IgA Nephropathy: Clinicopathological and Immunological Studies*

Vijitr Boonpucknavig, M.D.
Somnate Boonpucknavig, M.D.
Rachanee Udomsangpetch, M.Sc.

SUMMARY **Studies performed on 16 consecutive patients with IgA nephropathy showed no correlation between clinical severity, disease duration, histopathological and immunological findings. The only correlation observed was between the pattern and intensity of IgA deposits and mesangial alteration. Certain results of our immunological studies were similar to the reports of a group of Japanese authors.**

ASIAN PACIFIC J ALLERG IMMUN 1983;1 : 117-122.

In 1969 Berger[1] described a form of glomerular disease characterized by the deposition of IgA with a lower intensity of IgG, IgM and C3 in mesangial areas. The patients presented micro- or macroscopic haematuria without any systemic disease. This condition has been called IgA nephropathy or Berger's disease.

Since the original description, many investigations on the immunological status and immune regulation of this disease have been done.[2-7] However, the pathogenesis of this glomerular disease is still not clear. Moreover, the results of immunological studies in some groups of patients in one country may differ from those of others.[8,9]

The aim of the present study, therefore, was the detection of immunological abnormalities resulting from IgA nephropathy in Thai patients compared with both normal, healthy controls and the findings of studies in other countries. This investigation was also designed to find any correlation between clinical and histopathological manifestations and immunological activity.

MATERIALS AND METHODS

Patients

IgA nephropathy was diagnosed using renal biopsy studies in 136 patients between 1970 and 1982. None of these patients had any history or clinical findings with regard to systemic problems. Nine unselected male and seven female patients ranging in age from 12 to 38 years were included in the present study. All of the latter patients had micro- or macroscopic haematuria with or without proteinuria. Some of them had abnormal blood pressure at the time of biopsy.

Peripheral blood and sera from 20 healthy subjects were studied for control purposes.

Renal biopsy study

Specimens were fixed for light microscopy in Zenker's solution and embedded in paraffin. Sections were cut 2-4 μ in thickness and stained with haematoxylin and eosin, periodic acid-Schiff, periodic acid-silver methenamine and Masson's trichrome.

Immunofluorescence studies were performed on cryostat sections stained with fluorescent conjugated monospecific antisera against IgG, IgM, IgA, C3 and fibrinogen (Hyland). All antisera were absorbed by rat liver homogenate for removing the non-specific staining.

Serological studies

Auto-antibodies were detected by indirect fluorescence antibody technique using various antigenic substrate, i.e. nuclei of rat liver cells for organ nonspecific antinuclear antibodies (ON-ANA), nuclei of polymorphonuclear leukocytes for granulocyte-specific antinuclear antibodies (GS-ANA), epithelial cells of human thyroid gland (TECA), gastric parietal cells of rat stomach (APCA), smooth muscle cells of rat stomach (SMA) and mitochrondria of rat kidney tubular cells (AMA).

*From the Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, and the Immunopathology Division, Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

Serum diluted 1:16 was applied for half an hour to the substrate. After washing, the sections were treated for half an hour with fluorescent labelled polyvalent and monospecific antihuman globulin reagents directed against IgG, IgM or IgA. Then the sections were washed, mounted and examined under the fluorescent microscope. Cold-reaction of antinuclear factor (ANF) was detected using the same method as previously described.[10] Anti-DNA antibody was evaluated by radio-immunoassay using the Farr assay. Antibody to extractable nuclear antigens (anti-ENA) was detected by an enzyme-linked immunosorbent assay (ELISA).[11] Serum level of IgA was evaluated by single radial immunodiffusion technique.

