

T-LYMPHOCYTE SUBSETS IN PRIMARY AND SECONDARY GLOMERULONEPHRITIS

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Summary

T-lymphocyte subsets, using the monoclonal antibodies OKT3 (peripheral T-cells), OKT4 (helper/inducer T-cells) and OKT8 (suppressor/cytotoxic T-cells) were measured in peripheral blood from 110 patients with various forms of primary and secondary glomerulonephritis (GN) (Berger's disease, membranous GN, focal glomerulosclerosis, membranoproliferative GN, lupus nephritis and mixed essential cryoglobulinaemia with GN). We have found a significantly higher OKT4⁺/OKT8⁺ ratio in patients with Berger's disease and membranous GN and a rather low OKT4⁺/OKT8⁺ ratio in patients with lupus nephritis and mixed essential cryoglobulinaemia, due to a significant decrease in OKT4⁺ cells. Our results suggest an imbalance in immunoregulatory mechanisms in some forms of GN.

Introduction

The reports on impaired distribution and function of immunoregulatory T-cells in systemic diseases and, more recently, in some forms of primary glomerulonephritis (GN) [1-6] have suggested an immunoregulatory imbalance in the pathogenesis of these conditions.

The recent availability of monoclonal antibodies of the OKT series which react with distinct antigenic determinants of human T-cells has allowed a reliable measurement of their functional subsets [7]. In the present study the distribution of peripheral T-cell subsets was assessed in 110 patients with primary and systemic GN using monoclonal antibodies in an indirect immunofluorescence assay.

Patients and methods

Patients

One hundred and ten patients with various forms of GN and whose renal biopsies were studied between 1972 and 1981 were selected for this study; 58 were male and 52 female. Patients were divided into two groups.

Group I primary GN (77 patients): 30 IgA mesangial GN (IgA-GN), 22 membranous GN (MGN), 15 focal glomerulosclerosis (FGS) and 10 membranoproliferative GN (MPGN). None had systemic disease, liver cirrhosis or cancer, no steroid or immunodepressant drugs were administered at the time of the study or during at least eight weeks before. Clinical and biological data are presented in Table I.

TABLE I. Main clinical and biological data of patients with primary glomerulonephritis

	IgA-GN	MPGN	MGN	FGS
Number of patients	30	10	22	15
Age	39(12-64)	29(17-57)	45(21-71)	45(14-68)
Sex M : F	4 : 1	2 : 1	1 : 1	1 : 1
Serum creatinine (mg/100ml)	1.75(0.6-6.0)	1.8(0.8-5.0)	1.67(0.3-7.8)	1.48(0.9-3.6)
Patients with chronic renal failure % *	26	40	27	33
Patients with nephrotic syndrome %	0	30	50	20
High serum IgA (>350mg/100ml) %	32	0	0	0

* Serum creatinine >1.4mg/100ml

Group II this category included 18 patients with lupus nephritis (SLE-GN) and 15 patients with a mixed essential cryoglobulinaemia with glomerular involvement (EMC-GN). Fourteen of 18 patients with SLE presented a proliferative GN and four a membranous GN. All patients with EMC showed a membranoproliferative GN (Table II).

TABLE II. Main clinical data of patients with lupus nephritis and essential mixed cryoglobulinaemia with glomerular involvement

	SLE	EMC
Number of patients	18	15
Age	35(14-60)	57(41-72)
Sex M : F	1 : 8	1 : 2
Extrarenal involvement %	39	60
Renal involvement %	100	100
Therapy (corticosteroids and/or immunosuppressive drugs) %	83	60

The control group consisted of 25 hospital or laboratory staff members (14 men and 11 women, 40 ± 15 years old) with no history of immune disorders.

Renal biopsy All the renal biopsies were processed as previously described [8] and were examined by light and immunofluorescence microscopy.

Determination of lymphocyte subsets Mononuclear cells were purified from peripheral blood on a Ficoll-Hypaque gradient as previously described [9]. Lymphocyte viability was tested by trypan blue exclusion and was greater than 95 per cent in all samples. Contamination with monocytes was tested by latex particle phagocytosis. T-cell subsets were determined by incubating 10^6 cells in suspension with $5\mu\text{l}$ of monoclonal antibodies OKT3 (peripheral T-cells), OKT4 (helper/inducer T-cells) and OKT8 (suppressor/cytotoxic T-cells) (Ortho Pharmaceutical). The cells were then washed three times, resuspended in PBS and incubated with $50\mu\text{l}$ of FITC-conjugated anti-mouse IgG serum (Meloy) and finally washed three times [7]. B-cells (Ig-bearing cells) were measured using a FITC-conjugated F(ab)₂ fragment of goat anti-human total Ig anti-serum as previously described [9].

