

## Parallel alteration of monocyte Fc receptor binding capacity and splenic macrophage clearance function in systemic lupus erythematosus

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### Summary

The Fc-IgG receptor (FcR)-mediated function of mononuclear phagocyte system (MPS) was investigated in 22 patients with systemic lupus erythematosus (SLE) and 10 control volunteers. The FcR binding capacity of peripheral monocytes was measured by 125-I-labelled bovine serum albumine (BSA) anti-bovine serum albumine (aBSA) complex. The mean value of FcR-s was increased and the association constant ( $K_a$ ) decreased in SLE group compared to the controls but the differences did not reach statistical significance. The clearance half-time of 51-Cr labelled and anti-D IgG sensitized autologous red blood cells was significantly increased in the SLE group ( $P < 0.05$ ).

However, the analysis of the individual data of patients showed a correlation between *in vitro* and *in vivo* FcR mediated function ( $r = -0.54$ ,  $P < 0.01$ ). The prolonged clearance half-time was accompanied by lower FcR number of peripheral monocytes in the same patient. These data suggest that the normal or even elevated numbers of FcR-s are not able to compensate the diminished phagocyte function of MPS in SLE.

### Introduction

Impaired clearance of anti-D IgG sensitized, 51-Cr labelled erythrocytes was found in systemic lupus erythematosus (SLE) by several investigators. These erythrocytes are trapped by cells of mononuclear phagocyte system (MPS) in the spleen via their Fc-IgG receptors (FcR) [2, 4, 14].

In contrast to the functional defect of MPS observed *in vivo*, we and others found normal or elevated FcR numbers on the surface of monocytes from patients with SLE. Increased FcR function was demonstrated by the binding of IgG sensitized bovine erythrocytes, radiolabelled IgG monomers and oligomers [3, 6, 12].

The aim of the recent study was to analyse further the connection between Fc receptor function of peripheral monocytes and splenic macrophages with sensitive and quantitative methods in a fair number of SLE patients. We measured the *in vivo* clearance of anti-D IgG sensitized erythrocytes in parallel with FcR number and binding association constant ( $K_a$ ) of monocytes in 22 SLE patients and 10 control volunteers. The FcR number was measured by using bovine serum albumine (BSA) 125-I-labelled anti-BSA rabbit IgG complex (BSA-aBSA). Our results suggest a correlation between FcR binding capacity of monocytes and MPS clearance function in SLE.

### Patients and methods

#### Selection of patients

Twenty-two SLE patients (19 women and 3 men, their age ranging 19–66 years) were studied. Each patient had four or more of the American Rheumatism Association (ARA) preliminary criteria [1]. Eight of the patients were considered to have active disease and 14 of them were in inactive state. The clinical activity was defined on the basis of Lupus Activity Criteria Count [13].

The correlation with laboratory parameters was not an aim of attention in the recent study because we studied earlier the clearance rate of sensitized erythrocytes as a function of antibodies to ENA and immune complexes [7].

Patients received the following daily doses of prednisone: none, two patients; 1 to 10 mg, five patients; 11 to 20 mg, ten patients; 21 to 30 mg, four patients. One patient with active disease was receiving 50 mg azathioprine and 20 mg prednisone.

In addition, 10 volunteers (5 women and 5 men, 35—62 years of age) participated in the study as controls. They had no inflammatory or autoimmune diseases and were not receiving anti-inflammatory or cytotoxic therapy. Informed consent was obtained from all participants.

#### *Measurement of whole-blood clearance of IgG sensitized autologous red blood cells*

FcR-mediated MPS studies were performed with a slight modification of a previously described method by Lockwood et al. [8]. Six ml of citrated blood was collected from each person. After centrifugation 1 ml of packed red blood cells were incubated with 50  $\mu$ Ci of  $^{51}\text{Cr}$  ( $\text{Na}_2\text{CrO}_4$  from Poland) at 37 °C for 10 min, and then washed once with physiological salt solution (PSS). After washing the cells were incubated with 50  $\mu$ g of anti-D IgG (OHVI, Budapest) for 15 min at 37 °C and washed twice with PSS. The volume was adjusted to 5 ml with PSS before reinjection. The cells were injected intravenously over 60 sec. Starting three minutes after the midpoint time of the injection 5 ml blood samples were removed at five minutes intervals for 20 min. Radioactivity per unit volume of blood was measured in a gamma counter (Gamma, Budapest). The clearance half-time was calculated on the basis of exponential disappearance of labelled erythrocytes from the circulation.

