

MINIMAL CHANGE GLOMERULONEPHRITIS AND FOCAL GLOMERULOSCLEROSIS MARKERS AND 'IN VITRO' ACTIVITY OF PERIPHERAL BLOOD MONONUCLEAR CELL

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Summary

Serum concentrations of IgG, IgA and IgM and PBMC were investigated in 11 prevalently adult patients with idiopathic glomerulonephritis, five minimal change glomerulonephritis (MCGN) and six focal glomerulosclerosis (FGS) and nephrotic syndrome. Among the peripheral blood mononuclear cells (PBMC) E rosette forming cells (T-cells), surface immunoglobulin bearing cells (B-cells), and T-cells with IgG Fc receptor (T_{γ}) were determined. In the culture supernatants of PBMC stimulated with PWM, the concentration of secreted IgG, IgA and IgM was determined by a solid phase immunofluorescence assay. After stimulation with PWM and PHA, the 3H -TdR uptake from PBMC was evaluated.

In patients only the serum values of IgG were found significantly decreased. No difference was observed between patients and healthy age and sex matched controls, in percentage and absolute number of T and B-cells, whereas an increased number of T_{γ} was present in patients. In these patients after stimulation with PWM the production of IgG and IgA, but not IgM, and the 3H -TdR incorporation, were significantly lower than in healthy controls. These results suggest an imbalance in the cellular co-operation or an intrinsic B-cell defect in the synthesis or secretion of Ig in MCGN and FGS.

Introduction

In idiopathic nephrotic syndrome (INS) due to minimal change glomerulonephritis (MCGN) the hypogammaglobulinaemia is mainly characterised by low values of serum IgG, not fully explained by urinary losses, as the proteinuria is highly selective. It has been suggested that this hypogammaglobulinaemia might be caused by a reduced synthesis of IgG [1] from lymphocyte subsets. In patients with INS we [2] and, more recently, others [3], found an increase in the number of surface IgG bearing cells. In an attempt to identify some possible dysfunction in Ig production, we studied Ig synthesis 'in vitro' and the numbers of some

lymphocyte subsets identified by surface markers in patients with MCGN and FGS. The latter is considered by some [4] to be an unfavourable evolution of MCGN.

Material and methods

Patients studied

Eleven patients, seven males and four females aged from five to 60 years (mean 28.5) with INS, five with MCGN and six with FGS, determined by renal biopsy, were investigated. All patients had hypogammaglobulinaemia with significantly decreased values of IgG ($300 \pm 100\text{mg/dl}$), and normal IgA ($210 \pm 70\text{mg/dl}$), and IgM ($150 \pm 75\text{mg/dl}$), and highly selective heavy proteinuria ($>3.5\text{g/L/m}^2$). During the period of study no patient received steroid or immunosuppressive treatment. Healthy controls (C), age and sex matched, were also included. In these the normal values of serum Ig were: IgG 1234 ± 282 , IgA 208 ± 81 , IgM 194 ± 78 .

Test employed

PBMC were separated by the Ficoll-Isopaque gradient centrifugation method. E-rosettes (T-cell) were counted according to Aiuti et al [5], enriched by centrifugation twice on gradients, and then the number of T-cells bearing the Fc receptor for IgG (T_γ) as described by Ferrarini [6] was determined. B-cells were detected utilising a $F(ab)_2$ FITC-anti-human-gammaglobulin serum according to Loor et al [7]. The $^3\text{H-TdR}$ incorporation by PBMC cultured in RPMI 1640 + FCS 10 per cent in the presence of the polyclonal activators Phytohaemagglutinin (PHA), and Pokeweed mitogen (PWM), was determined on the fifth day by adding $^3\text{H-Thymidine}$ six hours before the end of culture. The results are expressed as the ratio of c.p.m. in stimulated to c.p.m. in unstimulated control cultures (stimulation index (SI)). In order to estimate the *in vitro* Ig synthesis 5×10^6 PBMC were cultured in 5ml of medium + $50\mu\text{l}$ of PWM. On the seventh day the supernatants were collected and the amount of IgA, IgG and IgM determined by a solid phase immunofluorescence assay (Immunofluor, Bio-Rad). The amount of Ig is expressed as $\text{ng}/1 \times 10^6$ PBMC and reported on a logarithmic scale utilising a geometric mean (gM). Statistical analysis was performed by Student's t test.

