Clin. exp. Immunol. (1990) **81**, 250–255

Rheumatoid factors and glomerulonephritis

M. MIYAZAKI, M. ENDOH, T. SUGA, N. YANO, T. KURAMOTO, Y. MATSUMOTO, K. EGUCHI, M. YAGAME, M. MIURA, Y. NOMOTO & H. SAKAI *Department of Internal Medicine, School of Medicine, Tokai University, Isehara, Kanagawa, Japan*

(Accepted for publication 9 February 1990)

SUMMARY

It is presently unknown whether rheumatoid factors have a pathogenic role in the development of various types of glomerulonephritis with immune deposits. Three isotypes of rheumatoid factors (RFs), which are autoantibodies to IgG, were measured using the solid-phase fluorescence immunoassay in sera from patients with diffuse proliferative lupus nephritis (DPLN), membranous lupus nephritis (MLN), IgA nephropathy (IgAN) and idiopathic membranous nephropathy (MN). RF activity of immunoglobulins deposited in the glomeruli from these patients was also studied by examining the binding of the FITC-conjugated human IgG and Fc portion of IgG to the glomeruli of renal biopsy specimens. IgG, IgA and IgM RFs were significantly increased in sera from patients with DPLN, and the increase was significantly lower in patients with MLN, IgAN and MN. Human IgG bound to immunoglobulin on the glomeruli only in DPLN, but not in MLN, IgAN or MN. The Fc portion of IgG was demonstrated to be involved in this reaction. It was suggested that RFs and IgG may play a major role in immune deposits on the glomeruli in DPLN and may be involved in the development of DPLN; however, this is not likely in MLN, IgAN or MN.

Keywords rheumatoid factor glomerulonephritis diffuse proliferative lupus glomerulonephritis

INTRODUCTION

Glomerulonephritis with immune deposits is presumed to be induced by depositions of immune complexes in the glomeruli. Deposition of immune complexes in the glomeruli is considered to induce glomerulonephritis including lupus nephritis, IgA nephropathy (IgAN) and idiopathic membranous nephropathy (MN).

In the development of lupus nephritis, DNA has been implicated as the antigen responsible for the formation of nephritogenic immune complexes (Koffler, Shur & Kunkel, 1967). IgAN has been recognized as an entity of primary chronic glomerulonephritis (Berger, 1969). Circulating IgA immune complexes have been demonstrated in patients with IgAN (Egido *et al.*, 1983). However, pathogenesis of IgAN and the antigens responsible for this disorder have not yet been identified. MN is also considered to be a prototype of immune complex-mediated disease because of its characteristic feature of granular immune deposits along the glomerular capillary walls, but antigens in this disease have not been defined except for hepatitis B (HB) antigen in HB nephropathy (Combes *et al.*, 1971). Recently, rheumatoid factors (RFs), which are autoantibodies to IgG, have been assumed to have some pathogeneic role in the development of some types of glomerulonephritis (Rossen *et al.*, 1975; McIntosh *et al.*, 1978).

Correspondence: Masanobu Miyazaki, MD, Department of Internal Medicine, Tokai University, Isehara, Kanagawa, 259-11, Japan.

The purpose of this study was to examine RF activities of immunoglobulins in sera and in the glomeruli of patients with lupus nephritis, IgAN and MN in order to elucidate the participation of RFs in the development of glomerulonephritis.

MATERIALS AND METHODS

Patients

Seven patients with lupus nephritis, five with primary IgAN, and five with idiopathic MN were studied. The diagnosis was confirmed by open renal biopsy, and biopsy specimens were examined by light microscopy, immunofluorescence staining and electron microscopy. All patients with lupus nephritis fulfilled the 1982 revised criteria for SLE (Tan *et al.*, 1982). Five of these patients had diffuse proliferative lupus nephritis (DPLN) and two had membranous nephropathy (MLN). None of the patients had received glucocorticoids or any other immunosuppressive drugs prior to the study.