Detection of B-lymphocytes and T-lymphocytes

Mononuclear leukocytes obtained from the peripheral blood were separated using the method that has been described.[12] Lymphocytes bearing surface immunoglobulin were detected by immunofluorescent staining of unfixed cell suspension. Each 0.1 ml containing 5 x 10^5 cells was incubated with 0.025 ml of conjugated monospecific goat antihuman IgG, IgM and IgA at 4°C. The positive cells were counted on a fluorescent microscope equipped with incident light (Ortholux II, Leitz). For the detection of lymphocytes containing immunoglobulins in the cytoplasm, the same method was used as previously described.[12] The evaluation of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes was performed using the binding of erythrocyte sensitized antibody and complement (EAC), the method described by Bianco *et al.*[13]

T-lymphocyte subsets were assessed by sheep erythrocyte rosette technique.

Immune complexes assays

Circulating immune complexes (CIC) were detected using the following methods: (a) inhibition of IgG-coated latex agglutination with rheumatoid factor (RF) as has been described,[14] (b) inhibition of complement-dependent lymphocyte rosette formation as described by Gluckman *et al.*, [15] and (c) rapid polyethylene glycol precipitation complement comsumption method (PEG-CC) as described by Brandslund *et al.*[16]

RESULTS

Table 1 shows that surface IgA-bearing lymphocytes were significantly increased in the peripheral blood of patients with IgA nephropathy as compared to normal controls ($P<0.05$), whereas the number of IgM-bearing lymphocytes was decreased significantly ($P<0.005$), and that of IgG-bearing lymphocytes did not show significant changes. In contrast, the number of cytoplasmic IgA-containing lymphocytes in patients was decreased significantly ($P<0.0005$). The absolute number of T-lymphocytes was significantly lower than in controls ($P<0.01$). The number of lymphocytes which bound EAC in the peripheral blood of patients was slightly higher than in the controls without being statistically sig-

Table 1 Immunological findings in IgA nephropathy.

Case No.	Lymphocyte bearing Ig on surface (No./mm³)			Lymphocyte containing Ig in cytoplasm (No./mm³)			T (E) (No./mm³)	EAC (No./mm³)	Autoantibodies (See text)				Circulating immune complexes			Serum IgA (mg/dl)
	IgG	IgM	IgA	IgG	IgM	IgA			ANF	SMA	AMA	APCA	Latex inhitition	EAC inhibition	PEG-CC	
1	53.8	35.8	39.2	0	7.8	0		50.4					0	neg	neg	1000
2	121.3	74.2	96.6	24.7	15.7	0	1312.2	20.2					1:64		neg	600
3	83.3	7.8	5.2	143.1	36.4	80.6	866.3	ND					1:32	+91.66%	+	110
4	149.2	8.5	4.2	14.9	4.2	6.4	527.7	226.8					0	neg	neg	470
5	ND	8.4	ND	30.8	16.8	5.6	546.9	165.5					1:16	neg	neg	440
6	35.4	5.9	1.9	25.5	13.7	3.9	204.4	145.5	+				1:8	+88.7%	+	560
7	34.5	4.9	0	162.7	69.0	44.4	1197.9	187.3					1:8	+43.71%	+	325
8	21.3	7.2	17.3	11.3	14.9	7.6	299.5	39.8	+				0	neg	neg	415
9	67.9	57.9	0	40.2	5.0	47.8	1432.4	151.0					1:16	neg	neg	345
10	97.3	18.0	43.2	7.2	28.6	19.8	956.0	126.1					0	neg	neg	
11	122.5	26.1	169.9	26.1	17.9	13.1	1045.8	214.1					0	neg	neg	
12	244.5	56.4	7.5	63.9	15.0	37.6	1023.2	357.4					1:16	+53.7%	+	500
13	244.6	44.9	26.9	5.9	11.9	5.9	1149.8	230.6					1:8	neg	neg	500
14	80.9	14.5	0	14.5	10.4	4.1	323.7	80.7					1:32	neg	neg	170
15	96.03	51.92	108.89	46.65	11.91	48.60	961.25	188.47					0	neg	neg	500
16	257.25	81.9	17.85	235.4	263.8	65.8	1670.2	92.0					0	+63.86%	+	
Mean	102.31	28.16	71.71	50.37	18.62	25.03	845.51	155.99								523.21
±SD	66.91	23.40	55.49	50.30	16.23	24.39	396.74	90.47								353.22
(Control)																
Mean±	97.93	60.09	29.56	66.0	29.9	59.2	1303.9	113.0					0	neg	neg	304.0
SD	49.35	34.47	19.13	51.1	24.0	42.4	700.8	84.2								120.0
P value*	NS	0.005	0.05	NS	NS	0.0005	0.01	NS								NS

