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The splenic extraction ratio of antibody-coated erythrocytes and its response to plasma exchange and pulse methylprednisolone

M. J. WALPORT,* A. M. PETERS,† K. B. ELKON,* C. D. PUSEY,* J. P. LAVENDER† & G. R. V. HUGHES* *Departments of *Medicine and †Diagnostic Radiology, Hammersmith Hospital, London, UK*

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SUMMARY

Splenic blood flow was measured in a series of normal subjects and patients with connective tissue diseases by measuring the rate of equilibration and the partition of ^{111}In-labelled autologous platelets between blood and spleen. These data were used to quantify the role of splenic blood flow in determining the splenic clearance of IgG coated erythrocytes (IgG-RBC) from the circulation. Previous studies have interpreted the clearance rates of IgG-RBC only in terms of splenic reticuloendothelial function. Splenic blood flow was increased in seven of eight patients with systemic lupus erythematosus (SLE), six of 10 patients with rheumatoid arthritis (RA) and in all five patients with essential mixed cryoglobulinaemia (EMC) compared with a series of thirteen normal subjects. Expressing the rate constant of clearance of IgG-RBC as a fraction of splenic blood flow gave a value for the 'extraction ratio' of IgG-RBC (a specific measurement of reticuloendothelial function, corrected for splenic blood flow). Normal splenic extraction ratio of IgG-RBC was calculated to be 32%. All the patients with SLE and with EMC had reduced extraction ratios (in seven out of 13 patients less than 10%). In RA the extraction ratio tended to be normal (average 27·3%) but variable (9–59%). Following plasma exchange in nine patients, a significant increase in IgG-RBC extraction ratio (average of 39% with respect to pre-exchange values, $P < 0{\cdot}05$) was found. In contrast there was no significant change in extraction ratio following pulse methylprednisolone therapy in a further nine patients. Although the rate constant of clearance of IgG-RBC decreased by an average of 33% ($P < 0{\cdot}01$) in the latter group, it was matched by an equal decrease of splenic blood flow (average 37%, $P < 0{\cdot}01$) and so extraction ratio showed no change. These data indicate that quantification of splenic reticuloendothelial function requires measurement of both IgG-RBC clearance and of splenic blood flow.

Keywords splenic extraction ratio plasma exchange methylprednisolone erythrocytes

INTRODUCTION

Study of the factors determining the fate of circulating immune complexes (IC) may be important in understanding the pathological mechanisms of immune complex disease. Following their injection into mice, pre-formed soluble IC are predominantly cleared from the circulation by the reticuloendothelial system (RES) of the liver (Benacerraf, Sebestyen & Cooper, 1959). With high

Correspondence: Dr M. J. Walport, MITI Unit, Medical Research Council Centre, Hills Road, Cambridge CB2 2QH, UK.

concentrations, the RES becomes saturated and IC are then deposited in many organs (Haakenstaad & Mannik, 1974).

It is an attractive hypothesis that in humans with immune complex disease, the RES, like in the experimental animal, may become saturated, but this has been difficult to directly test. Because pre-formed soluble IC are toxic, a model system initially designed to investigate mechansims of haemolytic anaemia has been used to quantify RES function in humans (Frank *et al.*, 1977)

In this system, the clearance rate by the spleen of erythrocytes coated with an IgG anti-Rh(D) antibody (IgG-RBC) is determined. This rate has been interpreted as reflecting splenic macrophage Fc receptor function (Frank *et al.*, 1979). Studies using this system have shown a decrease in the rate of clearance of IgG-RBC in patients with immune complex disease (Frank *et al.*, 1979; Lockwood *et al.*, 1979; Hamburger *et al.*, 1982; Parris *et al.*, 1982). In some studies (Frank *et al.* 1979; Hamburger *et al.*, 1982) (but not all, Parris *et al.* (1982)) this decrease has correlated with levels of circulating IC. Patients with systemic lupus erythematosus (SLE) with renal involvement are reported to have greater impairment of IgG-RBC clearance than those without renal disease (Parris *et al.*, 1982). After plasma exchange therapy the clearance rate of IgG-RBC increases very significantly, accompanied by a decrease in circulating IC, and this has been interpreted as reflecting 'de-saturation' of the RES (Lockwood *et al.*, 1979).