Serological studies

Levels of serum IgG, IgA, IgM, C4 and C3 were measured by laser nephelometry (Behring Institute, FRG). C1q-binding immune complexes (C1q-ICs) were determined by a solid-phase enzyme immunoassay (Special References Laboratories, Tokyo, Japan). Anti-nuclear factors (ANF) were determined by indirect immunofluorescence staining using chicken erythrocytes and human granulocytes as substrates. Anti-native DNA

Table 1. Amounts of proteinuria and haematuria, and serum levels of IgG, IgA, IgM, C3, and C4 in patients with glomerulonephritis (normal values in parentheses)

Case no.	Diagnosis	Proteinuria g/day (<0·01)	Haematuria* (<1)	Serum levels, mg/dl IgG (1110–1820)	IgA (140–350)	IgM (50–180)	C3 (70–110)	C4 (20–40)
1	DPLN	0·46	10–50	2446	229	151	27	6
2	DPLN	1·43	10–50	3071	179	223	13	4
3	DPLN	5·61	10–50	592	163	124	15	6
4	DPLN	0·18	1–5	2312	184	211	17	3
5	DPLN	0·80	1–5	2465	416	697	41	5
6	MLN	1·00	10–50	2804	135	118	30	8
7	MLN	7·72	50–100	262	159	116	23	8
8	IgAN	0·85	10–50	1408	489	100	96	56
9	IgAN	1·13	100	1785	601	126	74	23
10	IgAN	1·96	50–100	877	423	167	68	35
11	IgAN	0·47	10–50	1133	487	149	86	35
12	IgAN	2·25	10–50	896	496	376	74	64
13	MN	4·08	1–5	697	207	149	74	30
14	MN	6·00	1–5	431	143	77	77	56
15	MN	0·66	1–5	1315	200	102	71	38
16	MN	0·95	<1	1326	346	63	77	45
17	MN	3·87	1–5	257	116	159	71	32

DPLN, Diffuse proliferative lupus nephritis; MLN, membranous lupus nephritis; IgAN, IgA nephropathy; and MN, idiopathic membranous nephropathy.

* Erythrocytes/high power field.

antibodies were determined by indirect staining of the kinetoplast of *Crithidia luciliae* (Fluoro nDNA test, MBL Laboratories, Nagoya, Japan). Latex fixation tests (RA test) were performed by aggregation of latex beads coupled with Cohn fraction II (RA test, Eiken Chemicals, Tokyo, Japan). Haemagglutination of sheep erythrocytes fixed with denatured rabbit IgG fraction (RAHA test, Fujirebio, Tokyo, Japan) was also performed in all patients. IgG, IgA and IgM RFs were measured by a solid-phase fluorescence immunoassay (FIA) as previously described (Endoh, Suga & Sakai, 1985). Briefly, polyacrylamide beads (Bio-Rad, Richmond, CA) were coupled covalently with rabbit IgG fraction (Cappel Laboratories, Cochranville, PA). These immunobeads were incubated with the sera from the patients at 4°C overnight. Then, FITC-conjugated anti-human IgG, IgA or IgM antibody (Behring Werke) was incubated with the beads at 4°C overnight. After washing, the fluorescence intensities of these reagents were measured by a fluorescence spectrophotometer (Hitachi MPF-4, Japan). The fluorescence value was used as a titre of RF.

Rheumatoid factor activity within the glomeruli

Sections of renal biopsy specimens (4-μm thick) were placed on fluorescence-free slide glasses, and stained with FITC-conjugated human IgG (0·05 mg/ml, F/P ratio 2·0) solution at 4°C overnight. After washing, these samples were observed using a fluorescence microscope (Zeiss) and the fluorescence intensities of the combined human IgG were scored from 0 to 3+. Human IgG fraction used in this study was prepared from pooled sera of healthy adults by precipitation with sodium sulphate, followed by DEAE-cellulose chromatography and gel filtration on Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden). The prepared IgG did not contain any other immunoglobulins, as confirmed by immunoelectrophoresis and immunodiffusion. ELISA showed IgA and IgM contamination of below 10 ng/ml. This fraction was conjugated with FITC (Sigma, St Louis, MO) using the method of Kawamura (1977).

In four patients with lupus nephritis (Cases 1, 2, 4 and 6), slide glasses with tissue sections were incubated in PBS at 56°C for 30 min prior to staining with FITC- conjugated human IgG to exclude the possibility of fixation of IgG via complement components. In patients with DPLN, renal biopsy specimens were also stained with FITC-conjugated human serum albumin, $F(ab')_2$ of IgG and the Fc portion of IgG (obtained from Cappel Laboratories) to observe which portion is mandatory for binding.

