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# Mononuclear phagocyte system Fc-receptor function in patients with seropositive rheumatoid arthritis

S. LOBATTO, F. C. BREEDVELD*, J. A. J. CAMPS†, E. K. J. PAUWELS†, MARIE-LOUISE WESTEDT*, M. R. DAHA & L. A. VAN ES *Department of Nephrology, * Department of Rheumatology, † Department of Nuclear Medicine, University Hospital Leiden, The Netherlands*

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## SUMMARY

Mononuclear phagocyte system (MPS) Fc-receptor function in 20 patients with seropositive rheumatoid arthritis (RA) was investigated using radiolabelled autologous erythrocytes coated with an average of 5,800 molecules of anti-rhesus IgG (E. IgG). Although clearance times ($T_{\frac{1}{2}}$) of E. IgG tended to be longer in RA patients than those in healthy controls ($46 \pm 6$ min vs $38 \pm 5$ min, mean $\pm$ s.e.m., $P = 0{\cdot}38$), this did not reach statistical significance. Liver spleen uptake ratios (LS ratios) were increased in patients with RA ($13/100 \pm 1/100$ vs $7/100 \pm 1/100$, $P < 0{\cdot}05$). There was no correlation of $T_{\frac{1}{2}}$ or LS ratios with articular disease activity, vasculitis, ESR, IgM containing immune complex levels or Clq-binding immune complex levels. Although Clq-binding immune complex levels were significantly higher in patients with vasculitis than in those without ($P < 0{\cdot}01$), $T_{\frac{1}{2}}$ and LS ratios did not differ in these two groups of patients. The $T_{\frac{1}{2}}$ and LS ratios of E.IgG did not reveal a defect in MPS Fc-receptor function and did not correlate with one of the above-mentioned clinical and immunological parameters. We suggest that in order to establish a possible defect in Fc-receptor function correlating with disease activity and immune complex levels in RA patients, soluble immune complexes or immune complex-like material should be used as probes.

**Keywords** Fc-receptor function rheumatoid arthritis mononuclear phagocyte system anti-rhesus IgG

## INTRODUCTION

Immune complexes (IC) are removed from the circulation by the cells of the mononuclear phagocyte system (MPS). Theoretically, Fc-receptor mediated phagocytic capacity might be overloaded by an excess of IC in the circulation. The presence of circulating IC may lead to their deposition in tissues, causing local inflammation (Haakenstad & Mannik, 1974). Using radiolabelled erythrocytes coated with anti-rhesus (D) IgG (E.IgG), MPS Fc-receptor function has been tested in various diseases such as SLE (Frank *et al.*, 1979; Hamburger *et al.*, 1982) mixed cryoglobulinaemia (Hamburger *et al.*, 1979b), RA (Fields *et al.*, 1983; Maliase *et al.*, 1985; O'Sullivan, Walker & Williams, 1985), and glomerulonephritis (Hamburger *et al.*, 1979a; Solomon *et al.*, 1984). If IC levels and MPS Fc-receptor function influence each other, and this can be detected with E. IgG, it would be expected that the $T_{\frac{1}{2}}$ of E. IgG would increase with increasing IC levels. This was found for SLE (Frank *et al.*, 1979; Hamburger *et al.*, 1982), but not for the other diseases

Correspondence: S. Lobatto MD, Department of Nephrology, Building 1, C3P, University Hospital Leiden, 2333 AA Leiden, The Netherlands.

studied. In RA, one study (Fields *et al.*, 1983) showed an increased $T_{\frac{1}{2}}$ of E.IgG, without a correlation with disease activity or CIC levels. In another study (Malaise *et al.*, 1985), $T_{\frac{1}{2}}$ of E. IgG was similar to controls. Using a gamma camera, liver spleen imaging showed that the splenic uptake of E.IgG was significantly lower in RA patients than in controls. The resulting increased liver spleen ratio correlated with Clq-binding IC levels. To examine a possible relationship between CIC levels, disease manifestations and MPS Fc-receptor function, we measured $T_{\frac{1}{2}}$ and liver spleen uptake ratios using E.IgG labelled with $^{99m}$Tc in 20 patients with RA.

## MATERIALS AND METHODS

*Selection of patients.* The study was performed after approval was received from the hospital ethical committee and the patient's informed consent was obtained. Twenty patients (11 female, nine male), age 37 to 74 years, were studied. They were selected from the in- and outpatient services of the Department of Rheumatology, on the basis of a diagnosis of definite or classical RA according to ARA criteria (Ropes *et al.*, 1958) and a Rh-positive blood type. The Ritchie index (RI) (Ritchie *et al.*, 1968) was registered by the same investigator (FCB) on the day of study; five patients had histologically proven vasculitis within 3 months of the study. All patients were receiving one or more medications, including nonsteroidal anti-inflammatory drugs, corticosteroids, D-penicillamine, gold, azathioprine or cyclophosphamide. Normal values for E.IgG clearance and liver/spleen uptake of E.IgG were determined in 10 healthy volunteers.