At least 200 cells per slide were counted at the same high power field (1000X) both in phase contrast and fluorescence microscopy. All analyses were performed without knowledge of the source of specimen.

Statistics The significance of differences was analysed by 2-tailed Student t test.

Results

The relative percentage of B-lymphocytes and OKT3⁺ cells was normal in all groups.

T-lymphocyte subsets in primary GN Patients with Berger's disease showed a significant increase of OKT4⁺/OKT8⁺ ratio compared with the normal controls ($p < 0.001$); this was due to a decrease in the percentage OKT8⁺ cells ($p < 0.001$) and to an increase in OKT4⁺ cells ($p < 0.005$). Patients with MGN also showed a significant increase of OKT4⁺/OKT8⁺ ratio ($p < 0.001$) due to decreased OKT8⁺ cells ($p < 0.001$) and to an increased OKT4⁺ cell percentage ($p < 0.01$). Patients with FGS and MPGN did not show significant alterations in the OKT4⁺/OKT8⁺ ratio, nor in the percentage of OKT4⁺ and OKT8⁺ cells (Table III and Figure 1).

In MGN patients no significant correlation was found between T-cell subsets and the presence of nephrotic syndrome, likewise in IgA-GN patients between T-cell subsets and serum IgA values. On the contrary in patients with IgA-GN a significant correlation was found between T-cell subsets and renal function, namely the patients with normal GFR showed a higher OKT4⁺/OKT8⁺ ratio compared with the patients with impaired GFR ($p < 0.02$) (Figure 2).

T-lymphocyte subsets in secondary GN Patients with SLE and EMC showed a decreased OKT4⁺/OKT8⁺ ratio, though the differences were not significant

TABLE III. Percentage of T-cell subsets, all peripheral B-lymphocytes (S.m.Ig) and OKT4⁺/OKT8⁺ ratio in patients with primary and secondary glomerulonephritis

	OKT3 ⁺	OKT4 ⁺	OKT8 ⁺	OKT4 ⁺ /OKT8 ⁺	S.m.Ig(B-Cells)	Lymphocytes/mm ³
IgA-GN	76.2± 7.8	55.0± 8.6***	23.4± 6.6*	2.56±0.87*	12.2±2.8	2530±1312
MPGN	78.7± 5.6	50.4± 8.5	30.9± 8.8	1.84±1.04	11.2±3.7	2471± 878
MGN	75.6± 7.7	53.9± 9.5**	21.7± 4.9*	2.65±0.89*	12.3±3.7	2720±1284
FGS	74.1± 7.0	45.3± 9.7	27.5± 8.9	1.89±1.04	11.6±2.8	2734±1251
SLE	73.3±10.3	39.5±11.7**	37.0±13.1	1.22±0.54	14.0±4.1	2493±1510
EMC	74.5± 7.5	40.7±11.7**	34.8±12.0	1.39±0.77	13.8±5.7	1730± 840
Controls	76.5± 6.2	48.6± 4.8	31.8± 4.9	1.57±0.35	11.7±2.5	2345± 864

* p<0.001

** p<0.01

*** p<0.005

Mean ± SD (%)

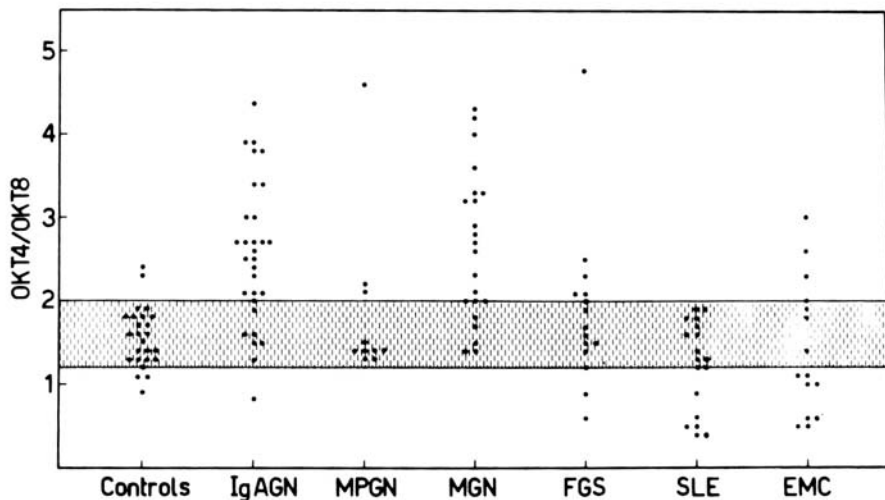


Figure 1. Representation of OKT4⁺/OKT8⁺ ratios in patients with GN and in control subjects. The shaded area represents the mean \pm SD of the normal controls

