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# Immunoregulation in glomerulonephritis, Henoch–Schonlein purpura and lupus nephritis

K. M. BANNISTER,* P. A. DREW,† A. R. CLARKSON* & A. J. WOODROFFE*
**Renal Unit, Royal Adelaide Hospital and †Department of Medicine, University of Adelaide, Adelaide, South Australia, Australia.*

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## SUMMARY

Immunoregulation was examined in normal controls and in patients with immune complex glomerulonephritis and lupus nephritis (SLE) using OKT monoclonal antibodies against helper (OKT4) and suppressor (OKT8) T cell subsets. Functional studies assessed T cell control of *in vitro* immunoglobulin synthesis by cultured peripheral blood mononuclear cells (PBMC). IgG and IgA synthesis was measured in unstimulated, pokeweed mitogen (PWM) stimulated and PWM + concanavalin A (Con A) stimulated cultures. Patients with primary membranous nephropathy (MN) and mesangial IgA nephropathy (IgA GN) were found to have elevated T4/T8 ratios secondary to a deficiency of the $T8^+$ subset. Patients with SLE had low T4/T8 ratios. B cell activation with high spontaneous immunoglobulin synthesis was present in cell cultures from patients with SLE, IgA GN and Henoch–Schonlein purpura (HSP). Defective Con A inducible suppression of *in vitro* immunoglobulin synthesis was found in SLE, HSP and to a lesser extent, primary MN. Functional Con A inducible suppressor defects correlated with elevated T4/T8 ratios only in patients with MN. All four disorders appear to share disturbances of cellular immune response with various degrees of defective immune suppression; however, it is not clear from these studies whether the defects are primary or secondary phenomena.

**Keywords** glomerulonephritis T cell subsets immunoglobulin production

## INTRODUCTION

Research into the pathogenesis of chronic glomerulonephritis mediated by immune complexes (IC) has centred primarily on the elucidation of humoral mechanisms of immune damage, antigen identification and measurement of circulating IC. However, it is clear from animal models that the immune response of the host is important in determining the type and extent of immunological glomerular injury. Disorders of the cellular control of immune regulation may result in abnormal antibody responses to ubiquitous antigens, perhaps the production of autoantibodies and the formation of nephritogenic IC.

We have used monoclonal antibodies against T cell subsets to examine immune regulation in patients with primary MN, mesangial IgA GN, HSP and lupus nephritis. In addition, we measured immunoglobulin synthesis *in vitro* and Con A inducible suppressor T cell activity in patients with these disorders.

Correspondence: Dr A. J. Woodroffe, Renal Unit, Royal Adelaide Hospital, Adelaide, South Australia 5000, Australia.

## PATIENTS

(A) T cell subsets were measured in: (i) 28 normal controls (17 males and 11 females; mean age $35 \pm 12$ years); (ii) 14 patients with primary MN (11 males and three females; mean age $51 \pm 13$ years) including six who were nephrotic (proteinuria > 3·5 g/day and serum albumin < 30 g/l); (iii) 10 patients with mesangial IgA GN (seven males and three females; mean age $45 \pm 14$ years); (iv) 15 patients with lupus nephritis (two males and 13 females; mean age $30 \pm 12$ years). Eleven were receiving steroid therapy at the time of testing and one patient was nephrotic; (v) two patients with HSP (B.V. female 42 years, F.T. male 59 years). Both were nephrotic.

(B) *In vitro* immunoglobulin synthesis was assessed in: (i) normal controls; (a) matched for the non-SLE patient group (IgG, $n = 23$; 15 male and eight female; IgA, $n = 22$; 15 male and seven female; mean age $47 \pm 11$ years); (b) matched for the SLE patient group (IgG, $n = 18$; four males and 14 females; mean age $32 \pm 10$ years; IgA, $n = 17$; four males and 13 females; mean age $32 \pm 11$ years); (ii) 13 patients with primary MN (nine males and four females; mean age $50 \pm 12$ years) including five who were nephrotic; (iii) 10 patients with mesangial IgA GN (eight males and two females; mean age $46 \pm 13$ years) including one nephrotic patient; (iv) three patients with HSP (B.V.: F.T.:J.W., male 49 years). All were receiving steroid therapy; (v) 10 patients with lupus nephritis (two males and eight females; mean age $31 \pm 14$ years). Nine were receiving steroid therapy at the time of testing and one was nephrotic.