However, not all observations made using this system can be easily interpreted solely on the basis of macrophage Fc receptor function. Thus rapid IgG-RBC clearance has been reported in primary biliary cirrhosis (Jaffe *et al.*, 1978). Systemic vasculitis (Dambuyant *et al.*, 1982) and essential mixed cryoglobulinaemia (EMC) without renal involvement (Hamburger *et al.*, 1979). However, these conditions are associated with splenomegaly, which has been shown to be accompanied by increased splenic blood flow (SBF) (Blendis *et al.*, 1970; Peters & Lavender, 1982, 1983), and IgG-RBC clearance obviously depends on both SBF and splenic macrophage function.

We have developed a non-invasive method of measuring SBF that is based on the equilibration of ^{111}In-labelled autologous platelets between circulating and splenic platelet pools following bolus intravenous injection (Peters & Lavender, 1982; Peters *et al.*, 1980). In this paper we have measured SBF in order to quantify its role in the clearance rate of IgG-RBC, and, as a result, have been able to express RES function as the splenic extraction efficiency for IgG-RBC.

MATERIALS AND METHODS

Subjects. Thirty-four Rhesus positive hospital in-patients (28 women and seven men, aged 18–70 years) under the care of the Rheumatology and Renal services, were studied. All gave informed consent to the investigations. Diagnoses were as follows: 10 with rheumatoid arthritis (RA) all of whom had definite or classical RA by American Rheumatism Association (1959) criteria; eight with SLE, all of whom fulfilled at least four preliminary classification criteria of the American Rheumatism Association (Cohen *et al.*, 1971), and five of whom had nephritis documented by renal biopsy. Five patients had essential mixed cryoglobulinaemia (EMC) and in each case a monoclonal IgM-k rheumatoid factor was demonstrated. The diagnoses of the other eleven patients were primary Sjögren's disease (two), adult Still's disease (one), polymyositis (one), Goodpasture's disease (one), cold agglutinin disease with seronegative polyarteritis (one), giant cell arteritis (one), polyarteritis nodosa (one), unclassified connective tissue disease (two), and diabetes mellitus (one).

Eighteen of these patients were studied twice; nine before and after pulse methylprednisolone therapy and nine before and after plasma exchange (see below).

In addition, 18 normal volunteers were studied. Of these, 13 (24–60 years) underwent ^{111}In-platelet kinetic studies including measurement of SBF, and the remaining five (28–34 years) measurement of IgG-RBC clearance.

Pulse methylprednisolone and plasma exchange. Six patients with rheumatoid synovitis, uncontrolled by conventional therapy, two with severe extra-renal manifestations of SLE and one with giant cell arteritis were treated with pulse methylprednisolone (MP). This was administered as three doses of 1 g MP on successive days. Each dose was given i.v. over 30 min. ^{111}In-platelet and IgG-RBC studies were performed during the 24 h before and after MP.

Four patients with EMC, two with rheumatoid vasculitis, one with SLE nephritis, one with Goodpasture's disease and one with cold agglutinin disease and seronegative arthritis were treated with plasma exchange. Four litre plasma exchanges were carried out on a Haemonetics Model 30 cell separator as previously described (Lockwood *et al.*, 1976). Kinetic studies were performed during the 24 h before and after 3 consecutive days of plasma exchange.

Cell labelling. Autologous platelets were labelled with ^{111}In or ^{113m}In using acetyl acetone as previously described (Peters & Lavender, 1982; Sinn & Silvester, 1979). Since both these indium isotopes are dispensed in 0·04 M HCl, they are interchangeable when labelling with acetylacetone. Autologous erythrocytes were labelled with ^{99m}technetium (^{99m}Tc) either entirely *in vitro* (Jones & Mollison, 1978) or partially *in vitro* (Armas, Thakur & Gottschalk, 1980).

Erythrocyte IgG coating. IgG anti-Rhesus (D) from a single donor (Avg) was kindly provided by Professor P. L. Mollison. Coating of erythrocytes was performed as previously described (Elkon *et al.*, 1982). The level of coating was chosen to give approximately 5,000 molecules of IgG on each red cell.

Imaging. The labelled platelets were given by bolus IV injection with the patient supine and positioned above a gamma camera (IGE, maxi-camera 400T), fitted with a medium-energy, parallel-hole collimator for ^{111}In imaging or a high-energy, parallel-hole collimator for ^{113m}In imaging, and on line to a computer (MDS A^2). After acquisition of background counts for 4 min, activity in the chest and upper abdomen was recorded dynamically for a further 30 min. Venous blood samples of 5 ml were obtained from the arm opposite the injection site before, 3 min after and 30 min after platelet re-injection.