Statistical analysis

Statistical analysis was performed by the Mann–Whitney *U*-test.

RESULTS

Results of serological studies are shown in Tables 1 and 2. Serum IgG levels were significantly decreased in patients with massive proteinuria including two patients with MN ($P<0·01$). Conversely, serum IgG levels were increased in five patients with lupus nephritis with mild proteinuria ($P<0·01$). Serum IgA levels were increased in all patients with IgA nephropathy ($P<0·01$) and in one patient with DPLN. Serum IgM levels were increased in three DPLN patients ($P<0·05$) and in a patient with IgA nephropathy. Levels of C3 and C4 were significantly decreased ($P<0·01$) and those of C1q-ICs were significantly increased ($P<0·01$) in all patients with lupus nephritis. Latex fixation tests were positive in two patients with DPLN. The rheumatoid arthritis haemagglutination test was positive in one patient with DPLN.

IgG, IgA and IgM RFs were measured by FIA. Normal values are shown in Table 2. Serum IgG RFs were significantly

Table 2. Amounts of anti-nuclear antibodies, immune complexes and rheumatoid factors in patients with glomerulonephritis

Case no.	Diagnosis	Anti-nDNA	ANF	C1q-IC (<1·5 μg/ml)	RA	RAHA (<40)	IgG RF (<400)	IgA RF (<60)	IgM RF (<40)
1	DPLN	+	+++ (diffuse)	9·7	−	<40	636	210	86
2	DPLN	++	+++ (shaggy)	51·0	+	<40	1100	230	130
3	DPLN	+	+++ (shaggy)	4·8	−	<40	1700	138	210
4	DPLN	++	+++ (shaggy)	11·8	−	<40	1318	270	116
5	DPLN	−	+++ (diffuse)	3·8	+	5120	700	110	70
6	MLN	+	++ (speckled)	24·6	−	<40	400	40	34
7	MLN	+	+ (speckled)	3·7	−	<40	355	28	60
8	IgAN	−	−	<1·5	−	<40	360	75	32
9	IgAN	−	−	<1·5	−	<40	384	24	54
10	IgAN	−	−	<1·5	−	<40	296	30	48
11	IgAN	−	−	<1·5	−	<40	306	54	38
12	IgAN	−	−	<1·5	−	<40	306	54	38
13	MN	−	−	<1·5	−	<40	86	20	48
14	MN	−	−	<1·5	−	<40	100	18	6
15	MN	−	−	<1·5	−	<40	240	30	32
16	MN	−	−	<1·5	−	<40	136	28	14
17	MN	−	−	<1·5	−	<40	38	6	46

Normal values are indicated in parentheses.
Intensity score: −, negative; +, slightly; ++, moderately; and +++, strongly positive.

Table 3. Immunofluorescence studies and bindings of FITC-conjugated human immunoglobulins on the glomeruli of renal biopsy specimens

Case no.	Diagnosis	Immunofluorescence studies*						Bindings of FITC-labelled IgG
		IgG	IgA	IgM	C1q	C3	Properdin	
1	DPLN	+++	+++	++	+++	++	+	++
2	DPLN	+++	++	++	++	++	+	++
3	DPLN	+++	+	+	+++	++	+++	++
4	DPLN	+++	+++	+++	+++	+++	++	++
5	DPLN	+++	++	++	++	++	+	++
6	MLN	+++	++	++	++	++	−	−
7	MLN	+++	+	+	+++	+++	+	−
8	IgAN	−	+++	+	+	++	+	−
9	IgAN	++	+++	++	+	+++	+	−
10	IgAN	+	+++	+	+	++	+	−
11	IgAN	++	+++	++	+	++	++	−
12	IgAN	+	+++	+	−	+++	+	−
13	MN	+++	+	++	−	+++	+	−
14	MN	+++	+	+	+	+	−	−
15	MN	+++	+	++	+	+	−	−
16	MN	+++	+	+	−	+	+	−
17	MN	+++	+	+	+	+	+	−