On the day of the study, blood samples were drawn and if necessary stored at $-70^{\circ}$C until the following studies could be performed: ESR, Rose Waaler agglutination and Latex fixation tests, ANA, CH50, determination of complement C3, C4, Clq by radial immunodiffusion and determination of circulating immune complexes by the Clq binding assay as described (Kauffmann *et al.*, 1979; 1980) and IgM containing immune complexes in the IgM-PEG assay (Baldwin *et al.*, 1982).

*Clearance studies.* MPS Fc-receptor function was tested as follows: anti-rhesus IgG containing plasma was obtained by plasmapheresis of a healthy volunteer who had previously been hyperimmunized. Plasma was shown to be free of HBsAg, bacterial contamination and pyrogens.

Clearance was studied as follows: the patient's erythrocytes were drawn into a heparinized syringe, the hematocrit was determined and two batches of the equivalent of 2 ml of erythrocytes were washed with isotonic saline. Both batches of red cells were sensitized with 1·5 ml of anti (Rh) IgG containing plasma. One batch of cells was set aside for determination of the degree of sensitization, the other batch of cells was radiolabelled with 400–500 $\mu$Ci $^{99m}$Tc using DTPA (labelling efficiency > 90%). The IgG- sensitized $^{99m}$Tc-labelled erythrocytes (E.IgG) were washed once with isotonic saline, and then resuspended in 12 ml of ice-cold isotonic saline.

Ten millilitres of this suspension (250–400 $\mu$Ci$^{99m}$Tc) were injected through an antecubital vein and erythrocyte survival was determined by timed serial bleeding over 2 h. Survival was calculated by determining the half-life of the cells.

*Determination of the degree of sensitization.* A 'standard' batch of erythrocytes was made by adding 30 ml of anti-rhesus IgG containing plasma to 40 ml of washed packed red cells from a volunteer. Aliquots of 1 ml E.IgG were stored at $-70^{\circ}$C after glycerolization. The degree of sensitization of these E.IgG was found to be 5,500 molecules of IgG per erythrocyte, as determined by Dr A. H. Merry (Merry *et al.*, 1982). The degree of sensitization of the autologous E.IgG injected was estimated by thawing and deglycerolization of a 'standard' E.IgG aliquot and measuring the binding of $^{125}$I-labelled rabbit anti-human gammaglobulin to serial dilutions of each set of cells. The number of molecules of IgG per autologous red cell was calculated from the ratio of binding of rabbit anti-human gammaglobulin to 'standard' cells as compared to autologous cells.

*Imaging and compartment analysis.* Splenic and hepatic uptake of $^{99m}$Tc was registered with the use of a Toshiba GCA 40 A gamma camera for 1 h at 1 s intervals. After 1 h an equilibrium was assumed to have been reached. Spleen size was determined by counting the number of pixels in the splenic region. Rate constants for reversible ($k_{12}$ and $k_{21}$) uptake, irreversible ($k_{13}$) spleen uptake and irreversible ($k_{14}$) liver uptake were determined, as described by van der Woude *et al.* (1986).

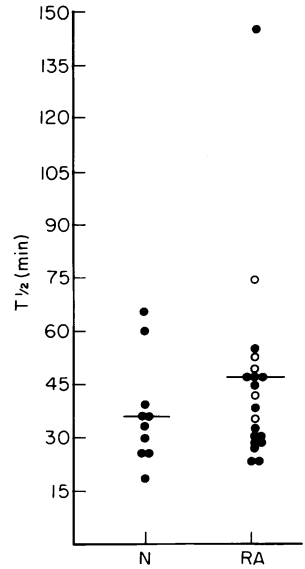


**Fig. 1.** The clearance of radiolabelled erythrocytes sensitized with anti-rhesus IgG ($T_{\frac{1}{2}}$) in healthy volunteers and in patients with RA. The difference in $T_{\frac{1}{2}}$ between the two groups is not statistically significant. (○) Patients with vasculitis; (●) patients without vasculitis.

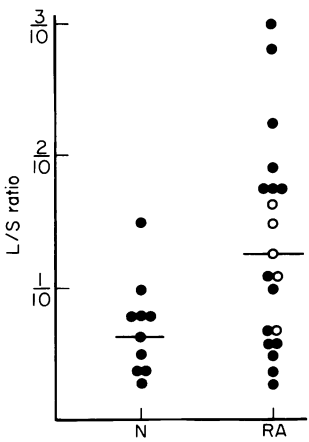


**Fig. 2.** Liver spleen uptake ratio (LS ratio) of radiolabelled erythrocytes sensitized with anti-rhesus IgG in healthy volunteers and in patients with RA. The difference in LS ratio was statistically significant ($P < 0{\cdot}05$, Student's *t*-test for unpaired samples). (○) Patients with vasculitis; (●) patients without vasculitis.