Following dynamic imaging, regions of interest were taken over the spleen, liver and cardiac blood pool, and time activity curves, based on an acquisition frame time of 1 min, were constructed. In the case of ^{113m}In which has a half-life of only 100 min, the recorded counts for each frame were corrected for isotope decay with respect to injection time.

An estimate of spleen size was made by drawing a region around the spleen on the computer VDU with a light pen and expressing the number of computer pixel points enclosed. No corrections were attempted regarding patient sex or age.

IgG coated erythrocyte clearance. On completion of SBF measurement, the IgG coated erythrocytes were injected as a bolus, after which 5 ml blood samples were taken into EDTA at 10-min intervals for 60 min.

Injected doses of isotope. ^{99m}Tc was given in a dose of 200–400 μCi, ^{111}In, 50–100 μCi, and ^{113m}In, 200–400 μCi. No subject received a total isotope dose that was the equivalent in terms of radiation dose of more than 250 μCi ^{111}In.

Blood sample analysis. Aliquots of 1 ml of whole blood were counted for ^{111}In ^{113m}In or ^{99m}Tc in a Packard gamma counter (Model 5360). Corresponding aliquots of 1 ml of cell-free plasma were also counted in order to correct for non-cell-bound activity. Indium standards were counted in order to calculate the 'cross over' of activity into the ^{99m}Tc 'window' from the previously injected ^{111}In or ^{113}In. The latter isotope had largely decayed by the time ^{99m}Tc counting was performed. ^{111}In 'cross over' into ^{99m}Tc was also minimal because relatively much lower doses were used.

Data analysis. Splenic blood flow was calculated as previously described (Peters *et al.*, 1980; Peters & Lavender, 1982). Briefly, this is based on measuring the rate of equilibration of labelled platelets between circulating blood and the splenic pool and on the ratio of platelets present in these compartments at equilibrium. The rate of constant uptake of activity in the spleen (K_s) was calculated using a computerized maximum likelihood estimate. The fraction, S, of the total circulating activity present in the spleen of equilibrium (the splenic pool) was calculated by comparing the count rate over the cardiac blood pool at equilibrium with the initial count rate, estimated on the assumption that, with respect to their equilibrium values, the splenic and cardiac blood-pool time activity curves are mirror images of each other. Correction for liver uptake and plasma indium activity has been described elsewhere (Peters & Lavender, 1982). SBF, in units of percent total (extrasplenic) blood volume per min, is then equal to the product K_sS, and the rate constant of clearance of platelets from the spleen (the reciprocal of mean intrasplenic platelet transit time) to $K_s - K_sS$.

IgG coated erythrocyte clearance was clearly monoexponential. Its time constant was calculated

from the visually fitted clearance redrawn on semilogarithmic paper. We have previously confirmed that our IgG coated RBC are removed from the circulation exclusively by the spleen (Elkon *et al.*, 1982; Peters *et al.*, 1984a). Since SBF based on platelets and IgG-RBC clearance are both expressed as the input into the spleen of a fraction of total blood volume they have the same units (per min), and their ratio is equal to the splenic IgG-RBC extraction ratio (i.e. the arterio-venous concentration difference divided by the arterial concentration).

Statistics. Non-parametric tests were applied to the data. Comparisons between groups of patients were made by Kruskall–Wallis one-way analysis of variance by ranks, in which the null hypothesis is that all the groups come from a single population (Kruskall & Wallis, 1952). Comparison of paired data from individual subjects was made by Wilcoxon signed rank test. Correlation analysis was by the Spearman rank correlation coefficient.

