR2 positivity was not therefore increased in our SLE population. As can be seen in Table 1, only four patients (Nos 4, 7, 9 & 10) with the DR2 phenotype underwent the Fc receptor function test: mean $T_{\frac{1}{2}}$ was 23·8, range 16–32 min.

Complement levels and other serological studies

Serum levels of both C3 and C4 were decreased in only one patient (No. 22) with major disease activity. In the other patients C3 and C4 levels were normal. There was no correlation between complement levels and clearance $T_{\frac{1}{2}}$ values. Titres of ANA and anti-dsDNA were higher in patients with active than in those with inactive disease (Table 1).

Table 1. Some clinical and laboratory characteristics of 23 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‡	ANA	Anti-dsDNA	HLA type	$T_{\frac{1}{2}}$§
1	19, f	1,5,7,8	none	none	1:10	negative	A1A11B8Bw35Bw6Cw4DR1	not done
2	24, m	1,3,4,7,8,10, 11,14	none	predn (50/0 mg)	1:100	negative	A1B8Bw6DR3	19
3	24, f	1,4,6,7,8,10,11	none	none	1:10	negative	A1A3Bw35Bw6Cw4DR1DRw6	not done
4	31, f	3,4,5,7,8,10, 11,14	none	none	1:10	negative	A9(Aw29)Bw22Bw35CW4DR2DR5	16
5	33, f	1,7,8,9,10,11, 12,13,14	none	none	1:1,000	negative	A2B12(Bw44)B40(Bw60)DR4DR8	18
6	33, f	1,2,3,4,5,6,7,8	none	predn ($7\frac{1}{2}$/0 mg)	1:100	negative	A2B7(Bw60)Bw6Cw3DR2DRw6	not done
7	38, f	1,3,5,6,7,8,14	none	none	1:10,000	negative	A1A3B8Bw35Bw6Cw4DR2DR3	32
8	45, f	1,4,7,8	none	none	1:10,000	1:1,000	A2A9B18Bw35Cw4DR5DR7	32
9	46, f	4,5,6,7,8,14	none	chloroquine (250 mg)	1:1,000	1:10	A2B8Bw6DR2DR3	22
10	47, f	1,3,5,7,8	none	none	1:1,000	negative	A2B40Bw6Cw2Cw3DR2DR5	25
11	59, m	1,2,3,5,10,11	none	none	1:1,000	negative	A1A3B7Bw35Bw6Cw4DR1	48
12	64, m	7,8,10,11	none	none	negative	negative	A1Aw19B8Bw6DR3Dw9	21
13	27, f	3,4,5,7,8	minor	none	1:10,000	negative	A1Aw4B8B27Cw2DR1DR3	15
14	32, f	1,2,3,4,5,6,8, 10,11,13,14	minor	predn (20/0 mg)	1:10,000	1:1,000	A1A11Bw35Bw6Cw3DR3DR4	46
15	34, f	1,4,7,8,12	minor	predn ($7\frac{1}{2}$/0 mg)	1:100	negative	A2A3B7Bw6DR1DR2	not done
16	38, m	5,7,8,11,14	minor	ibuprofen (1,100 mg)	1:10,000	negative	A3A11B15(Bw62)B17Bw4Bw6DR4DR7	15
17	46, f	1,2,3,5,6,7,8	minor	ibuprofen (600 mg)	1:1,000	negative	A1A3B7B8DR1DR3	23
18	49, f	3,5,7,8	minor	none	1:10	negative	A1Aw19B8Bw21DR1DR3	20
19	50, f	1,2,3,4,5,6,7,8	minor	none	1:100	negative	A2A3B7Bw41DR1DR6	26
20	56, m	3,4,5,7,8,12	minor	predn (20/5 mg)	1:10,000	negative	A9Aw19B5Bw35Cw4D4DR7	37
21	25, f	1,5,6,7,8,12,14	major	predn (40 mg) azapropazon (600 mg)	1:10,000	1:10	A1A2B7B15DR1DR2	not done
22	33, f	3,4,5,6,7,8,10, 11,12,13,14	major	predn ($7\frac{1}{2}$/0 mg) cyclo-phosphamide (50/0 mg)	1:10,000	1:1,000	A2B5Bw41DR5DR7	61
23	54, f	1,3,4,5,6,7,8, 10,11,14	major	predn (10/0 mg)	1:1,000	1:100	A1B8DR3	70

*,† ARA criteria with corresponding number and criteria for assessing disease activity are given under patient selection.

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

§ The $T_{\frac{1}{2}}$ of the blood disappearance curve of the injected IgG coated red cells.

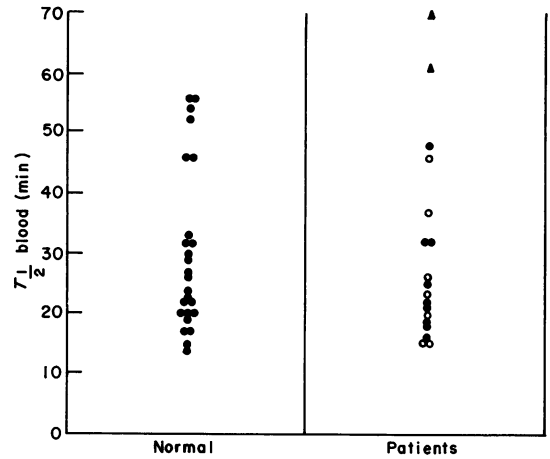

Fig. 1. $T\frac{1}{2}$ of the blood disappearance curve of the IgG coated cells in normals and patients. ● = not active disease; ○ = minor disease activity; ▲ = major disease activity.