*Statistical analyses.* Student's *t*-test for unpaired samples and linear regression analysis were used.

## RESULTS

The mean degree of sensitization of E.IgG was $5{,}850 \pm 452$ molecules per erythrocyte (mean $\pm$s.e.m.) in RA patients, in the same range as healthy controls ($5{,}826 \pm 578$ molecules per erythrocyte). Figure 1 shows the $T_{\frac{1}{2}}$ of the RA patients compared to the $T_{\frac{1}{2}}$ of healthy volunteers. As a group, $T_{\frac{1}{2}}$ of RA patients was longer, but not significantly different from controls, $46 \pm 6$ min vs

**Table 1.** Individual patients' $T_{\frac{1}{2}}$, LS ratio, medication and IC levels

| Patient | $T_{\frac{1}{2}}$ | LS ratio (×/100) | Medication (mg/day) | Clq binding assay μg eq AIgG/ml | IgM PEG assay % binding |
|---|---|---|---|---|---|
| 1 | 31·7 | 16 | Nivaquine 100, indomethacin 175 | 0 | 10 |
| 2* | 47·9 | 13 | prednisone 10<br>ibuprofen 1600 | 621 | 63 |
| 3 | 28·5 | 10 | piroxicam 20 | 19 | 40 |
| 4* | 34·9 | 17 | acetylsalicylic acid 3600 | 208 | 54 |
| 5* | 42·5 | 11 | None | 367 | 42 |
| 6 | 55·3 | 11 | prednisone 7·5, indomethacin 150 | 196 | 70 |
| 7 | 47·2 | 6 | Auromyose 50 mg/month | 98 | 16 |
| 8 | 29·3 | 3 | prednisone 10, indomethacin 100<br>Auromyose 120 mg/month | 18 | 38 |
| 9 | 23·6 | 6 | prednisone 12·5<br>sodiumdiclofenac 150<br>azathioprine 50 | 0 | 40 |
| 10 | 39·6 | 28 | azathioprine 50, indomethacin 175 | 219 | 53 |
| 11 | 29·5 | 23 | Nivaquine 100, indomethacin 25 | 77 | 58 |
| 12 | 36·4 | 17 | D-penicillamine 750<br>ibuprofen 2000 | 36 | 48 |
| 13 | 47·4 | 4 | azathioprine 100<br>paracetamol 1000<br>sodiumdiclofenac 100 | 314 | 59 |
| 14 | 45·1 | 19 | D-pencillamine 250, naproxen 250 | 0 | 24 |
| 15 | 23·3 | 7 | prednisone 5, Nivaquine 200<br>acetylsalicylic acid 600 | 50 | 37 |
| 16* | 74·5 | 15 | prednisone 40<br>azathioprine 150 | 127 | 46 |
| 17 | 144·5 | 30 | ibuprofen 1500 | 30 | 33 |
| 18 | 32·3 | 7 | indomethacin 50 | 81 | 40 |
| 19* | 52·7 | 7 | prednisone 50<br>cyclophosphamide 150 | 166 | 62 |
| 20 | 48·2 | 5 | Auromyose 200 mg/month, sodium-diclofenac 150 | 38 | 40 |

* Patients with vasculitis.

38 ± 5 min, respectively (mean ± s.e.m.). The $T_{\frac{1}{2}}$ of E.IgG did not show a correlation with articular disease activity ($r=0{\cdot}25$, $P>0{\cdot}1$) CIC levels as measured by the Clq binding assay ($r=0{\cdot}23$, $P=0{\cdot}17$), the IgM-PEG assay ($r=0{\cdot}34$, $P=0{\cdot}08$) or the ESR ($r=0{\cdot}058$, $P>0{\cdot}1$). The $T_{\frac{1}{2}}$ in five patients with vasculitis did not differ significantly from that in 15 patients without vasculitis, although Clq binding IC levels were significantly higher in patients with vasculitis than in patients without vasculitis ($P<0{\cdot}01$).