RESULTS

Rate constant (K_A) of IgG-RBC clearance

The mean value of K_A in five normal subjects was 0·014/min (corresponding to a $T_{\frac{1}{2}}$ of 40 min) with a range of 0·01–0·018/min (corresponding to 39–69 min). Values of K_A in subjects with SLE, RA and EMC are compared in Fig. 1a. In six of eight patients with SLE, K_A was below the range in normals. In five of 10 patients with RA and two of five with EMC, K_A was above the range in normals. Kruskall–Wallis one-way analysis of variance by ranks gave an H statistic of 10·46 ($P < 0·01$) suggesting that on the basis of K_A, the members of the four samples (normal, RA, SLE, EMC) were

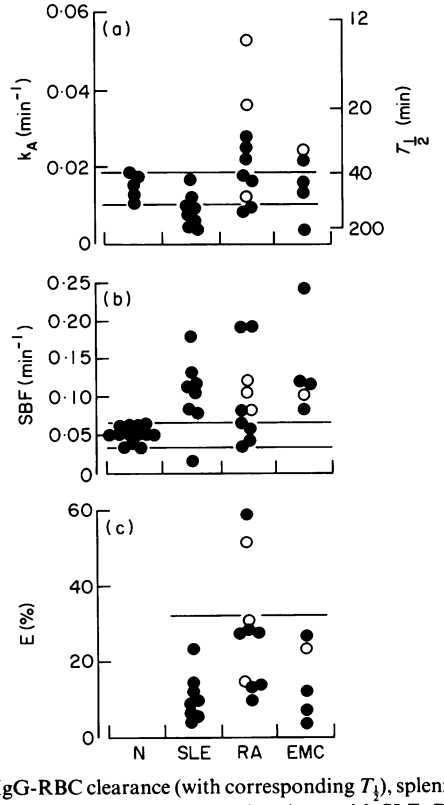

Fig. 1. Rate constant (K_A) of IgG-RBC clearance (with corresponding $T_{\frac{1}{2}}$), splenic blood flow (SBF) and splenic IgG-RBC extraction ratio (E) in normal subjects (N) and patients with SLE, RA and EMC. Horizontal lines indicate the normal range (a, b) and normal average (c). ○: patients with splenomegaly; ●: subjects without splenomegaly.

not all from a single population. A Spearman rank correlation coefficient of 0·59 ($P < 0·001$) suggested an association between K_A and the two dimensional scintigraphic estimate of spleen size.

Splenic blood flow (SBF)

Mean SBF in 13 normal subjects was 0·049/min; i.e. 4·9% total blood volume per min (range, 0·033–0·066). Seven of eight patients with SLE, six of 10 with RA and all five with EMC had SBF above the range in normals (Fig. 1b). One patient with SLE had a value of SBF lower than the range in normals. Kruskall–Wallis analysis gave an H statistic of 12·17 ($P < 0·01$). Like K_A (the IgG-RBC clearance rate constant), SBF correlated significantly with spleen size ($r_s = 0·47$, $P < 0·01$), although in a number of patients, SBF was markedly elevated without an accompanying increase in spleen size.

Splenic IgG-RBC extraction ratio (K_A/SBF)

Values of extraction ratio in SLE, RA and EMC are shown in Fig. 1(c), which also shows an estimate (32%) of the mean normal IgG-RBC extraction ratio based on the means of SBF and K_A in the normal subjects. Since D_A and SBF were not simultaneously measured in any normal subjects, it was not possible to express a normal range. IgG-RBC extraction ratio was similar in patients with

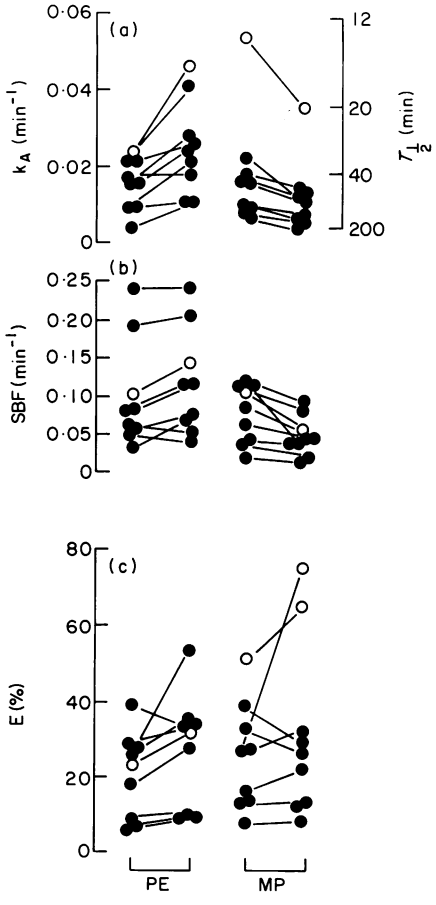

Fig. 2. Changes in K_A, SBF and splenic IgG-RBC extraction ratio (E) in response to plasma exchange (PE) and pulse methylprednisolone (MP). ○: splenomegaly. Significance levels for changes after PE: K_A (<0·01), SBF (<0·05), E (<0·05); for changes after MP: K_A (<0·01), SBF (<0·01), E (>0·05).

