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Defective splenic reticuloendothelial function in idiopathic membranous nephropathy

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SUMMARY

We studied splenic macrophage reticuloendothelial function in 18 patients with idiopathic membranous nephropathy and eight patients with mesangio-capillary glomerulonephritis by measuring the clearance from the circulation of technetium labelled, autologous, antibody coated erythrocytes (IgG cells) or heat damaged erythrocytes (HDE). Nine of 15 rhesus-D positive patients with membranous nephropathy had delayed clearance of the IgG cells and the defect did not correlate with disease activity. Circulating immune complexes were not detected in any of the patients with membranous nephrophathy. The defect in clearance of the IgG cells in the membranous patients was associated with the presence of HLA-B8 and DR3. In the mesangio-capillary glomerulonephritis patients, the clearance rates of the IgG cells were fast-normal in six and enhanced in two, and correlated with the clearance of HDE. The clearance rates of the cells in this group correlated with the degree of hypocomplementaemia but not with the disease activity nor HLA haplotype.

Keywords reticuloendothelial function glomerulonephritis HLA B8-DR3

INTRODUCTION

The glomerular localization of immune complexes (IC) is thought to be of primary importance in the pathogenesis of some human glomerulopathies (Wilson & Dixon 1974). IC are normally cleared from the circulation by the reticuloendothelial system (RES) and defects of RES function could pre-dispose to the continued circulation and enhanced tissue deposition of these complexes (Mannik, 1979). Using recently developed techniques of study (Frank *et al.*, 1977), defects in splenic RES function have been described in various forms of IC disease (Frank *et al.*, 1979; Hamburger *et al.*, 1979; Lawley *et al.*, 1981) including acute glomerulonephritis (Lockwood *et al.*, 1979). We used these techniques to determine whether there was any defect in splenic RES function in patients with chronic glomerulopathies.

The first technique measures the clearance from the circulation of technetium (Tc^{99m})-labelled autologous erythrocytes coated with Rhesus anti-D IgG (IgG cells). These cells are cleared by their interaction with Fc receptors of splenic macrophages (Lobuglio, Cotran & Jandl, 1967), which are also important in the clearance of circulating IC (Mantovani, Rabinovitch & Nussenzweig, 1972).

The second technique, which measures the blood clearance of Tc^{99m}-labelled autologous heat damaged erythrocytes (HDE) is not immune specific but it is also a test of splenic macrophage function (Crome & Mollison, 1964).

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We studied 18 patients with idiopathic membranous nephropathy (IMN) and eight patients with mesangio-capillary glomerulonephritis (MCGN). IMN is strongly associated with the HLA B8-DR3 haplotype (Klouda *et al.*, 1979), and this haplotype has also been associated with defective RES Fc receptor specific function (Lawley *et al.*, 1981). We extended our study to determine whether there was any association between Fc receptor function and HLA antigens in the patients studied.

MATERIALS AND METHODS

Patients. Eighteen patients (four females and 14 males) with IMN were studied. All had biopsy proven membranous nephropathy with no underlying disease. The mean age of the patients was 49 years (range 23–73 years) with duration of disease ranging from 2 months to 21 years (mean 4 years). Renal function varied, seven patients having progressive renal failure, four having non-progressive mild renal failure, and seven patients having a normal creatinine clearance. All the patients had significant proteinuria and 15 were Rhesus-D positive. We also studied eight patients with MCGN (five males and three females), three patients with type II (dense deposit disease) and five patients with type I. The mean age of this group was 30 years (range 16–59 years) with a mean duration of illness of 3·5 years. All had renal failure and six had progressive renal disease. All had proteinuria and all were Rhesus-D positive. Controls for the clearance studies consisted mainly of young healthy volunteers but included five elderly patients with no glomerular disease. A total of 19 controls were studied by one or other technique.

Preparation of Tc^{99m}-labelled IgG coated erythrocytes. Approximately 12 ml of heparinized blood was collected from each patient. The haemoglobin was determined and the volume of blood containing 1 ml packed cells calculated. This volume was centrifuged at 1,500 r/min for 10 min and the serum and buffy coat removed. The cells were washed twice in 0·9% saline then incubated at room temperature with 50 μCi Tc^{99m}. A volume of freshly prepared stannous chloride was added and the mixture allowed to stand for 5 min. The cells were washed twice in 0·9% saline. The packed cells were then incubated for 20 min at 37°C with 0·4 ml standard rhesus anti-D IgG (kindly supplied by Dr R. Mitchell, West of Scotland BTS). The cells were then washed twice with 0·9% saline and resuspended to a volume of 5 ml before reinjection.