* All except for properdin, from antisera were obtained from Behringwerke (Marburg, FRG), Kent Laboratories, North Vancouver, Canada. Intensity score as in Table 2.

increased in all patients with DPLN ($P<0·01$). IgA RFs were significantly increased in all patients with DPLN ($P<0·01$) and in one patient with IgAN. IgM RFs were also significantly increased in all patients with DPLN ($P<0·01$), in one patient with MLN, in three patients with IgAN and in two with MN. No correlation was observed between the levels of serum RFs and serum immunoglobulin. The degrees of immunofluorescence staining of kidney biopsy specimens are shown in Table 3. IgG was the dominant immunoglobulin class in depositions on the glomeruli in patients with DPLN, MLN and MN, but IgA was the dominant class in patients with IgAN. However, depositions of other immunoglobulin classes were present to a lesser degree and complement components were observed with different intensities in all patients examined in this study.

FITC-labelled human IgG was bound to the glomerular sections from all patients with DPLN (Table 3, Fig. 1). The FITC-conjugated Fc portion of human IgG was also bound to the glomeruli of DPLN (Fig. 2), but both FITC-conjugated human serum albumin and FITC-conjugated F(ab')2 of IgG did not bind to immunoglobulins on the glomeruli. These bindings

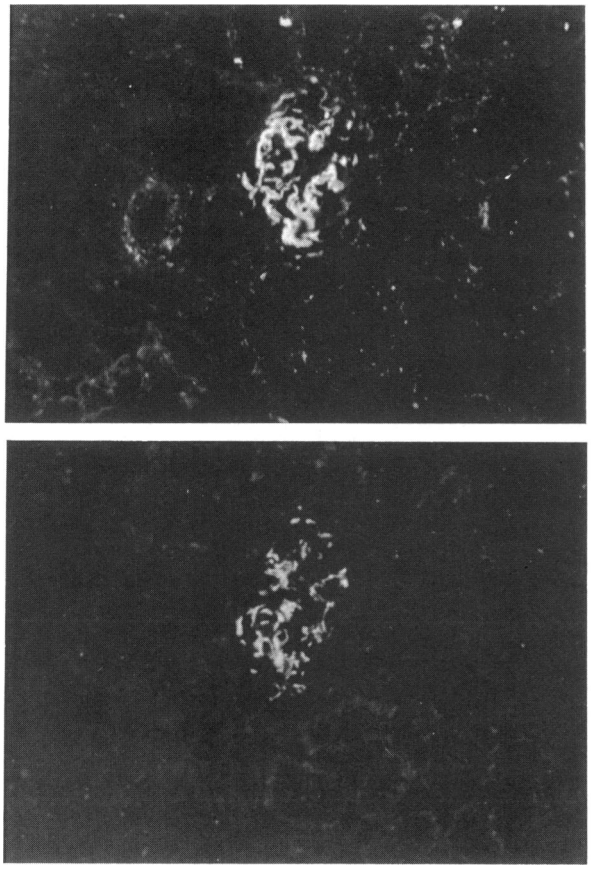

Fig. 1. Top: kidney biopsy specimen from a patient with DPLN stained with FITC-conjugated anti-human IgG antiserum; bottom: biopsy specimen from the same patient stained with FITC-conjugated human IgG fraction. The staining pattern was similar to that stained with anti-IgG serum shown in the top.