SLE (mean 10%, range 4·2–23) and EMC (mean 14·3%, range 3·3–26). In contrast, mean extraction ratio in RA was 27·3% (range 9–59). Kruskall–Wallis analysis gave an H statistic of 8·3 ($P < 0·02$). There was no significant correlation between extraction ratio and spleen size.

Relationship between the IgG-RBC clearance rate constant (K_A) and parameters of platelet kinetics

K_A correlated significantly ($r_s = 0·41$, $P < 0·02$) with the splenic platelet pool capacity (expressed as a fraction of the total circulating platelet population).

A relationship was also found between extraction ratio of IgG-RBC and the rate constant of clearance of platelets from the spleen (i.e. the reciprocal of mean intrasplenic platelet transit time, see 'data analysis') with a Spearman rank correlation coefficient of 0·51 ($P < 0·01$).

Response to plasma exchange and pulse methylprednisolone therapy (Fig. 2)

The IgG-RBC clearance rate constant (K_A) increased in all nine patients after plasma exchange by an average of 0·0094/min (69% with respect to the initial value, range 6–157%; $P < 0·01$). SBF increased in six patients, fell slightly in two and did not change in one; overall there was a significant increase of 0·017/min (25% with respect to the initial value, range −23–94%; $P < 0·05$). This increase was not as great as the increase in K_A and so extraction ratio increased significantly by an average of 6·7% (39% with respect to the initial value, range −13–93%; $P < 0·05$).

In contrast, K_A decreased following pulse methylprednisolone in all patients by an average of 0·0058/min (−33% with respect to the initial value, range −45–−20%; $P < 0·01$). This fall could be accounted for by the fall in SBF that was observed (−0·027/min; −37% with respect to the initial value, range −76–7·5%; $P < 0·01$) and so extraction ratio was not found to change significantly.

DISCUSSION

In this study, data are presented which, for the first time, relate splenic blood flow to the clearance rate of IgG-RBC in patients with a wide variety of connective tissue diseases. The method of splenic bood flow measurement, which is based on measurement of the equilibration of radiolabelled platelets between the splenic platelet pool and circulating blood, has been validated in two recent studies. In anaesthetized dogs, SBF, calculated using indium-labelled platelets as in the current study, correlated with SBF recorded by electromagnetic flowmetry, although blood flow was underestimated by about 50% (Peters *et al.*, 1983). This discrepancy was accounted for by evidence which suggested that the platelet technique specifically measures splenic pulp flow. In the second study, SBF, measured from compartmental analysis of platelets between the spleen and blood agreed with and correlated closely with SBF measured from analysis of the first-pass ^{111}In-platelet time–activity curve recorded over the spleen (Peters *et al.*, 1984b).

Splenic blood flow was found to be increased in the majority of patients with SLE, RA and EMC, although only four patients appeared to have splenomegaly. There have been few previous studies of SBF in humans. Using intra-arterial ^{133}xenon, Williams *et al.* (1968) reported increased SBF in patients with hepatic cirrhosis and with haemolytic anaemia, but found no correlation between SBF and spleen weight. The same group subsequently described an increase in SBF in 20 patients with splenomegaly due to blood dyscrasia or diseases involving the reticulo-endothelial system, including two subjects with Felty's syndrome (Blendis *et al.*, 1970).

We have shown previously a correlation between SBF, measured by our method, and splenic size determined scintigraphically (Peters & Lavender, 1982). In this study we again found this relationship. However, the correlation was much weaker due to the presence in this series of patients with markedly elevated splenic blood flow without splenomegaly. This finding suggests that elevated blood flow may precede splenomegaly and may be one of its causes in patients with connective tissue diseases.