#### *Preparation of monocytes*

Monocytes were isolated by using the Ficoll-Hypaque gradient centrifugation, followed by adherence [9]. In brief, the monocyte rich fraction was incubated in tissue culture polystyrene plates (Linbro 76-003-05) for 60 min at 37 °C in  $\text{CO}_2$  atmosphere ( $2 \times 10^6$  cells/well). After removing the non-adherent cells, the adherent ones were positive esterase-stained (85—90%), viable (95—98%) monocytes ( $3\text{--}6 \times 10^5$ /well).

#### *Binding of immune complexes by monocytes*

The adherent cells were incubated with various doses of BSA-aBSA complex (containing 50 to 300 ng IgG) in 100  $\mu$ l Parker medium balanced with HEPES (pH 7.4) containing 0.5% ovalbumine for 4 hours at 4 °C. The immune complex (IC) was obtained from BSA and anti-BSA-rabbit IgG prepared at a molar ratio 1 : 1, which represented antigen-antibody complex formed in moderate antibody excess. The anti-BSA was labelled with  $^{125}\text{I}$  to a specific activity of 0.5 mCi/mg by the iodogen method. In parallel wells cold IgG was put at least 100-fold molar excess over the labelled ligand. Other parallel wells were set up containing labelled BSA-aBSA but no cells. Assays were done in duplicate. After incubation supernatants were aspirated and the washed cell pellets were dissolved in 0.5 N NaOH for quantification of  $^{125}\text{I}$  and cell count [9]. Binding data, corrected for nonsaturable component, were analyzed by the method of Scatchard [10].

#### *Statistical analysis*

The statistical significance (P) of the differences between the groups was calculated using Student's *t* test. The correlation coefficient (r) and its significance (P) were calculated from straight line equation.

## *Results*

#### *Fc receptor-mediated immune clearance*

A significant difference was found between Fc receptor-mediated immune clearance of SLE patients and normal controls as shown in *Table I*.

Values of clearance half-time measured in subsets of patients with inactive ( $24 \pm 10.2$  min) and active disease ( $34.38 \pm 26.4$  min) tended to increase with disease activity (*Fig. 1.*). Only one of 10 control volunteers had suppressed MPS function.

Table I.

Clearance half-time of labelled and sensitized red blood cells, FcR number and binding association constant ( $K_a$ ) of peripheral monocytes measured by BSA-aBSA in 22 patients with SLE and 10 control volunteers

Measured data	Units	Pts with SLE (22) mean $\pm$ SD	Controls (10) $\pm$ SD	Significance
Clearance half-time	min	27.6 $\pm$ 18	17.22 $\pm$ 6	P < 0.05
Fc receptor number	n/cell $\times 10^5$	2.98 $\pm$ 2.1	2.47 $\pm$ 0.76	NS
$K_a$ of BSA-aBSA binding to monocytes	$\times 10^7$ $M^{-1}$	4.81 $\pm$ 1.95	5.73 $\pm$ 2.3	NS

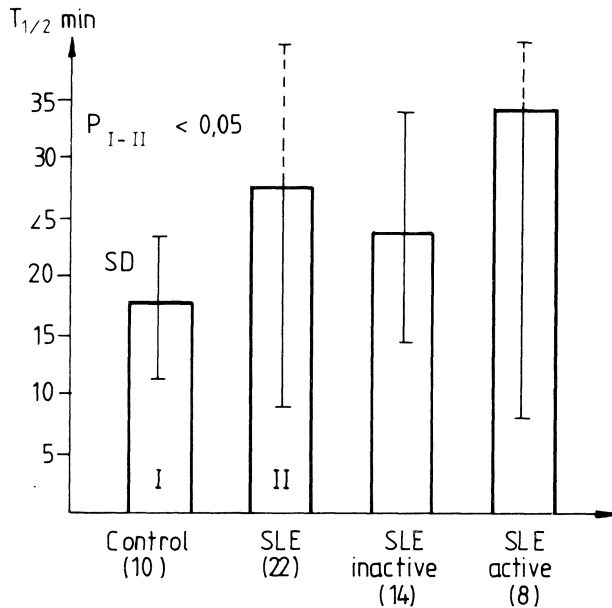


Figure 1. Clearance half-time ( $T_{1/2}$ ) of  $^{51}\text{Cr}$  labelled, anti-D IgG sensitized red blood cells in 10 control volunteers and 22 SLE patients. The patients with SLE were divided in inactive (14) and active (8) groups

#### Immune complex binding by Fc receptor of monocytes

As it shown in Table I the monocytes from SLE patients tended to have increased FcR number but no significant difference was found between the SLE and control group. The mean association constant value for FcR binding of BSA-aBSA complex was decreased in the SLE group but did not differ significantly from the normals (Table I). Reverse correlation was found between the FcR number and the  $K_a$  either in the SLE group and the control group but they did not reach the level of statistical significance (Table II).