Results

No difference was observed between patients and controls in the number of leucocytes (mean \pm SE = 6932 ± 602 versus 6604 ± 437) and the percentage of T and B cells. The mean value of T_γ cells was significantly increased (mean \pm SE = $18.95 \pm 3.43\%$ versus $10.49 \pm 1.35\%$; $p < 0.025$), although these cells were increased only in some patients (Figure 1). The DNA synthesis in patients was lower than in controls in response to PWM (SI = $55.6 \pm \text{SE } 12.11$ versus 122.2 ± 18.17 ; $p < 0.05$), while the response to PHA was normal (SI = $149.5 \pm \text{SE } 35.98$ versus 144.3 ± 28.02) (Figure 2). After stimulation of PBMC by PWM the con-

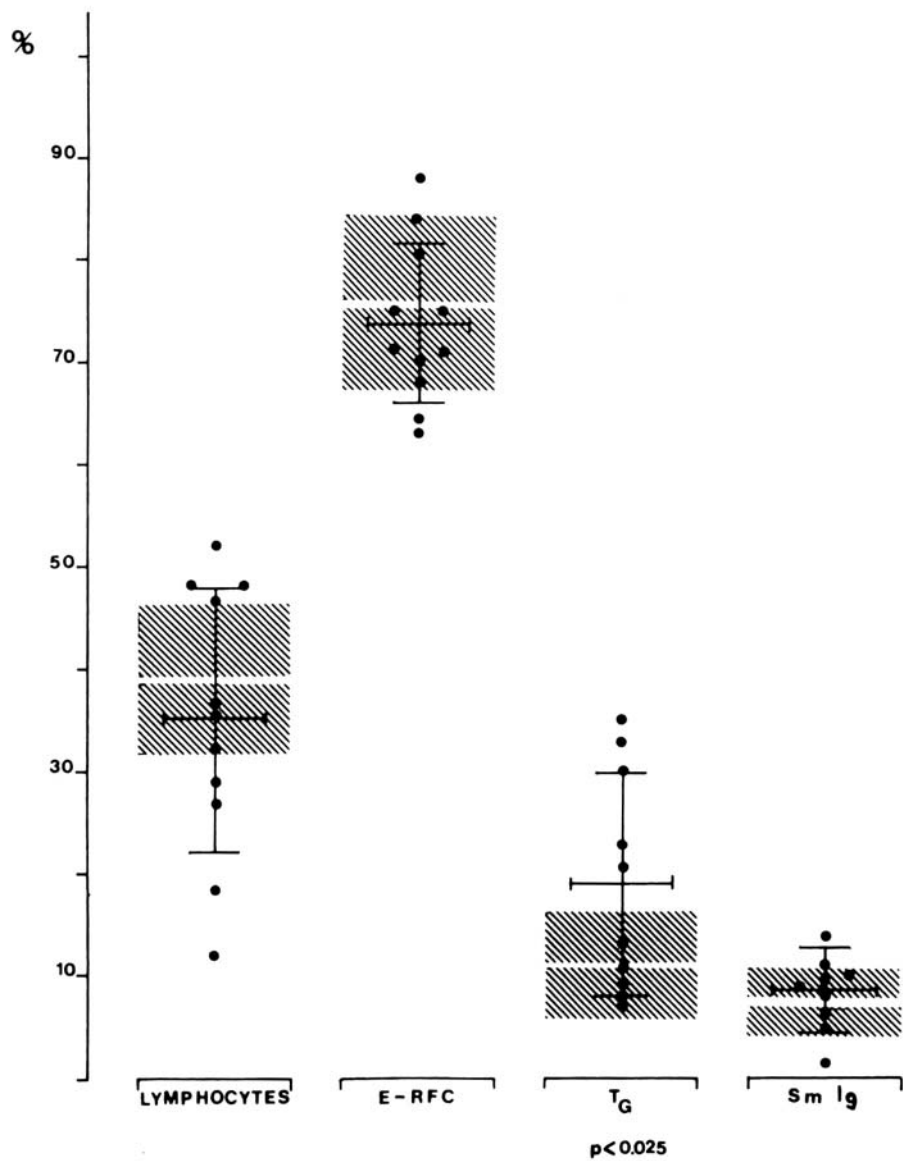