The reduction in the rate of clearance of IgG-RBC in six of eight patients with SLE and the increase in this rate in two of five patients with EMC confirms the findings of previous workers (Hamburger *et al.*, 1979). Our finding of an increased IgG-RBC clearance rate constant in five of ten patients with RA has not been previously described. Hamburger *et al.* (1980) found normal or only

slightly reduced IgG-RBC clearance in 50 patients with RA. Others (Williams, Lockwood & Pussil, 1979; Henderson *et al.*, 1981; Gordon *et al.*, 1981) have described impaired splenic reticuloendothelial function in RA based on reduced clearance of heat damaged erythrocytes. However, we previously found that there was a poor correlation between the clearance rates of simultaneously injected heat damaged erythrocytes and IgG-RBC and a good correlation between the rate of clearance of heat damaged erythroctyes and splenic blood flow (Peters *et al.*, 1984a). We concluded from that study that SBF was the principal determinant of the rate of clearance of heat damaged erythrocytes. As part of that comparative study two patients with RA were studied and both were found to have increased rates of clearance of IgG-RBC and heat damaged erythrocytes.

In the present study we found a highly significant correlation between the IgG-RBC clearance rate constant (K_A) and spleen size in spite of the semi-quantitative approach to the determination of the latter and in spite of the variable patient blood volumes for which we did not make a correction. Although this relationship is not surprising, it nevertheless underlines the insensitivity of a single marker (IgG-RBC) as an indicator purely of Fc receptor function. Significant relationships were also found between K_A and spleen platelet pool capacity, between the latter and spleen pool size (which is well known) and between splenic blood flow and spleen size. These observations may explain the findings of Jaffe *et al.* (1978) who found an increased clearance rate of IgG-RBC in patients with primary biliary cirrhosis and with chronic hepatitis, conditions which have been shown to be associated with increased splenic blood flow (Williams *et al.*, 1968).

When K_A was divided by SBF to give the splenic IgG-RBC extraction ratio, interesting differences emerged between the patients with SLE or EMC and those with RA. Extraction ratio was lower in SLE and EMC than in RA (and the estimated normal value of 32%) suggesting suppression of Fc receptor function in SLE and EMC. A possible alternative explanation for reduced extraction ratio, unrelated to Fc receptor function, is elevated splenic perfusion (i.e. SBF per unit volume) which might result in reduced time available for Fc receptor–IgG-RBC interaction. Thus this may explain the relationship observed between splenic IgG-RBC extraction ratio and the intrasplenic platelet transit time, a parameter which we have previously shown to be inversely related to splenic perfusion (Peters & Lavender, 1983). It would appear that the splenic compartment in which platelets pool is the same as that in which the IgG-RBC are cleared.

The changes that occured in SBF and the IgG-RBC clearance rate constant (K_A) after plasma exchange and pulse methylprednisolone provide further insights into the relationship between these parameters. After plasma exchange both SBF and K_A increased. However, the increase in SBF was not great enough to account for the increase in K_A, suggesting that Fc receptor function was more efficient after plasma exchange, i.e. the extraction ratio increased and did so in spite of the increase in SBF, which, as discussed above, might have tended to reduce it. This supports the conclusions of Lockwood *et al.* (1979), who also observed an increase in the rate of IgG-RBC clearance after plasma exchange.

Opposite changes occurred after pulse methylprednisolone. The fall in SBF was sufficient to account for the fall in K_A and as a result extraction ratio remained unchanged. The haemodynamic effects of methylprednisoone have recently been studied in six male renal allograft recipients (Warren & Smith, 1983). After an infusion of 30 mg/kg of methylprednisolone, cardiac index fell by 15%, and peripheral resistance increased by 37%. Similar haemodynamic effects were not, however, observed in normal subjects (Warren & Smith, 1983).

Corticosteroids have previously been shown to depress the rate of clearance of IgG-RBC in guinea pigs (Atkinson, Schreiber & Frank, 1973) and of artificial immune complexes in mice (Haakenstaad, Case & Mannik, 1975). *In vitro* studies have demonstrated depression of a number of monocyte functions by steroid therapy (Rinehart *et al.*, 1974). Fries, Brickman & Frank (1983) have shown a reduction in the number of Fc receptors on peripheral blood monocytes after glucocorticoid therapy although fixed macrophages may not show the same phenomenon. However, our data suggest that after high dose methylprednisolone, the predominant acute effect is haemodynamic and that changes in the rate of clearance of IgG-RBC may be secondary.

In conclusion, the data presented here suggest that splenic blood flow may be an important influence on the rate of clearance of IgG-RBC. Measurements of the extraction ratio of IgG-RBC do, however, suggest that the clearance of IgG-RBC is impaired in patients with SLE or EMC, but

not in those with RA. Plasma exchange was found to improve this defect. Further studies will be needed to distinguish whether this defect is due to abnormal splenic perfusion or to an underlying abnormality of tissue macrophages.