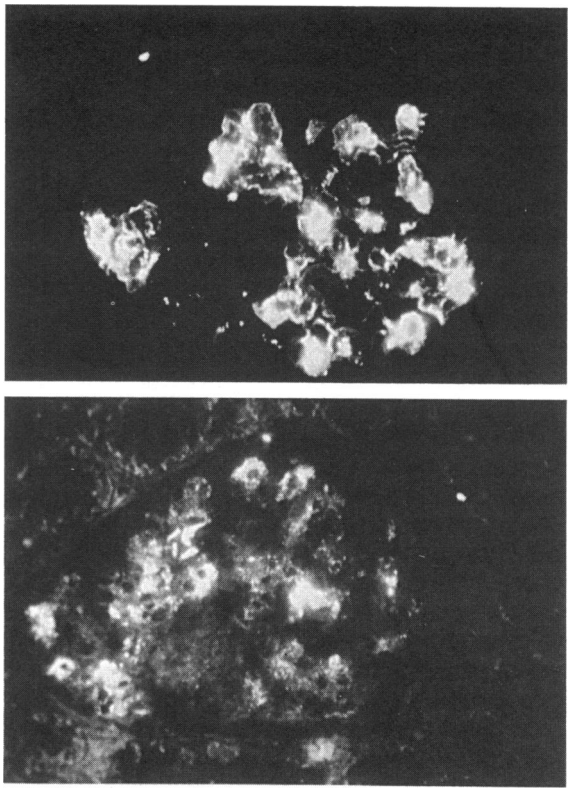

Fig. 2. Top: deposits of FITC-conjugated human IgG were shown in the glomeruli of kidney biopsy specimen from a patient with DPLN; bottom: biopsy specimen from the same patient stained with FITC-conjugated Fc portion of human IgG. Both human serum albumin and $F(ab')_2$ fragment of IgG did not bind to immunoglobulins in the glomeruli from patients with DPLN.

were observed even in glomeruli previously heated at 56°C for 30 min. Binding of FITC-human IgG was observed in the same areas in which IgG was already deposited as immune complexes (Figs 1, 2). FITC-IgG was not combined with immunoglobulins on the glomeruli from patients with MLN, IgAN or MN (Figs 3, 4).

DISCUSSION

The precise mechanism of the development and exacerbation of chronic glomerulonephritis has not been elucidated. Immune complexes which are circulating or formed *in situ* have been assumed to be major mediators in the development of glomerulonephritis. However, antigens responsible for the formation of immune complexes have not been fully elucidated except for DNA in lupus nephritis (Koffler *et al.*, 1967), streptococcal antigen in acute post-streptococcal glomerulonephritis (Seegal *et al.*, 1965; Michael *et al.*, 1966), and HB antigen in HB-related membranous nephropathy (Combes *et al.*, 1971). Recently, it has been postulated that RF may play a pathogenic role in the development of some types of glomerulonephritis. McIntosh *et al.*, (1978) demonstrated localization of anti-IgG in the kidneys of patients with acute post-streptococcal glomerulonephritis. Sugisaki *et al.*, (1982) reported that the deposits in renal grafts in *de novo* membranous nephropathy were composed of IgG and anti-IgG immune complexes. It was also reported that glomerular immune deposits in patients with cryoglobulinaemia glomerulonephritis showed RF activity (Maggiore *et al.*, 1982; Sinico *et al.*, 1988). Furthermore, Czerkinsky *et al.*, (1986) found that serum IgA-RF was elevated in IgAN.

In this study we demonstrated that sera from patients with DPLN showed high titres of IgG, IgA and IgM RFs. Immunoglobulins deposited in the glomeruli from these patients also bound to allogenic IgG *in vitro*. These binding activities may be due to anti-IgG activities of immunoglobulins deposited in the glomeruli because these phenomena were also observed in specimens previously heated at 56°C for 30 min to inactivate complement activities. Moreover, only whole human IgG and the Fc portion of IgG, but not $F(ab')_2$ of IgG or human serum albumin were bound to immunoglobulins on the glomeruli. These findings suggested that immunoglobulins deposited in the glomeruli with DPLN have anti-IgG activity, particularly antibody activity for the Fc portion of IgG.

Rossen *et al.* (1975) demonstrated that FITC-IgG was bound to immune deposits in the glomeruli from some patients with glomerulonephritis, including patients with DPLN. They showed that severity of the disease as judged by renal function and glomerular histology was correlated with the presence of glomerular-fixed anti-globulins. In our study, RF activities of immunoglobulins deposited on the glomeruli were specific to DPLN rather than to the disease activity. Kawasumi *et al.*