Preparation of Tc^{99m}-labelled HDE. Approximately 12 ml heparinized blood was collected from each patient. The haemoglobin was determined and the volume containing 2 ml packed cells calculated. After twice washing with 0·9% saline, the packed cells were damaged by heating for

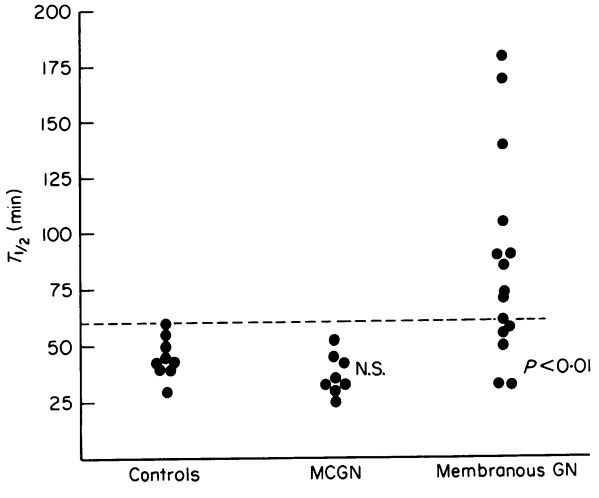

Fig. 1. Splenic RES Fc specific clearance ($T\frac{1}{2}$) in normal subjects and patients with primary glomerulopathies

exactly 30 min in a water bath at 50°C. After washing with saline to remove free haemoglobin, the cells were labelled with 50 μCi Tc^{99m}, as described above. The cells were resuspended in saline to a volume of 5 ml before reinjection.

Measurements of whole blood clearance of IgG coated erythrocytes and HDE. This was done using previously described methods (Lockwood *et al.*, 1979). Half-life clearance values were determined, and the results were expressed as the time in which 50% of the cells were removed from the circulation ($T\frac{1}{2}$; in min).

Circulating IC. These were detected by Clq binding and rheumatoid factor binding assays as previously described (Lockwood *et al.*, 1977; Schifferli *et al.*, 1981), and kindly performed for us in Professor Peters' Unit, Hammersmith Hospital, London.

HLA and DR antigens. The HLA antigens were identified on peripheral blood lymphocytes separated from heparinized whole blood using Ficoll-hypaque. A standard microtoxicity technique was used covering most of the major specificities using 150 sera. B cells were separated using a neuraminidase rosetting technique (Kissmeyer-Neilsen & Dick, 1979). The DR antigens tested for were DRI to DR8.

Statistics. Statistical significance was measured using the paired Wilcoxon's Rank sum test.

RESULTS

Whole blood clearance of the IgG coated cells

The $T\frac{1}{2}$ blood clearance values of the IgG cells in the normal controls ranged between 31 and 60 min. Nine of the 15 Rhesus-D positive patients with IMN had a delayed clearance of the IgG cells. The difference in the clearance rates between the controls and the membranous group was statistically significant ($P > 0{\cdot}01$) (Fig. 1). The delay in clearance did not correlate in this group with age, renal function at the time of study, nor degree of proteinuria. Six patients with MCGN had normal clearance of the IgG cells, which tended to be at the more rapid end of normal and two patients had enhanced clearance of the IgG cells. Clearance rates did not correlate with disease activity nor proteinuria in this group.

Whole blood clearance of HDE

Measurements of clearance of HDE were made in 12 of the 15 patients with IMN studied by the IgG cell method. A further three Rhesus-D negative patients were studied (Fig. 2). In contrast to the defect in clearance of the IgG cells, there was no defect in clearance of HDE in the patients with

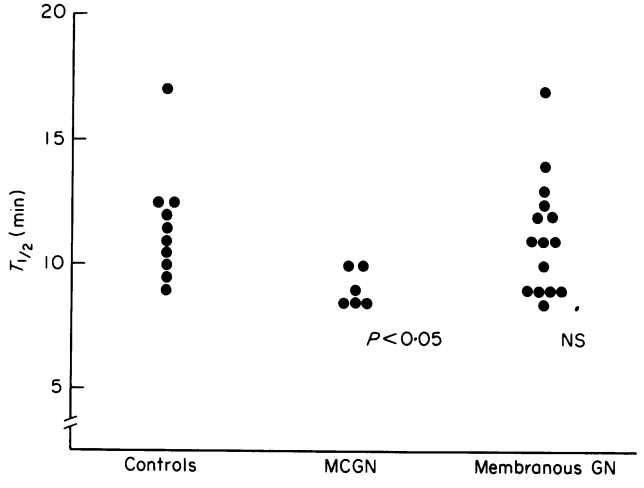

Fig. 2. Splenic RES clearance ($T\frac{1}{2}$) of HDE in normal subjects and patients with primary glomerulopathies.