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REFERENCES

AMERICAN RHEUMATISM ASSOCIATION COMMITTEE (1959) Diagnostic criteria for rheumatoid arthritis: 1958 revision. M. W. Ropes (chairman). *Ann. Rheum. Dis.* **18,** 49.

ARMAS, R.R., THAKUR, M.L. & GOTTSCHALK, A. (1980) A simplified method of selective spleen scintigraphy with Tc-99m labelled erythrocytes – clinical applications. *J. Nucl. Med.* **21,** 413.

ATKINSON, J.P., SCHREIBER, A.D. & FRANK, M.M. (1973) Effects of corticosteroids and splenectomy on the immune clearance and destruction of erythrocytes. *J. clin. Invest.* **52,** 1509.

BENACERRAF, B., SEBESTYEN, M. & COOPER, N.S. (1959) The clearance of antigen-antibody complexes from the blood by the reticuloendothelial system. *J. exp. Med.* **82,** 131.

BLENDIS, L.M., BANKS, D.C., RAMBOER, C. & WILLIAMS, R. (1970) Spleen blood flow and splanchnic haemodynamics in blood dyscrasia and other splenomegalies. *Clin. Sci.* **38,** 73.

COHEN, A.B., REYNOLDS, W.E., FRANKLIN, E.C., KULKA, J.P., ROPES, M.W., SHULMAN, L.E. & WALLACE, S.L. (1971) Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21,** 643.

DAMBUYANT, C., THIVOLET, J., VIALA, J.-J., VILLE, D. & BOYER, J. (1982) Clearance mediated by splenic macrophage receptors for immune complexes in cutaneous vasculitis. *J. Invest. Dermatol.* **78,** 194.

ELKON, K.B., FERJENCIK, P.P., WALPORT, M.J., PETERS, A.M., LEWIS, S.M. & HUGHES G.R.V. (1982) Evaluation of methods for testing reticuloendothelial function in man. *J. Immunol. Meth.* **55,** 253.

FRANK, M.M., SCHREIBER, A.D., ATKINSON, J.P. & JAFFE, C.J. (1977) Pathophysiology of immune hemolytic anaemia. *Ann. Intern. Med.* **87,** 210.

FRANK, M.M., HAMBURGER, M.I., LAWLEY, T.J., KIMBERLEY, R.P. & PLOTZ, P.H. (1979) Defective reticuloendothelial system Fc receptor function in systemic lupus erythematosus. *New Engl. J. Med.* **300,** 518.

FRIES, L.F., BRICKMAN, C.M. & FRANK, M.M. (1983) Monocyte receptors for the Fc portion of IgE increase in number in autoimmune hemolytic anaemia and other hemolytic states and are decreased by glucocorticoid therapy. *J. Immunol.* **131,** 1240.

GORDON, P.A., DAVID, P., RUSSELL, A.S., COATES, J.E., ROTHWELL, R.S. & LE CLERCQ, S.M. (1981) Splenic reticuloendothelial function in patients with active rheumatoid arthritis. *J. Rheumatol.* **8,** 490.

HAAKENSTAAD, A.O. & MANNIK, M. (1974) Saturation of the reticuloendothelial system with soluble immune complexes. *J. Immunol.* **112,** 1939.

HAAKENSTAAD, A.O., CASE, J.B. & MANNIK, M. (1975) Tissue localisation of soluble immune complexes. *J. Immunol.* **114,** 1153.

HAMBURGER, M.I., GOREVIC, P.D., LAWLEY, T.J., FRANKLIN, E.C. & FRANK, M.M. (1979) Mixed cryoglobulinaemia: association of glomerulonephritis with defective reticuloendothelial system Fc receptor function. *Trans. Am. Assoc. Phys.* **92,** 104.

HAMBURGER, M.I., FIELDS, T.R., BENNETT, R.S., LAWLEY, T.J., HALL, R.P., KARSH, J.R., KLIPPEL, J., PLOTZ, P.H. & FRANK, M.M. (1980) Reticuloendothelial system Fc receptor function in rheumatoid arthritis (abstract). *Clin. Res.* **29,** 158A.

HAMBURGER, M.I., LAWLEY, T.J., KIMBERLEY, R.P. & PLOTZ, P.H. (1982) A serial study of splenic reticuloendothelial system FC receptor functional activity in systemic lupus erythematosus. *Arthr. Rheum.* **25,** 48.