#### Correlation between MPS clearance and binding of BSA-aBSA by monocytes

Studying the SLE group we noted that a significant negative correlation was present between clearance half-time and FcR number of peripheral monocytes

Table II.  
Correlation between FcR number (FcR), binding association constant ( $K_a$ ) of monocytes and clearance half-time ( $T_{1/2}$ ) of labelled and sensitized red blood cells in 22 SLE patients and 10 control volunteers

Correlation coefficients (r)	SLE patients (22)	Controls (10)
r FcR— $K_a$	-0.400 NS	-0.488 NS
r FcR— $T_{1/2}$	-0.544 $P < 0.01$	-0.59 NS
r $K_a$ — $T_{1/2}$	0.426 $P < 0.05$	0.388 NS

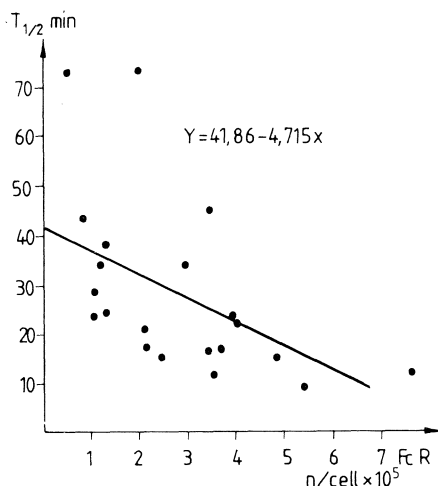


Figure 2. Correlation between FcR number of peripheral monocytes and clearance half-time ( $T_{1/2}$ ) of sensitized red blood cells ( $r = -0.54$ ,  $P < 0.01$ )

(Table 2, Fig. 2). Further investigation of this phenomenon detected a significant positive correlation between clearance half-time and association constant (Table II). In the control group similar suggestive correlations were found but these did not reach statistical significance (Table II).

### Discussion

In the present experiment the FcR-mediated function of MPS was investigated in vitro and in vivo in SLE and control groups. The qualitative and quantitative properties of FcR appeared characteristic of FcR ligand binding. In our study the FcR binding capacity of peripheral monocytes was measured by using BSA-aBSA complexes because the number of FcR sites for immune complexes (IC) were much higher than for IgG monomers [3]. The in vitro interaction between BSA-aBSA and monocytes might be a model of in vivo interactions of circulating IC and monocytes in SLE patients.

The mean value of FcR number was higher in our SLE group than in controls but the difference was not significant. At the same time a decreased mean value of the association constant (Ka) was revealed. Similar tendency was described by others used monomer and oligomer IgG [3]. Decreased FcR-ligand binding was published by using aggregated IgG as ligand [5]. This discrepancy may be related to the different behaviour of IgG monomer, oligomer and aggregated IgG.

The in vivo clearance of sensitized and labelled erythrocytes was measured parallelly in the same patients. Similarly to earlier findings [2, 4] a significantly prolonged clearance half-time ( $T_{1/2}$ ) was revealed in the SLE group. Furthermore our data suggest that this method can be useful to estimate the disease activity of patients with SLE.

The SLE group with elevated or normal FcR numbers of peripheral monocytes had prolonged  $T_{1/2}$ . However, comparing these two data in each patient we could show that the longer  $T_{1/2}$  was associated with lower FcR number of peripheral monocytes in the same patient. As it was shown there was a reverse correlation between the FcR number and  $T_{1/2}$  in SLE. The tendency was similar in the control group.

This analysis of individual data may help to resolve the paradox findings observed by us and others namely that decreased FcR-mediated MPS clearance exists in SLE despite normal or elevated FcR numbers of the monocytes [3, 12].

On the other hand it was published recently that the FcR-mediated in vitro internalisation of sensitized erythrocytes by SLE monocytes was decreased comparing to controls [11, 12]. This and our data suggest that the elevation of FcR numbers can not compensate the decreased phagocyte function of MPS in SLE. Furthermore, the alteration of phagocyte function may not be caused by circulating IC saturation of FcR-s.

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