Figure 1. Shaded area: mean \pm SD of 20 controls

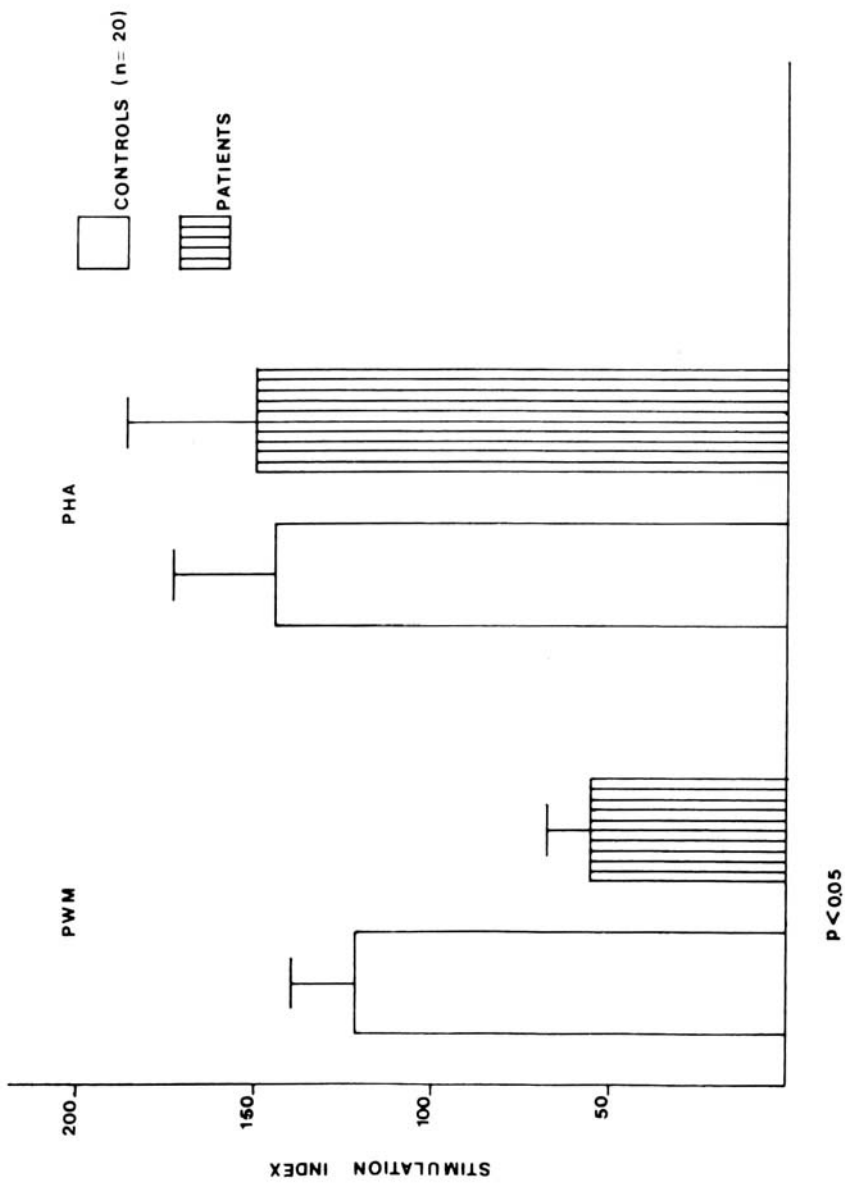


Figure 2. Mean \pm SE

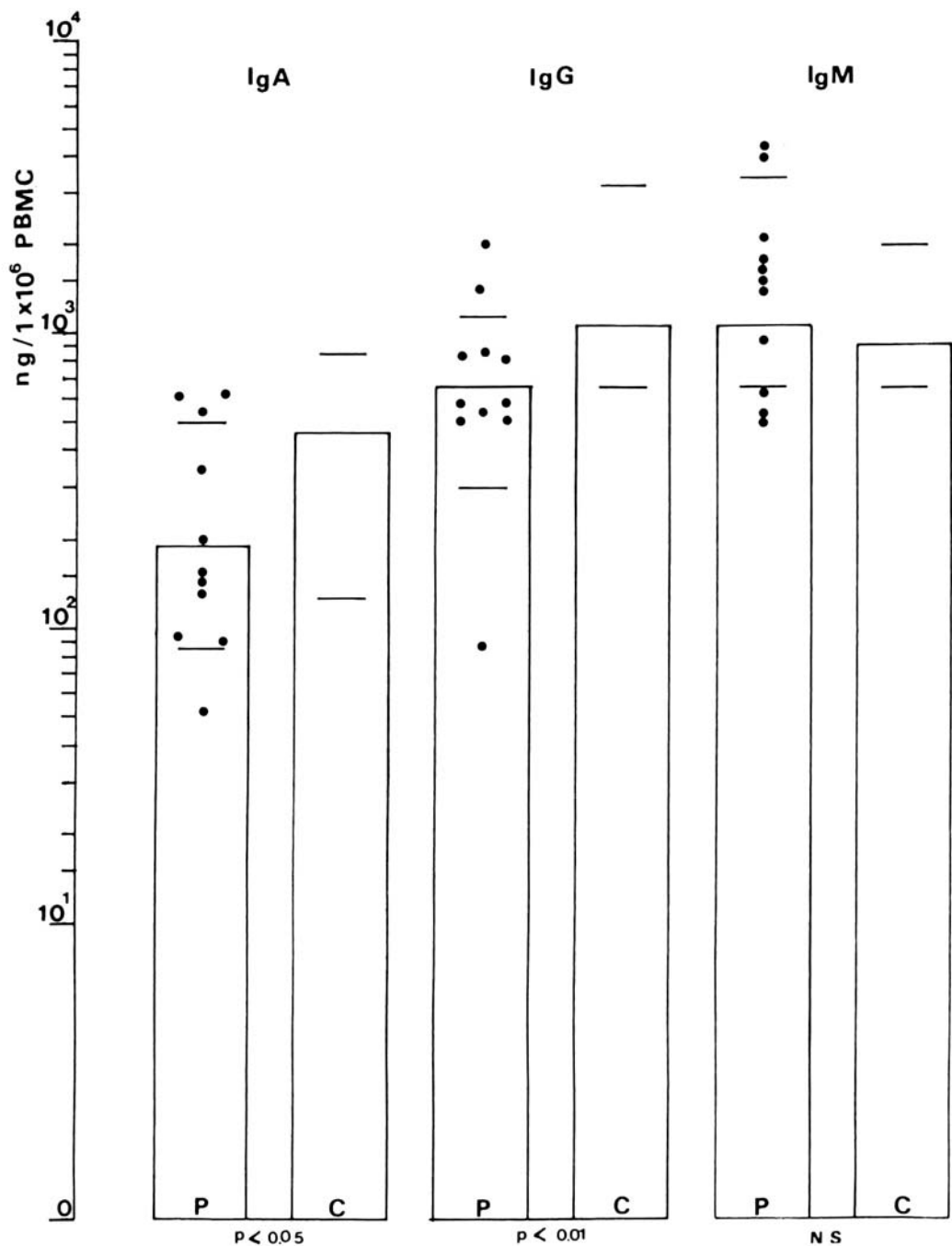


Figure 3. 'In vitro' Ig synthesis. Geometric mean \pm SD; P = patients (n = 11); C = controls (n = 31)

centrations of IgG and IgA in the culture supernatants of patients were significantly lower (IgG = 464 ± 2.29 versus 1024 ± 2.22 , $p > 0.01$; IgA = 151 ± 2.15 versus 288 ± 2.54 , $p > 0.05$); while IgM concentrations were slightly increased (1038 ± 2.14 versus 840 ± 1.90 , values expressed as $\text{gM} \pm \text{SD}$) (Figure 3).

Conclusions

This study shows that in patients with INS there is decreased ability of PBMC to produce normal amounts of IgG and IgA in culture after stimulation by PWM. In the serum of these patients only IgG was significantly decreased (the IgA and IgM being normal). This difference in Ig synthesis (in vivo and in vitro) could be due to the different environment. We observed in some patients an increased number of T_γ cells which have a suppressor activity in the PWM-activated system [8]. The reduced PBMC response to PWM as shown by DNA synthesis and IgA and IgG production might be due to either a T-cell suppression on B-cell differentiation or an intrinsic B-cell defect in the synthesis or in the release of Ig.

References

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