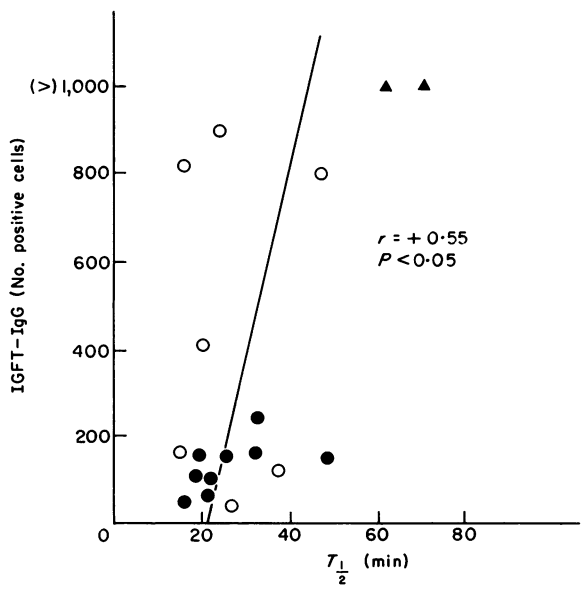

Fig. 2. Correlation between $T\frac{1}{2}$ of the blood disappearance curve and CIC (IGFT) in 18 SLE patients. ● = inactive disease; ○ = minor disease activity; ▲ = major disease activity.

Five patients (Nos 9, 11, 12, 17 & 18) had a positive rheumatoid factor test; the only two patients (Nos 9 & 18) with Sjögren's syndrome were within this group. A positive Coombs test was found in eight patients without relation to other parameters of disease activity (Nos 4, 5, 7, 10, 11, 16, 19 & 21). Clearance time did not correlate with ANA, a-dsDNA, rheumatoid factor, Coombs test or Sjögren's syndrome.

DISCUSSION

Our data show that a defective reticuloendothelial Fc receptor function in active SLE can be demonstrated with clearance curves of radioisotope labelled IgG-sensitized erythrocytes. This confirms and extends previous reports (Frank *et al.*, 1979; Lockwood *et al.*, 1979; Parris *et al.*, 1982).

There was no disease related shift in organ localization of the injected cells, as we demonstrated with heat damaged red blood cells (van der Woude *et al.*, 1983) in another group of SLE patients. Clearance of heat damaged erythrocytes does not therefore always parallel the clearance of IgG coated red cells, a finding that was first reported by Elkon *et al.* (1980, 1982).

There was no relation between HLA-DR2 or B8 DR3 positivity and Fc receptor function in our SLE patients. A prolonged $T_{\frac{1}{2}}$ of injected IgG coated red cells in normal subjects with the HLA-B8 DR3 phenotype was originally reported by Frank's group (Lawley *et al.*, 1981). Recently, a similar phenomenon has been described for the DR2 phenotype (Kimberley *et al.*, 1983). $T_{\frac{1}{2}}$ values in the normal subjects in our study do not differ from the findings in these two reports. The six subjects with the longest $T_{\frac{1}{2}}$ within our control group, however, were B8 DR3 and DR2 negative. The absence of an association between B8 DR3 positivity and Fc receptor function *in vivo* in SLE patients has been previously reported (Parris *et al.*, 1982). The reasons for the discrepancies between different studies remain unclear. Our data do not support the concept that a genetic HLA linked defect in reticuloendothelial Fc receptor function predisposes the B8 DR3 or DR2 positive individual to SLE.

Disease activity did relate to two of the three performed IC tests (PEG precipitation and IGFT). The IGFT does detect complexes in antibody excess (van der Giessen, unpublished observation). Only this test related statistically significantly to RES Fc receptor function, when all patients of this study were included. We are cautious in interpreting these results, because there is no such relationship if we exclude two patients with severe disease activity who were on potent cytotoxic agents. Perhaps the different results obtained thus far, concerning the correlation between IC and RES Fc receptor function, can be attributed to different sensitivities and specificities of the IC test used (Hamburger, 1983). If one favours the hypothesis of an IC-mediated RES Fc receptor dysfunction, the immunoglobulin content of the complexes could be of importance. However, the fact that the $T_{\frac{1}{2}}$ of the RES Fc receptor test was only prolonged in SLE patients with major disease activity also favours the alternative hypothesis that both IC levels and Fc receptor function are related to disease activity. In this study some patients with minor disease activity had elevated CIC levels, but not a defective Fc receptor function (Fig. 2). We conclude that the Fc receptor dysfunction in SLE is possibly disease related. A genetic HLA linked defect seems unlikely.

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