*significant difference from normal at 95% level; NS = non significant.

Table 2 Summary of clinical, histological and immunopathological findings.

Case No.	Sex/age (yr)	Clinical features	Duration of disease prior to biopsy (month)	B.P.	Urine		Renal function		No. of glomeruli with					Glomerular deposition					Pattern of IgA deposition	
					Protein DS/g in 24 h	RBC /HPF	BUN (mg/dl	Cr (mg/dl)	N	MC	C	FS	OB	IgG	IgM	IgA	C3	Fibrin	Mes	GCW
1	F/23	MH	36	N	3+	numerous	24	1.5	0	14	1	0	3	1+	neg	2+	2+	neg	H	+
2	M/27	oedema	1	N	3+	few	24.5	1.5	0	16	0	0	2	1+	Tr	2+	2+	neg	H	+
3	M/12	MH	48	N	2+	6-8	N	N	0	0	6	2	0	1+	Tr	2+	1+	neg	G	+
4	F/21	MH	1 wk	N	1+/<1	10-12	N	N	0	17	0	0	1	1+	neg	3+	2+	neg	H	neg
5	F/19	MH	unknown	N	1+/<1	15-20	N	N	0	19	3	0	3	1+	1+	3+	2+	neg	H	neg
6	M/23	MH	12	N	2+/1.13	>30	2	1.0	0	30	0	0	3	2+	Tr	3+	2+	+	H	++
7	F/30	oedema	1	N	4+/<3	10-12	24	2.3	0	17	1	0	0	2+	Tr	3	2+	neg	H	+
8	M/27	oedema	1	190/100	3+	few	N	N	0	2	0	0	5	1+	1+	2+	2+	neg	H	++
9	F/27	MH	10	N	1+	numerous	18	1.2	6	0	0	1	0	1+	Tr	2+	2+	neg	G	neg
10	F/38	GH	unknown	N	3+/0.98	numerous	12	1.4	0	7	0	0	1	neg	neg	2+	2+	neg	H	+
11	F/26	GH	8	N	2+/1.39	numerous	18	1.2	0	3	0	3	1	2+	1+	2+	2+	neg	G	+
12	F/50	oedema*	3	130/100	3+/0.8	5-10	16	1.6	0	25	0	1	3	1+	neg	2+	2+	neg	G	+
13	M/30	MH	2	N	2+	10-20	18	1.2	0	8	0	1	0	2+	neg	3+	2+	neg	H	neg
14	M/21	GH	2	N	2+	30-40	12	1.1	0	8	0	3	0	2+	1+	3+	1+	neg	G	++
15	M/29	MH	10 yr	N	1+	5-6	15	1.2	0	8	0	0	1	2+	Tr	3+	3+	Tr	G	+
16	F/32	oedema	2	N	2+/3.49	>30	N	N	0	7	0	3	0	1+	1+	2+	1+	Tr	G	+

*with diabetes mellitus
DS = dip stick

GH = gross haematuria
MH = microhaematuria

N = normal

C = creascence
Cr = creatinine

MC = mesangial change
FS = focal sclerosis
OB = obsolescence

H = homogeneous; G = granular
Tr = trace; + = mild; ++ = moderate; +++ = severe
Mes = mesangial
GCW = glomerular capillary wall

nificant.