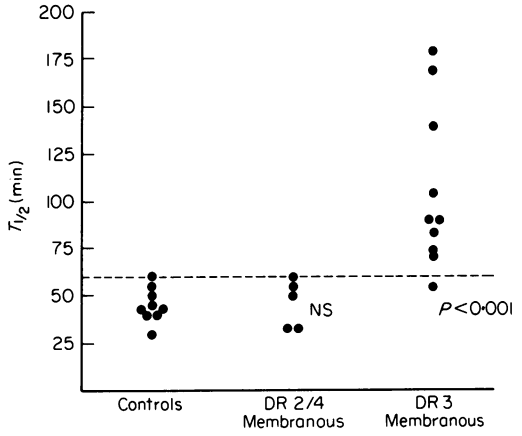

Fig. 3. Splenic RES Fc receptor specific clearance in normal subjects and patients with idiopathic membranous nephropathy related to the presence of specific HLA-DR antigens.

IMN. There was also no correlation between the rate of clearance of the IgG cells and the rate of clearance of the HDE in individual patients in this group. The clearance rates of the HDE was slightly enhanced in the patients with MCGN as a group ($P > 0{\cdot}05$) and the clearance rates correlated with the clearance rates of the IgG cells.

Circulating IC

None of the 18 patients with IMN had detectable IC in the circulation either by rheumatoid factor binding assay (18 measured) or Clq binding (10 measured). One patient with MCGN had IC detected by rheumatoid factor binding assay (40·5%: normal 15%) and had an enhanced clearance of both the IgG cells ($T\frac{1}{2} = 24$ min) and HDE ($T\frac{1}{2} = 8{\cdot}5$ min).

HLA and DR antigens

Ten of the 15 Rhesus-D positive patients with IMN possessed the DR3 antigens, in association with the presence of HLA B8 and/or B18. Nine of these patients had delayed clearance of the IgG cells ($P > 0{\cdot}001$) (Fig. 3). Five patients with IMN with DR2 or DR4 antigens had normal clearance of the IgG cells. Three patients with MCGN were DR3 positive and had either normal (two), or enhanced, (one) clearance of the IgG cells.

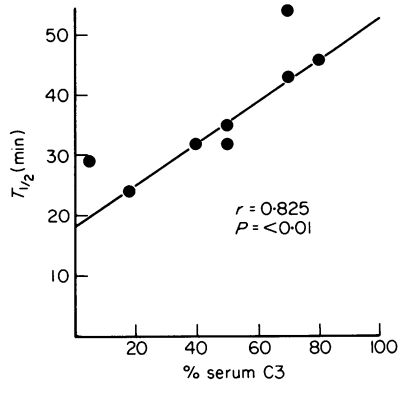

Fig. 4. Correlation between splenic Fc receptor specific clearance and serum complement C3 levels, expressed as a percentage of normal value, in patients with mesangio-capillary glomerulonephritis.

Correlation between RES function and serum C3 levels in MCGN

The clearance rates of the IgG cells and HDE correlated significantly with the serum C3 levels in the patients with MCGN ($r = 0{\cdot}825$ for IgG cells, $r = 0{\cdot}825$ for HDE cells) (Fig. 4). The lower the serum level of C3, the more rapid the clearance of the cells. Three of the most hypocomplementaemic patients had C3b detected on the surface of their circulating red cells. Only one patient in this group had detectable IC in the circulation.

DISCUSSION

Many glomerulopathies are immunologically mediated disorders. Animal studies have established the importance of IC deposition within the renal glomeruli as a pathogenetic mechanism in both acute and chronic glomerulonephritis. The identification of immunoglobulin and/or complement in the glomeruli of biopsies of patients with IMN and MCGN has been interpreted as evidence that the glomerular localization of IC are important in the pathogenesis of these disorders (Wilson & Dixon, 1974). An important function of the RES is the clearance of IC from the circulation, failure of which may promote deposition of complexes in the glomeruli or other tissues (Haackenstad & Mannik, 1974). The RES is a complex system composed of circulating and tissue fixed mononuclear cells on the surface of which are Fc and C3b receptors (Huber *et al.*, 1968). Methods of evaluating the functional activity of Fc receptors have recently been described and they have been used to demonstrate defects of reticuloendothelial function in diseases of IC origin (Frank *et al.*, 1979) including acute nephritis (Lockwood *et al.*, 1979). In this study we examined RES Fc receptor function of patients with IMN and MCGN to determine whether there was any correlation between disease activity and RES function.