All patients had renal biopsy documented glomerulonephritis. With three exceptions, serum creatinine concentrations were < 0·25 mmol/l (normal range 0·05–0·12 mmol/l): patient B.V. with HSP who required dialysis during the course of her acute illness but recovered normal renal function and two patients with mesangial IgA GN had creatinine concentrations of 0·39 mmol/l and 0·60 mmol/l at the time of testing. Serum IgA levels were measured in all group B patients with mesangial IgA GN and in two patients (F.T.: J.W.) with HSP.

## MATERIALS AND METHODS

*T cell subsets.* These were measured using Ortho monoclonal antibodies OKT4 and OKT8 and indirect immunofluorescence with a fluorescein conjugated goat anti-mouse second antibody and flow cytometry (Becton-Dickinson, FACS IV). Cells were detected by low angle scatter with light excitation of 500 mW output and 488 nm wavelength. A 530 nm light filter removed light of the exciting wavelength.

*Functional studies.* After density gradient separation peripheral blood mononuclear cells (PBMC) were cultured at a concentration of $1 \times 10^6$ cells/ml in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine and 100 u/ml penicillin and 100 $\mu$g/ml streptomycin, in microtitre trays with 200 $\mu$l of cell suspension added to each of five wells together with 25 $\mu$l of either culture medium, PWM, or PWM + Con A.

Cell cultures were incubated in a 5% $CO_2$ incubator at 37°C for 8 days and the supernatant was collected and stored at −70°C until the IgG and IgA could be assayed using a solid phase radioimmunoassay sensitive to 10 ng/ml of immunoglobulin.

*Mitogens.* PWM (Sigma; Lot No. 100F-9655) was used at a final concentration of 0·1 $\mu$g/ml and Con A (Pharmacia; Lot No. FK 18607) at a final concentration of 5 $\mu$g/ml. Preliminary experiments established that these concentrations of PWM and Con A induced maximal stimulation and suppression of immunoglobulin synthesis respectively. The percentage suppression of PWM stimulated immunoglobulin synthesis by Con A was calculated using the formula:

Con A suppression =

$$\frac{\text{PWM stimulated Ig synthesis} - \text{PWM} + \text{Con A stimulated Ig synthesis}}{\text{PWM stimulated Ig synthesis}} \times 100.$$

Serum IgA levels were determined using laser nephelometry ($n = 12$).

*Statistical analysis.* Student's *t*-test was employed to compare the means of two groups. Spearmans rank correlation coefficient ($r_s$) was used where indicated. Results are expressed as means ± standard error of the mean.

## RESULTS

*T cell subsets and helper/suppressor (T4/T8) ratios* (Table 1)
The mean T4/T8 ratio of the normal control group was $3{\cdot}0 \pm 0{\cdot}2$. This is higher than has been reported in other centres and may relate to the use of fluorescent microscopy by other workers (Chatenoud & Bach, 1981) or to different gating criteria for the FACS (Morimoto *et al.*, 1980). No significant difference in T4/T8 ratios was found between normal controls aged less than 30 years ($3{\cdot}1 \pm 0{\cdot}2$) or greater than 40 years ($2{\cdot}9 \pm 0{\cdot}5$) or between males ($3{\cdot}0 \pm 0{\cdot}3$) and females ($3{\cdot}1 \pm 0{\cdot}2$). Patients with primary MN and IgA GN demonstrated a significantly elevated mean T4/T8 ratio ($P < 0{\cdot}05$ for both) due to an absolute reduction in T8 positive cells (MN $P < 0{\cdot}01$, IgA GN $P < 0{\cdot}10$). In contrast, patients with SLE showed a highly significant ($P < 0{\cdot}005$) mean reduction in T4/T8 ratios secondary to both a depression of mean T4 values ($P < 0{\cdot}005$) and elevation of mean T8 values ($P < 0{\cdot}01$). There was no correlation between the presence of the nephrotic syndrome and T4/T8 ratios in any disease group.

*Functional studies* (Figs 1 & 2)

*Membranous nephropathy*. No significant difference was present between MN patients and controls in the amount of IgG and IgA produced by PWM stimulated and unstimulated PBMC.