CIC were detected in nine out of the total 16 patients (56.25%) using latex inhibition, in five out of the 15 (33.3%) using EAC inhibition and in six out of the total (37.5%) using PEG-CC. The over-all results from these techniques showed CIC in 62.5 per cent of patients' sera. The mean concentration of IgA in their sera was 523.21 mg/dl which was higher than that of the controls.

Antibodies to double-stranded DNA, ENA, ON-ANA, GS-ANA and cold-reaction ANF were not detected in the patients' sera. Anti-smooth muscle antibodies were present in cases No. 6 and 8 but not in the control sera.

The clinical, histological and immunopathological findings are summarized in Table 2. All biopsies showed glomerular lesions predominantly in the mesangial areas. Mesangial enlargement with or without mesangial hypercellularity (Figs. 1 and 2) was observed in 10 cases with extensive homogeneous deposits of IgA (Fig. 3). Normal or mild mesangial cell proliferation (Fig. 4) appeared in six cases with

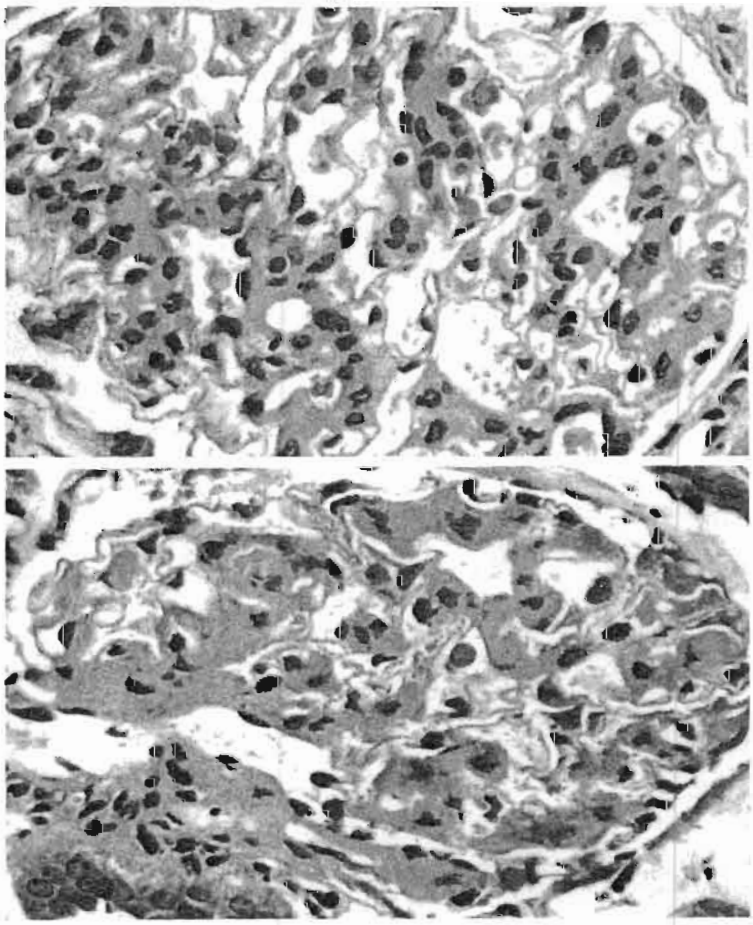

Fig. 1 Mesangial cells hyperplasia with increasing matrix in a glomerulus (case No. 5).

Fig. 2 A glomerulus with widening of mesangial areas due to increasing matrix and deposits of hyaline material (case No. 1). (Haematoxylin-eosin x 200).

Fig. 3 Same case as Fig 2, showing dense homogeneous deposits of IgA in mesangial areas. Deposits are seen along certain capillary walls. (x 200).