There was a significant shift in liver spleen uptake ratios (L/S ratio) of E.IgG in RA patients when compared to controls (Fig. 2), 13/100 ± 1/100 vs 7/100 ± 1/100 (mean ± s.e.m., $P<0{\cdot}05$). In controls and RA patients a significant correlation between $T_{\frac{1}{2}}$ and L/S ratio was found ($r=0{\cdot}65$ and 0·46, respectively, $P<0{\cdot}02$). L/S ratios in RA patients were not correlated to the RI, IC levels in the Clq binding assay or IgM-PEG assay. Spleen size as determined by number of pixels was not correlated to $T_{\frac{1}{2}}$ or L/S ratio in healthy controls or in patients ($P>0{\cdot}1$). Medication had no apparent influence on $T_{\frac{1}{2}}$ or L/S ratios (Table 1). In Table 2, rate constants for organ uptake are shown for patients and volunteers. Although the L/S ratio differs significantly in patients from volunteers, an

**Table 2.** Rate constants for reversible ($k_{12}$ and $k_{21}$) uptake, irreversible ($k_{13}$) spleen uptake and irreversible ($k_{14}$) liver uptake

| | *n* | $k_{12} \cdot 10^{-4}$ ($min^{-1}$) Mean (±s.d.) | $k_{21} \cdot 10^{-4}$ ($min^{-1}$) Mean (±s.d.) | $k_{13} \cdot 10^{-4}$ ($min^{-1}$) Mean (±s.d.) | $k_{14} \cdot 10^{-4}$ ($min^{-1}$) Mean (±s.d.) |
|---|---|---|---|---|---|
| Volunteers | 10 | 349 (633) | 615 (465) | 271 (87) | 111 (122) |
| RA patients | 20 | 202 (140) | 499 (435) | 297 (178) | 54 (51) |

explanation for this cannot be found in significant differences in rate constants for either irreversible liver or spleen uptake of E.IgG.

## DISCUSSION

In this study, we found that although clearance times of E.IgG were generally prolonged in patients with RA, this prolongation was not significant when compared to controls. Theoretically an existing difference in $T_{\frac{1}{2}}$ of E.IgG between RA patients and volunteers may be obscured by the induction of Fc receptor expression on phagocytic cells after injection of E.IgG. However, evidence exists that this is not likely. Experiments *in vitro* with polymorphonuclear cells (PMN) from patients with RA and volunteers, have shown that the percentage of PMN showing rosette formation with rabbit IgG anti-ox erythrocytes as a measure of Fc receptor expression, is decreased in RA patients when compared to healthy controls. After activation, the relative increase in Fc receptor expression is less than in healthy controls (Breedveld *et al.*, 1984). Therefore, if induction of Fc receptor expression takes place, it may be expected that this would be more pronounced in HC than in patients with RA.

In analysing the results of L/S uptake ratios, the significant correlation between $T_{\frac{1}{2}}$ and L/S uptake ratios found in both volunteers and RA patients suggests that Fc receptors on Kupffer cells may be less efficient at removing E.IgG from the circulation than those on phagocytic cells of the spleen. Fc receptor function of the MPS was abnormal, as indicated by an increased L/S uptake ratio in the RA patients. This change in L/S uptake ratios, suggesting an increase in the relative importance of Kupffer cells of the liver, could not be explained by a change in spleen size in these patients, as spleen size in patients and controls was similar. Walport *et al.* (1985) reported a good correlation between splenic blood flow and spleen size. Because spleen size was comparable in healthy controls and patients, this suggests that the change in L/S uptake ratio is a result of a change in MPS Fc receptor function, and not a change in blood flow. The increased L/S ratio might be explained by activation of Kupffer cells when splenic phagocytic capacity decreases, but analysis of rate constants for organ uptake do not support this theory. Our findings confirm and extend some earlier findings by others, in which some patients were found to have increased $T_{\frac{1}{2}}$ (Fields *et al.*, 1983), and others were found to have an increase in L/S uptake ratios (Malaise *et al.*, 1985). A correlation of $T_{\frac{1}{2}}$ with disease activity or Clq-binding IC levels was not present in either study. In the latter study, however, a correlation between LS ratios and Clq-binding IC levels was encountered. We found no correlation between $T_{\frac{1}{2}}$ and articular disease activity or CIC levels, or between LS ratio and disease activity or CIC levels.

The results of our study provide additional evidence that measuring the clearance of E.IgG is not helpful in establishing a possible functional defect in MPS Fc-receptor function which relates to disease activity, circulating immune complex levels or vasculitis in rheumatoid arthritis. Because most patients in our study had substantial levels of CIC as measured with the Clq-binding assay, it appears that in RA these IC do not inhibit the Fc receptor function of the MPS as measured with E.IgG. Another possibility is that measuring the clearance of E.IgG does not reflect the capacity of

the MPS to remove CIC. This theory is supported by the finding that although patients with vasculitis have higher levels of CIC than patients without vasculitis, $T_{\frac{1}{2}}$ of E.IgG does not differ significantly in these two groups of patients (Table 1 and Fig. 1). We suggest that other probes need to be found in order to establish a defect in MPS-function in RA correlating with disease activity and IC levels.

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