**Table 1.** T cell subsets (mean percentage ±s.e.) and helper/suppressor (T4/T8) ratios in controls and patients with MN, IgA GN and lupus nephritis

| | *n* | T4 | T8 | T4/T8 |
|---|---|---|---|---|
| Controls | 28 | $52{\cdot}6 \pm 1{\cdot}5$ | $18{\cdot}9 \pm 0{\cdot}96$ | $3{\cdot}0 \pm 0{\cdot}2$ |
| MN | 14 | $51{\cdot}5 \pm 2{\cdot}9$ | $14{\cdot}8 \pm 1{\cdot}3$† | $4{\cdot}0 \pm 0{\cdot}6$* |
| IgA GN | 10 | $52{\cdot}1 \pm 2{\cdot}6$ | $15{\cdot}9 \pm 2{\cdot}4$ | $4{\cdot}5 \pm 1{\cdot}2$* |
| SLE | 15 | $46{\cdot}0 \pm 1{\cdot}2$‡ | $24{\cdot}6 \pm 2{\cdot}3$† | $2{\cdot}1 \pm 0{\cdot}2$‡ |

*$P < 0{\cdot}05$; †$P < 0{\cdot}01$; ‡$P < 0{\cdot}005$.

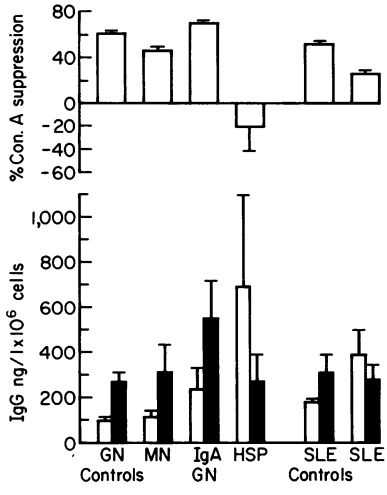


**Fig. 1.** Mean amount (±s.e.) of IgG synthesized by PBMC from controls and patients with MN, IgA GN, HSP and lupus nephritis spontaneously (□) and after PWM stimulation (■). Also shown is the % Con A suppression of PWM stimulated IgG synthesis.

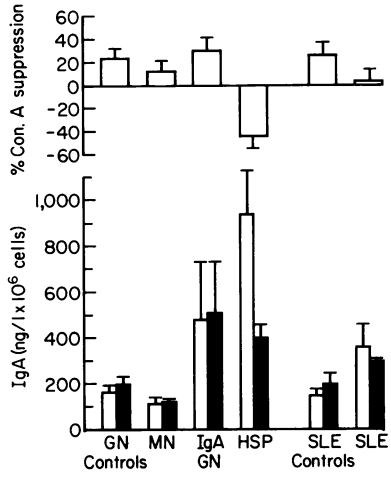


**Fig. 2.** Mean amount (±s.e.) of IgA synthesized by PBMC from controls and patients spontaneously (□) and after PWM stimulation (■). Also shown is the % Con A suppression of PWM stimulated IgA synthesis.

However, the Con A inducible suppression of IgG synthesis differed significantly ($P < 0{\cdot}05$) between the MN patients (46 ± 8%) and controls (63 ± 4%). A significant correlation ($r_s = -0{\cdot}65$, $P < 0{\cdot}05$) was also found between T4/T8 values in MN patients and functional IgG suppressor activity (Fig. 3). A difference was found in Con A inducible suppression between nephrotic (IgG 40 ± 20%; IgA 8·5 ± 15%) and non-nephrotic (IgG 54 ± 7%; IgA 17·7 ± 11%) patients but this was not statistically significant.

*IgA nephropathy.* Patients with IgA GN had high spontaneous IgG (six of 10; $P < 0{\cdot}0125$) and IgA (five of 10; $P < 0{\cdot}05$) synthesis compared to the control group. PWM stimulations caused further significant elevations of IgG (six of 10; $P < 0{\cdot}025$) and IgA (six of 10; $P < 0{\cdot}05$) production. However, the degree of Con A-induced suppression was not significantly different from the control population. Although five of 12 patients with mesangial IgA deposits had both elevated serum and *in vitro* IgA levels, the correlation was not significant ($r_s = 0{\cdot}17$).