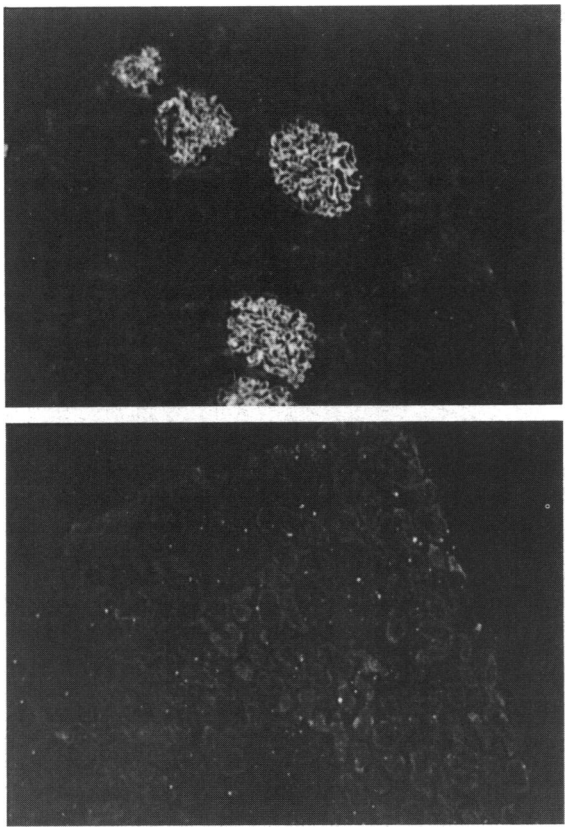

Fig. 3. Top: kidney biopsy specimen from a patient with idiopathic MN stained with FITC-conjugated anti-human IgG. Granular deposits along the capillary walls were demonstrated; bottom: the FITC-conjugated human IgG did not bind to the glomeruli.

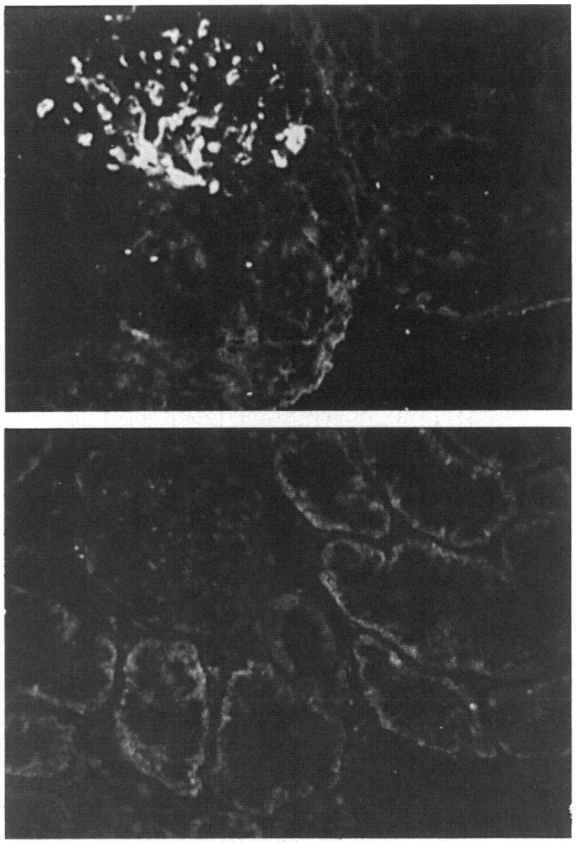

Fig. 4. Top: IgA-dominant deposits in patients with IgA nephropathy; bottom: the FITC-conjugated human IgG did not bind to glomeruli from these patients.

(1982) also demonstrated RF activity of renal immune deposits in patients with lupus nephritis, MN, and membranoproliferative glomerulonephritis using a dissociation technique with the addition of a high dose of IgG to the immune deposits as an excess antigen. Maggiore *et al.* (1981) showed IgM deposited in the glomeruli had anti-immunoglobulin activity in some cases of glomerulonephritis, which interfered with the direct immunofluorescence testing for HB surface antigen deposits in glomeruli. In (NZB/W)F1 mice, immune complex deposits in the glomeruli were shown to consist of IgG class anti-nuclear antibody and IgG class RF (Takase *et al.*, 1983). Rauch *et al.* (1986) demonstrated that certain human monoclonal antibodies have anti-DNA and RF activities. Furthermore, RFs exhibited multiple reactivities with other autoantigens, including dsDNA and histones (Rubin *et al.*, 1984). It is considered that immunoglobulins deposited in the glomeruli from patients with DPLN react not only DNA but also autologous IgG. Not only DNA–anti-DNA antibody complexes, but also RF and IgG complexes might be implicated in some way in the pathophysiology of DPLN. Our present data favour the previous observation that anti-IgG–IgG complexes may be one component of immune deposits in patients with DPLN. However, high RF activity in sera or rheumatoid activity of immunoglobulins on the glomeruli could not be demonstrated in patients with MLN and MN in this study. Such results could be due to occupied antigen-binding sites of immunoglobulins on the glomeruli from patients with MLN and MN, which may also contribute to the size and localization of immune complexes on renal glomeruli with membranous lesions. It remains unknown why RF activity of glomerular Igs was detected only in DPLN. It was suggested that the pathophysiology of DPLN may be different from that of other diseases.