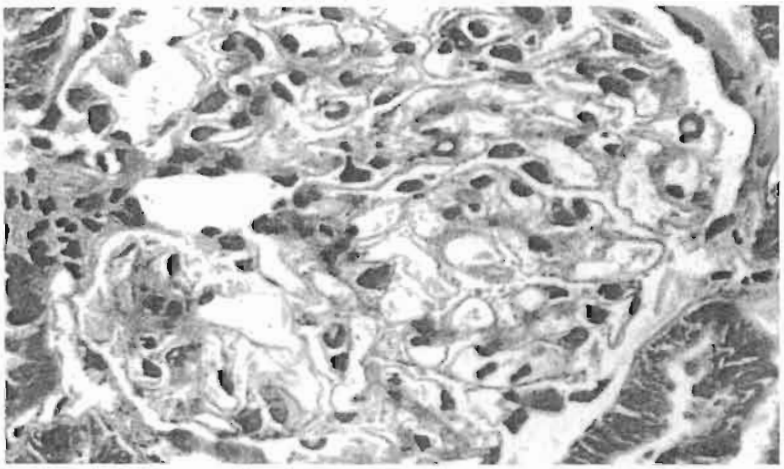

Fig. 4 A glomerulus from case No. 9 showing no definite mesangial alteration. (Haematoxylin-eosin x 200).

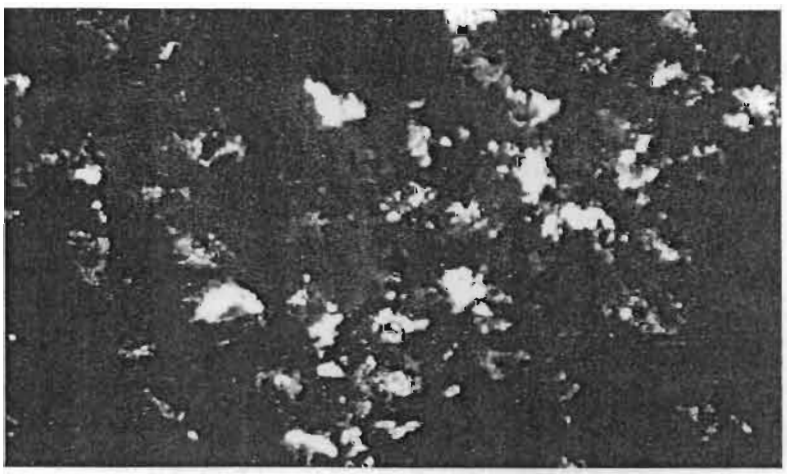

Fig. 5 Granular deposition of IgA in mesangial areas in a glomerulus of case No. 9. (x 200).

granular IgA deposits (Fig. 5). Frequently, thickening of certain capillary walls with focal or segmental sclerotic lesions was observed (Fig. 6). In such cases, deposits of IgA along the capillary walls were also noted (Fig. 7). Thin, crescentic, extracapillary proliferation occurred in four cases. Ten biopsies also presented obsolescent glomeruli (Fig. 8).

DISCUSSION

IgA nephropathy has been diagnosed in about 8.6 per cent of the biopsy studies of primary glomerular disease in Thai adults.[17] In our 16 unselected patients we failed to establish any correlation between clinical severity, disease duration, histopathological and immunological findings. The histological appearances were relatively correlated with the pattern and intensity of IgA deposits. However, the features of histological alteration in these cases made it difficult to perform histological grading as has been reported.[18] The results of the present immunological studies were more closely compareable with the findings on patients studied in Japan than those of Western countries.

The precise pathogenesis of this glomerular disease is still not clear. The reported incidence of CIC varies with the method of measurement used by different authors. They were demonstrated by Gluckman *et al*[19] to be in 74.5 per cent of their cases; by Woodroffe *et al*[2] in 43.6 per cent; and by Cairns *et al*[20] to be in 100 per cent. The over-all incidence in our present study was 62.5 per cent. IgA-CIC have been detected in about 50 per cent of patients by Stachura *et al*,[5] and Hall *et al*.[21] Moreover, IgA-CIC levels have been shown to be correlated with clinical and histological activity.[22] The detection of CIC and the presence of IgA concomitant with IgG and C3 granular deposits in the glomeruli thus provide suggestive evidence that immune complexes play some role in the pathogenesis