HENDERSON, J.M., BELL, D.A., HARTA, M. & CHAMBERLAIN, M.J. (1981) Reticuloendothelial function in rheumatoid arthritis: correlation with disease activity and circulating immune complexes. *J. Rheumatol.* **8,** 486.

JAFFE, C.J., VIERLING, J.M., JONES, E.A., LAWLEY, T.J. & FRANK, M.M. (1978) Receptor specific clearance by the reticuloendothelial system in chronic liver disease: demonstration of defective C^{3b}-specific clearance in primary biliary cirrhosis. *J. clin. Invest.* **62,** 1069.

JONES, J. & MOLLISON, P.L. (1978) A simple and efficient method of labelling red cells with ^{99m}Tc for determination of red cell volume. *Br. J. Haematol.* **38,** 141.

KRUSKALL, W.M. & WALLIS, W.A. (1952) Use of ranks in one-criterion variance analysis. *J. Am. Statist. Assoc.* **23,** 525.

LOCKWOOD, C.M., REES, A.J., PEARSON, T.A., EVANS, D.J. & PETERS, D.K. (1976) Immunosuppression and plasma exchange in the treatment of Goodpasture's Syndrome. *Lancet*, **i,** 711.

LOCKWOOD, C.M., WORLLEDGE, S., NICHOLAS, A., COTTON, C. & PETERS, D.K. (1979) Reversal of impaired splenic function in patients with nephritis

or vasculitis (or both) by plasma exchange. *New Engl. J. Med.* **300,** 524.

PARRIS, T.M., KIMBERLEY, R.P., INMAN, R.D., MCDOUGAL, J.S., GIBOVSKY, A. & CHRISTIAN, C. (1982) Defective Fc receptor-mediated function of the mononuclear phagocyte system in lupus nephritis. *Ann. Intern. Med.* **97,** 526.

PETERS, A.M. & LAVENDER, J.P. (1982) Factors controlling the intrasplenic transit of platelets. *Eur. J. Clin. Invest.* **12,** 191.

PETERS, A.M. & LAVENDER, J.P. (1983) Platelet kinetics with indium-111 platelets: comparison with chromium-51 platelets. *Sem. Thromb. Hemost.* **9,** 100.

PETERS, A.M., KLONIZAKIS, I., LAVENDER, J.P. & LEWIS, S.M. (1980) Use of ^{111}In-labelled platelets to measure spleen function. *Br. J. Haematol.* **46,** 587.

PETERS, A.M., MATHIE, R.T., WALPORT, M.J., BELL, R.N., REAVY, H.J. & LAVENDER, J.P. (1983) Measurement of splenic blood flow in the anaesthetised dog with electromagnetic flowmetry and indium labelled platelets. *Cardiovasc. Res.* **17,** 710.

PETERS, A.M., WALPORT, M.J., ELKON, K.B., REAVY, H.J., FERJENCIK, P.P., LAVENDER, J.P. & HUGHES, G.R.V. (1984a) Comparative blood clearance kinetics of heat damaged and antibody coated erythrocytes. *Clin. Sci.* **66,** 55.

PETERS, A.M., WALPORT, M.J., BELL, R.N. & LAVENDER, J.P. (1984b) Measurement of splenic blood flow and intrasplenic platelet transit time in man using ^{111}In labelled platelets. *J. Nucl. Med.* **25,** 86.

RINEHART, J.J., BALCERZAK, S.P., SAGONE, A.L. & LOBUGLIO, A.F. (1984) Effects of corticosteroids on human monocyte function. *J. clin. Invest.* **54,** 1337.

SINN, H. & SILVESTER, D.J. (1979) Simplified cell labelling with indium-111 acetylacetone. *Br. J. Radiol.* **52,** 758.

WARREN, D.J. & SMITH, R.S. (1983) High dose prednisolone. *Lancet*, **i,** 594.

WILLIAMS, B.D., LOCKWOOD, C.M. & PUSSEL, B.A. (1979) Inhibition of reticuloendothelial function by gold and its relation to post-injection reactions. *Br. Med. J.* **1,** 235.

WILLIAMS, R., CONDON, R.E., WILLIAMS, H.S., BLENDIS, L.M. & KREEL, L. (1968) Splenic blood flow in cirrhosis and portal hypertension. *Clin. Sci.* **34,** 441.