IgAN has been recognized as an entity of primary chronic gomerulonephritis characterized by IgA-dominant immune depositions in the mesangial area of the glomerulus (Berger, 1969). The levels of serum IgA, circulating IgA immune complexes and IgA RF have been shown to be increased in this disorder (Tomino *et al.*, 1982; Egido *et al.*, 1983; Czerkinsky *et al.*, 1986), although antigens in the immune complexes have not been elucidated. Some patients with IgA nephropathy showed increases in some isotypes of serum RFs in this study. However, the degrees were much lower than those in DPLN. In this study, immunoglobulins on the glomeruli showed no RF activities in these patients. Patterns of immune deposition of the glomeruli in patients with IgAN were similar to those in DPLN except for the fact that the dominant class of immunoglobulins deposited in the glomeruli was IgA in patients with IgAN. However, FITC-IgG could not bind to immunoglobulins on the glomeruli from these patients with IgAN. These results suggested that IgA–IgG complexes might not be involved in the immune deposits on the glomeruli from patients with IgAN. Immunoglobulins deposited on the glomeruli are detected in many kinds of glomerulonephritis, including lupus nephritis, idiopathic MN and IgAN in immunofluorescence studies. Immune complexes, circulating or formed *in situ*, are considered to be associated

with development of glomerulonephritis, but they might have various characteristics and play different roles in the pathogenesis of each type of glomerulonephritis.

It was suggested in this study that RFs and IgG complexes may be of the major components in the immune deposits on the glomeruli from patients with DPLN, but probably not in patients with MLN, MN and IgAN.

ACKNOWLEDGMENTS

This study was supported in part by grants from the Ministry of Health and Welfare, and the Ministry of Education, Science and Culture of Japan.

REFERENCES

BERGER, J. (1969) IgA glomerular deposits in renal disease. *Transplant. Proc.* **1,** 939.

COMBES, B., STASTNY, P., SHOREY, J., EIGENBRODT, E.H., BARRERA, A., HULL, A.R. & CARTER, N.W. (1971) Glomerulonephritis with deposition of Australia antigen–antibody complexes in glomerular basement membrane. *Lancet*, **ii,** 234.

CZERKINSKY, C., KOOPMAN, W.J., JACKSON, S., COLLINS, J.E., CRAGO, S.S., SCHROHENLOHER, R.E., JULIAN, B.A., GALLA, J. H. & MESTECKY, J. (1986) Circulating immune complexes and immunoglobulin A rheumatoid factor in patients with mesangial immunoglobulin A nephropathies. *J. clin. Invest.* **77,** 1934.

EGIDO, J., SANCHO, J., BLASCO, R., RIVERA, F. & HERNANDO, L. (1983) Immunopathogenic aspects of IgA nephropathy. *Adv. Nephrol.* **12,** 103.

ENDOH, M., SUGA, T. & SAKAI, H. (1985) IgG, IgA and IgM rheumatoid factors in patients with glomerulonephritis. *Nephron*, **39,** 330.

KAWAMURA, A. (1977) Preparation of labelled antibody. In *Fluorescent Antibody Techniques and their Application* (2nd edn) p.44. University of Tokyo Press, Tokyo.

KAWASUMI, H., TAKASE, S., SHIBATA, T., SAITO, K., UCHIDA, J., YAMAMOTO, J., KITAZAWA, K., SATO, S., YONEKURA, M., ITO, S., SHIWACHI, S., KAN, K. & SUGISAKI, T. (1982) A study on immune complex deposits in glomeruli of membranous nephropathy, membranoproliferative glomerulonephritis nephritis and lupus nephritis. *Jpn. J. Nephrol.* **24,** 625.