*Henoch/Schonlein purpura.* Spontaneous IgG and IgA synthesis was markedly elevated in all three patients with HSP (IgG 280, 1,520, 280; IgA 1,320, 740, 775 ng/l × $10^6$ cells, respectively). The addition of PWM caused a reduction of both IgG and IgA synthesis in all three patients. Paradoxically, the addition of Con A to PWM stimulated cultures resulted in enhancement of IgG (two of three) and IgA (three of three) synthesis. Both HSP patients in whom serum IgA was

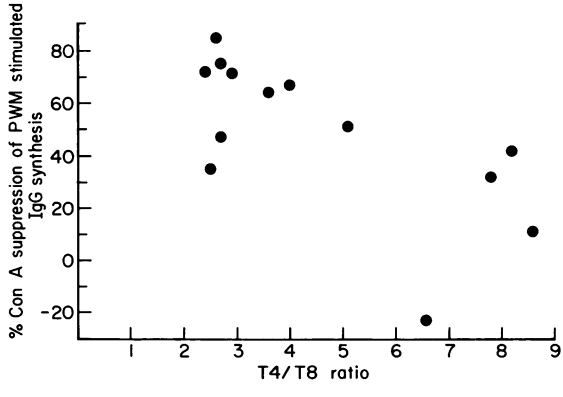


**Fig. 3.** Correlation between T4/T8 and *in vitro* Con A inducible suppression of IgG in patients with primary MN ($r_s = -0{\cdot}65$; $P < 0{\cdot}05$).

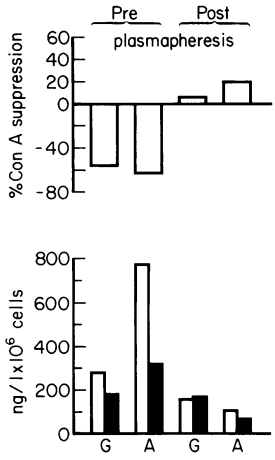


**Fig. 4.** Spontaneous (□) and PWM stimulated (■) IgG and IgA synthesis by PBMC from HSP patient J.W. before and after plasma exchange therapy. Also shown is the % Con A suppression of PWM stimulated immunoglobulin synthesis.

measured had elevated levels. One patient (J.W.) with severe disease involving skin, joints, gut and kidneys was treated with plasma exchange therapy in combination with steroids. Fig. 4 shows immunoglobulin synthesis *in vitro* by PBMC from this patient, collected immediately before and after the completion of seven plasma exchanges. The return of spontaneous and PWM stimulated IgG and IgA synthesis to normal, corresponded with a dramatic and sustained improvement in his clinical status.

*SLE.* Increased B cell activation was present in this group of patients with highly significant elevations of spontaneous IgG (nine of 10; $P < 0{\cdot}0025$) and IgA (seven of 10; $P < 0{\cdot}0125$) synthesis compared with age and sex matched controls. In contrast to the control group, the addition of PWM caused a reduction in the amount of immunoglobulin produced. A significant decrease ($P < 0{\cdot}005$) in the mean Con A inducible suppression of IgG synthesis (but not IgA synthesis) was found in the SLE group ($27 + 8\%$) compared with controls ($56 \pm 6\%$).

## DISCUSSION

This study has attempted to dissect the cellular control arm of the immune response in patients with IC glomerulonephritis and lupus nephritis.

Two approaches were employed to examine possible disturbances of immune regulation. The use of monoclonal antibodies against T cell subsets with or without flow cytometry has been used by other workers (Bach & Bach, 1981) to define alterations in T cell subpopulations in immune disorders. Functional studies of cultured mononuclear cells from our patients relied on established methods of tissue culture and the ability of Con A to induce suppressor cells (Dwyer & Johnson, 1981). These functional assays are not yet standardized between laboratories, the characteristics of the cells involved in suppression are not clearly defined and these cells appear to be heterogeneous (Damle & Gupta, 1982). Patient variables such as age (Hallgren & Yunis, 1977) and renal function (Lortan *et al.*, 1982) need also to be considered.