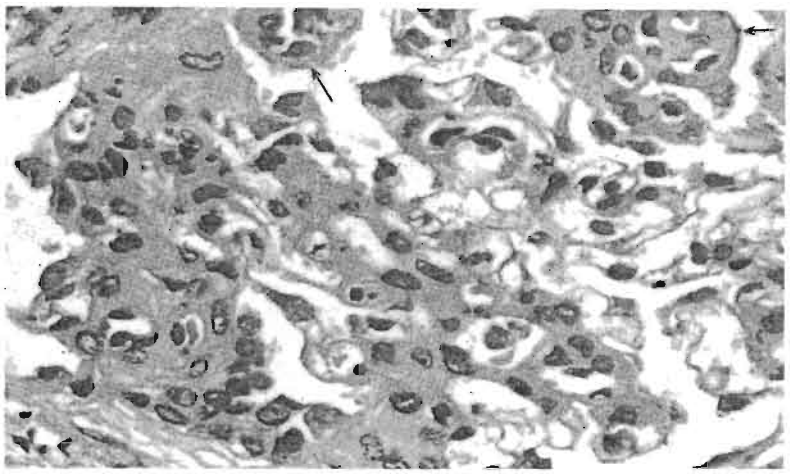

Fig. 6 Illustrating mesangial cell hyperplasia with thickening of certain capillary walls (arrows) and focal sclerosis of a glomerulus in case No. 11. (Haematoxylin-eosin x 200).

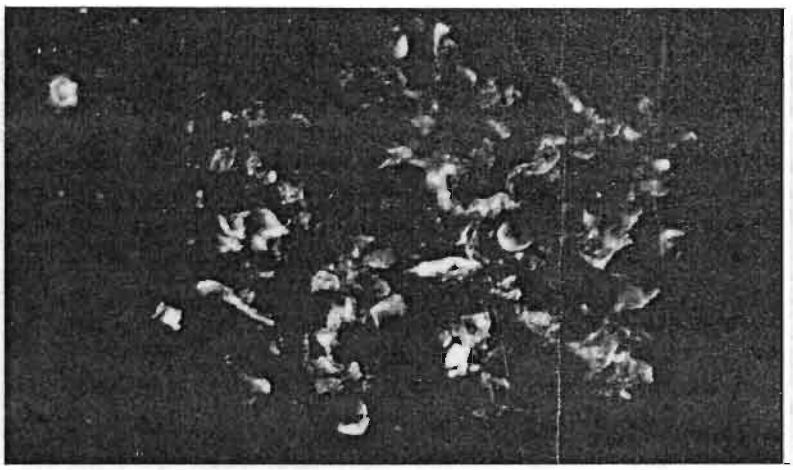

Fig. 7 Same case as Fig. 6 with IgA deposits along most of the capillary walls. (x 200).

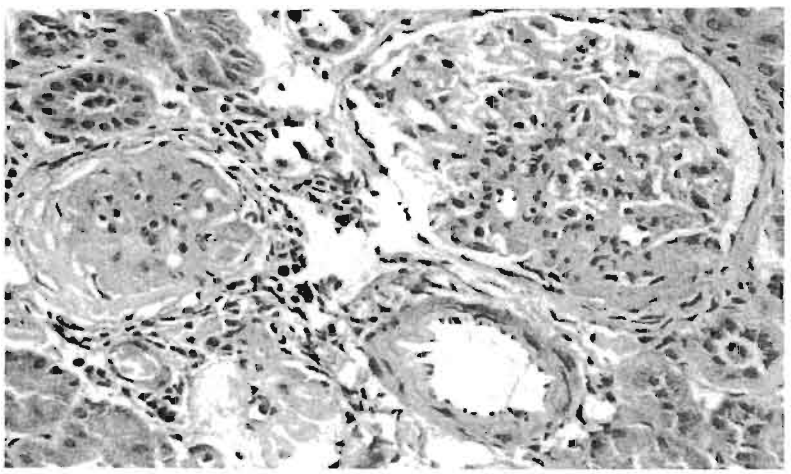

Fig. 8 Two obsolescent and one focal sclerotic glomeruli in case No. 12. (Haematoxylin-eosin x 50).