We have developed and assessed a method of studying splenic RES Fc receptor function which should make the technique available for general use. The basic technique is to measure the clearance from the blood of autologous radiolabelled erythrocytes with Rhesus anti-D IgG antibody. Previous studies have used antibody taken from a single donor which is of limited availability. We used anti-D IgG taken from a pool of donors which is readily available from blood transfusion centres and was usable as supplied. Since this test examines splenic macrophage Fc receptor function, we also studied the clearance of autologous radiolabelled HDE, a test which also measures splenic macrophage function but is not dependent on the Fc receptor. This test also has the advantage in that it can be used in Rhesus negative patients.

The major findings of our study were the defect in clearance of IgG sensitized cells in nine of 15 patients with IMN and the normal clearance of HDE in all patients with IMN. The Fc receptor defect was associated with the presence of HLA B8 and DR3 ($P > 0{\cdot}001$) and did not correlate with disease activity. Circulating IC were not detected in these patients.

The cause of the delay in clearance of IgG cells in patients with IMN is unlikely to be due to reduced splenic flow since it was not matched by delay in clearance of HDE. Our own kinetic studies have shown that where there is a defect in blood flow to the spleen, there is a matched defect in clearance of IgG cells and HDE (to be published). Saturation or blockage of Fc receptors is also unlikely since no IC were detected in any patients with IMN, nor was the defect correlated with disease activity as judged by serum creatinine or protenuria. It is possible that complexes which cause IMN may not be detected by the tests used, but most other studies have also failed to detect complexes consistently in these patients (Border, 1979). A more likely hypothesis is that the defect is inherited and predates the onset of the disease and represents a reduced expression of Fc receptors on tissue macrophages. A recent study demonstrated defective Fc receptor function in patients with Dermatitis herpetiformis (DH), and some normal subjects all of whom were HLA B8-DR3 positive (Lawley *et al.*, 1981). As in our study, the defect did not correlate with disease activity nor with the level of circulating IC in patients with DH. Therefore, it is very likely that patients with IMN who possess the DR3 antigen have a primary defect of Fc receptor function and that the gene coding for Fc receptors is in linkage disequilibrium with the DR3 gene. The further study of normal HLA-DR3 subjects will help resolve this question.

The clearance of both IgG cells and HDE was slightly accelerated or at the more rapid end of

normal in patients with MCGN. Interestingly, there was a close correlation between clearance times and serum C3 concentrations. Intravascular C3 activation is known to occur in MCGN (Kourilsky *et al.*, 1974) and C3b may be bound to red cells through interaction with C3b receptors on the surface of the cells. The three patients with the most rapid clearances had C3b detected on the surface of their red cells by direct Coombes test, but no IgG. Complement has been shown to augment the clearance of red cells (Frank *et al.*, 1979) and it could be that complement components on the surface of these patients cells are responsible for the accelerated RE clearance which we found. This distortion of results of splenic functional tests may occur in any group of patients with intravascular complement activation and requires further study.

The importance of the defect of RES Fc receptor function in the pathogenesis of IMN is unknown. If IMN is caused by the deposition from the circulation of IC of a type which can be deposited on the epithelial side of the GBM (Cochrane & Koffler, 1973), then the defect in Fc receptor function may be of prime importance in permitting these complexes to circulate and thus be available to cause glomerular injury. If, however, IMN is the result of *in situ* formation of IC in the GBM for which there is evidence derived from the studies of passive Heymann's nephritis (Couser & Salant, 1980), an animal model for human IMN, then the defect in Fc receptor function is unlikely to be directly responsible for the development of IMN. It is possible that the gene coding for Fc receptors on splenic macrophages is in linkage disequilibrium with both the DR3 gene and a gene which codes for another immune function which is important in the pathogenesis of IMN. For example, this immune response gene could code for macrophage handling of antigen, or the antibody response to an as yet unidentified antigen. Immune response genes which code for antibody production, and responsiveness to certain antigens have been shown to be closely associated with the histocompatibility locus in animals (Benacerraf & McDevitt, 1972). It is only recently that specific immune responses have been linked with DR antigens in man (Legrand, Hors & Dausset, 1980), and further study of the genetic control of immune functions should enhance our understanding of many immunological diseases.

This study has shown that patients with IMN who possess HLA B8 and DR3 antigens have defective splenic macrophage Fc receptor specific function. The importance of this defect in the pathogenesis of IMN has yet to be determined.

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