Elevated helper to suppressor T cell ratios in patients with MN have been reported by others (Chatenoud & Bach, 1981; Cagnoli *et al.*, 1982; Short *et al.*, 1982) and were confirmed in this study. In addition, our studies of Con A suppression of IgG synthesis *in vitro* showed suppressor defects which correlated with the elevation of the T4/T8 ratio. No significant difference was found in the amount of IgG or IgA synthesized by PBMC from MN patients compared to controls. This is in contrast to the findings of Ooi *et al.* (1980) showing diminished IgG and IgM production in MN patients and to Heslan *et al.* (1982) who reported impaired IgG synthesis in patients with the

nephrotic syndrome secondary to a variety of glomerulopathies. We did not find a significant difference in immunoglobulin synthesis between MN patients with and without the nephrotic syndrome. Our data raises the possibility that defective immune suppression may be important in the pathogenesis of MN. In some cases this disturbance may lead to autoantibody production. We have found anti DNA antibodies in PEG precipitates from four of 14 patients with primary MN (unpublished observations) and Roberts & Lewis (1978) demonstrated the presence of anti-DNA antibodies in a cryoprecipitate from a patient with MN. In addition, Nomoto & Sakai (1979) demonstrated that presence of cold reactive IgG antibody to extractable nuclear antigen (ENA) in patients with primary MN. Finally, there is a significant association between MN and the autoimmunity associated HLA DR3 antigen (Klouda *et al.*, 1979).

Increased T4/T8 ratios were also present in patients with mesangial IgA GN confirming an earlier report by Chatenoud & Bach (1981). Although double labelling of normal cells with OKT4 and OKT8 reagents is low (Reinherz *et al.*, 1980), the possibility that cells from disease states may be doubly labelled cannot be entirely excluded in our study.

Functional tests showed B cell hyperactivity with high spontaneous and PWM stimulated IgG and IgA synthesis by PBMC from patients with IgA GN suggesting defective suppressor cell activity. However, Con A inducible suppression of IgG and IgA synthesis was similar to controls. Reports by other workers have included normal *in vitro* IgA production (Cosio *et al.*, 1982), increased IgA production after PWM stimulation compared to controls (Egido *et al.*, 1982) and an increase in IgA bearing lymphocytes (Nomoto & Sakai, 1979) in patients with IgA GN. The latter group were also able to show a decrease in IgA specific T suppressor cell activity in these patients (Sakai, Nomoto & Arimari, 1979) and demonstrated the presence of cold reactive IgM antibody to ENA in 27 of 33 patients with IgA GN, suggesting a disturbance of immune regulation with autoantibody production (Nomoto & Sakai, 1979).

Henoch–Schonlein purpura is a disease closely allied with IgA GN with mesangial deposition of IgA and complement and frequent elevations of serum IgA and circulating IC (Levinsky & Barratt, 1979). Marked disturbances of immunoglobulin synthesis were found in three patients with HSP in the present study. All had high levels of spontaneous IgG and IgA synthesis. The addition of PWM to the cultures caused a significant decrease in immunoglobulin production, a phenomenon also seen in the SLE cultures. The presence of Con A in the PWM cultures enhanced immunoglobulin production suggesting a marked suppressor defect in the patients with HSP. Beale *et al.* (1982) reported similar findings in cultures of PBMC from five HSP patients and found that the addition of allogeneic normal T cells resulted in a decline in immunoglobulin synthesis. The reduction of spontaneous IgG and IgA synthesis and improvement of the suppressor defect in our patient J.W. following plasma exchange therapy is unexplained but may reflect removal during plasma exchange of soluble factors (e.g. anti T cell antibodies or IC) which modulate lymphocyte function.

Studies in patients with SLE by other workers have demonstrated reduced suppressor cell function (Bresnihan & Jasin, 1977; Sagawa & Abdou, 1978; Fauci *et al.*, 1978). Our results in patients with lupus nephritis confirmed these findings and also showed highly significant alterations in T cell subsets with elevated T8 fractions and decreased T4 positive cells resulting in a low T4/T8 ratio. Other workers (Chatenoud & Bach, 1981; Smolen *et al.*, 1982) have reported similar findings. Subtle alterations of cell subpopulations necessary for suppression within the $OKT4^+$ and the $OKT8^+$ subsets (Yachie *et al.*, 1982; Thomas *et al.*, 1982) may be important in producing the low ratio in SLE patients.

Although further work using separated T cell subpopulations and coculture experiments needs to be performed in MN, IgA GN and HSP, the alterations in T cell subsets and the functional defects of *in vitro* immunoglobulin synthesis suggest that these disorders and SLE share similar disturbances of immune regulation and that defective suppression and/or autoimmunity may be important in the pathogenesis of these disorders. Serial studies and measurements in relatives of patients with primary glomerulonephritis and HSP are required to determine whether the observed defects are primary or secondary events.

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