KOFFLER, D., SHUR, P. & KUNKEL, H.G. (1967) Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. exp. Med.* **126,** 607.

MAGGIORE, Q., BARTOLOMEO, F., L'ABBATE, A. & MISEFARI, V. (1981) HBsAg glomerular deposits in glomerulonephritis: fact or artifact? *Kidney Int.* **19,** 579.

MAGGIORE, Q., BARTOLOMEO, F., L'ABBATE, A., MISEFARI, V., MARTORANO, C., CACCAMO, A., DI BELGIOJOSO, G.B., TARANTINO, A & COLASANTI, G. (1982) Glomerular localization of circulating antiglobulin activity in essential mixed cryoglobulinemia with glomerulonephritis. *Kidney Int.* **21,** 387.

MCINTOSH, R.M., GARCIA, R., RUBIO, L., RABIDEAU, D., ALLEN, J.E., CARR, R.I. & RODRIGUEZ-ITURBE. (1978) Evidence for an autologous immune complex pathogenic mechanism in acute poststreptococcal glomerulonephritis. *Kidney Int.* **14,** 501.

MICHAEL, A.F., DRUMMOND, K.N., GOOD, R.A., VERNIER, R.L., OPSTAD, A.M. & LOUNBERG, J.E. (1966) Acute poststreptococcal glomerulonephritis: immune deposit disease. *J. clin. Invest.* **45,** 237.

RAUCH, J., TANNENBAUM, H., STRAATON, K., MASSICOTTE, H. & WILD, J. (1986) Human–human hybridoma autoantibodies with both anti-DNA and rheumatoid factor activities. *J. clin. Invest.* **77,** 106.

ROSSEN, R.D., REISBERG, M.A., SHARP, J.T., SUKI, W.N., SCHLOEDER, F.X., HILI, L.L. & EKNOYAN, G. (1975) Antiglobulins and glomerulonephritis: classification of patients by the reactivity of their sera and real tissue with aggregated and native human IgG. *J. clin. Invest.* **56,** 427.

RUBIN, R.L., BALDERAS, R.S., TAN, E.M., DIXON, F.J. & THEOFILOPOULOS, A.N. (1984) Multiple autoantigen binding capabilities of mouse monoclonal antibodies selected for rheumatoid factor activity. *J. exp. Med.* **159,** 1429.

SEEGAL, B.C., ANDRES, G.A., HSU, K.C. & ZABRISKIE, J.B. (1965) Studies on the pathogenesis of acute and progressive glomerulonephritis in man by immunofluorescein and immunoferritin techniques. *Fed. Proc.* **24,** 100.

SINICO, R.A., WINEARLS, C.G., SABADINI, E., FORNASIERI, A., CASTIGLIONE, A. & D'AMICO, G. (1988) Identification of glomerular immune deposits in cryoglobulinemia glomerulonephritis. *Kidney Int.* **34,** 109.

SUGISAKI, T., KANO, K., BRENTJENS, J.R., ANTHONE, R., ANDRES, G.A. & MILGROM, F. (1982) Immune complexes in a renal allograft with de novo membranous nephropathy. *Transplantation*, **34,** 90.

TAKASE, S., SOEDA, K., KAWASUMI, H., SAITO, K., SHIBATA, T., UCHIDA, J., KITAZAWA, K., YAMAMOTO, J., YONEKURA, M., ITO, S., SHIWACHI, S. & SUGISAKI, T. (1983) A study of immune complex deposits (IgG–IgG–ANA/rheumatoid factor) in glomeruli of NZB/W F1 mice. *Jpn. J. Nephrol.* **25,** 949.

TAN, E.M., COHEN, A.S., FRIES, J.F., MASI, A.T., MCSHANE, D.J., ROTHFIELD, N.F., SCHALLER, J.G., TALAL, N. & WINCHESTER, R.J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25,** 1271.

TOMINO, Y., SAKAI, H., ENDOH, M., KANESHIGE, H. & NOMOTO, Y. (1982) Detection of immune complexes in polymorphonuclear leukocytes by double immunofluorescence in patients with IgA nephropathy. *Clin. Immunol. Immunopathol.* **24,** 63.