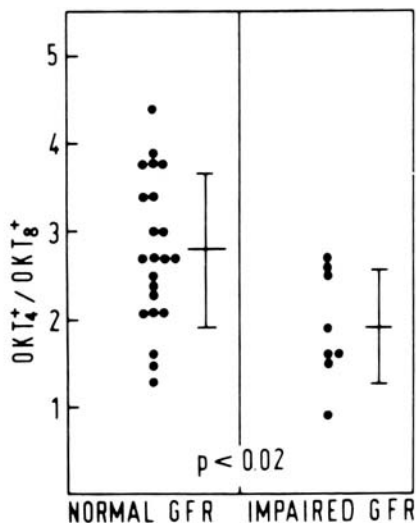


Figure 2. Representation of OKT4⁺/OKT8⁺ ratio in patients with Berger's disease with normal or impaired GFR

when compared with normal controls. In both groups of patients a significant decrease in OKT4⁺ cell percentage was found ($p < 0.01$) (Table III) and Figure 1).

Discussion

Experimental and clinical evidence has suggested that a defective immune response to unidentified agents may explain the generation and the glomerular deposition of immune complexes in primary GN as well as in GN associated with systemic disease (SLE, essential mixed cryoglobulinaemia, Henoch-Schönlein syndrome).

The new insights into the regulation of the immune response by lymphocyte subpopulations have suggested that a T-cell imbalance may be involved in the pathogenesis of GN.

Recently monoclonal antibodies have become available for detection of lymphoid T-cell subsets by immunofluorescence; this has prompted us to assess the distribution of T-cell subsets in the peripheral blood of patients with GN.

In primary chronic GN we have found an increased OKT4⁺/OKT8⁺ ratio in IgA-GN and MGN, suggesting a prevalence of helper/inducer over the suppressor/cytotoxic T-cells in these diseases. Our results are similar to those of Chatenoud and Bach [1] and correlate well with the decrease in IgA-specific suppressor T-cell activity described by Sakai et al [2] in IgA-GN, though our patients with high serum IgA did not show a significant decrease in OKT8⁺ cells compared with patients with normal serum IgA. Moreover in Berger's disease we have found a higher OKT4⁺/OKT8⁺ ratio in patients with a normal GFR in comparison to patients with impaired GFR, suggesting a disappearance of T-cell aberration from the onset of renal failure.

In patients with EMC a depressed mitogenic reactivity to phytohaemagglutinin and Con-A has been described [6]. This finding supports the hypothesis that in this disease a qualitative and/or quantitative T-cell defect may be operating.

Studies of patients with SLE have frequently shown diminution in function and number of suppressor cells [4–6], although results discordant with this pattern have been reported [10].

In EMC and SLE we have found a rather low OKT4⁺/OKT8⁺ ratio, also in patients not on steroid therapy, due to a significant decrease of OKT4⁺ cell percentage. These results are in contrast to the low proportion of T γ -cells reported by some authors in SLE [6] but it is possible that immune complexes modulate the expression of Fc γ in T γ -cells.

Great caution should be exercised in the interpretation of studies concerning T-cell subsets, as their peripheral distribution does not always correlate with their functional capacity and we suggest that in some cases an increased number of a determinate population may reflect a numerical compensation to a decreased function.

In conclusion, our findings support the evidence of an immunoregulatory imbalance in some forms of GN. Prospective studies on T-cell subsets analysed by both surface markers and functional in vitro assay are now necessary to further support this hypothesis.

Acknowledgment

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Open Discussion

GABRIEL (London) May I ask if you have made any observations with your monoclonal antibody technique in siblings of those patients living in the same homes as those who presented with acute glomerular disease because they should be enjoying the same environment and one may learn just that little more? This is also relevant to the previous paper and probably the next.

FORNASIERI No, we have not looked at this aspect.

THAYSEN (Chairman) Dr Fornasieri have you studied patients with SLE nephritis before they were treated and then after to compare their status?

FORNASIERI Yes, we have studied two patients with SLE before treatment and after treatment. After treatment with methylprednisolone in these patients there was an increase of OKT⁺4/OKT⁺8 ratio but this in acute terms. Our patients are on low doses of drugs and recently evidence has shown that methylprednisolone does not have this effect in low dosage.