of this glomerular disease. To support this view, deposition of IgA along the blood vessel walls of the skin[23,24] and muscle[25] of patients with IgA nephropathy has been reported. The recurrence of IgA mesangial deposits in transplanted kidneys has been observed.[26] Both classical and alternative complement pathways were proven to be involved in disease processes.[27] The finding of an increase of surface IgA-bearing lymphocytes in our study was similar to the observation of Japanese patients.[4] However, normal numbers of this subpopulation of circulating lymphocytes have been recorded in Western patients.[8,9] On the other hand, the number of cytoplasmic IgA-containing cells and T-lymphocytes in our study was significantly lower than those detected in normal controls. Although most of our patients had a higher level of serum IgA than the mean level of normal controls, the figure did not reach statistical significance as has been shown in other reports.[6,28] The polymeric IgA level was found to be increased and was demonstrated in mesangial deposits[29,30] in certain groups of patients. However, these findings were not confirmed by any other study.[2]

The cold-reacting ANF that was detected in 81.8 per cent of the patients reported on by Nomoto and Sakai[10] was not observed in our study. The presence of anti-smooth muscle antibody in two of our 16 patients is interesting; this antibody has been demonstrated in about 49 per cent of Thai children with dengue viral infection.[31] In spite of the fact that IgM and IgG immune complex glomerulonephritis occurred in these children,[32] the relationship between viral infections in adults and IgA nephropathy should receive very close attention. Moreover, one needs to be aware also of the fact that certain HLA antigens were significantly associated with IgA nephropathy.[33] The genetic factor[34] among the many abovementioned factors may also be in-

volved in the development of IgA nephropathy.

ACKNOWLEDGEMENTS

The fluorescence and light microscopes used in this study were donated by the German Agency for Technical Cooperation and the Kidney Foundation of Thailand respectively. The secretarial work of Miss Peejin Rodphungpha is greatly appreciated.

REFERENCES

1. Berger J. IgA glomerular deposits in renal disease. Transplant Proc 1969; 1:939-44.
2. Woodroffe AJ, Gormly AA, McKenzie PE, et al. Immunologic studies in IgA nephropathy. Kidney Int 1980; 18:366-74.
3. Lopez Transcasa M, Egido J, Sancho J, Hernando L. IgA glomerulonephritis (Berger's disease): evidence of high serum levels of polymeric IgA. Clin Exp Immunol 1980; 42:247-54.
4. Nomoto Y, Sakai H, Arimori S. Increase of IgA-bearing lymphocytes in peripheral blood from patients with IgA nephropathy. Am J Clin Pathol 1979; 71:158-60.
5. Stachura I, Singh G, Whiteside TL. Immune abnormalities in IgA nephropathy (Berger's disease). Clin Immunol Immunopathol 1981; 20:373-88.
6. Sakai H, Nomoto Y, Arimori S. Decrease of IgA-specific suppressor T cell activity in patients with IgA nephropathy. Clin Exp Immunol 1979; 38:243-8.
7. Doi T, Kanatsu K, Sekita K, Yoshida H, Nagai H, Hamashima Y. Circulating immune complexes of IgG, IgA, and IgM classes in various glomerular diseases. Nephron 1982; 32:335-41.
8. Cosio FG, Lam S, Folami AO, Conley ME, Michael AF. Immune regulation of immunoglobulin production in IgA-nephropathy. Clin Immunol Immunopathol 1982; 23:430-6.
9. Fiorini G, Fornasieri A, Sinico R, et al. Lymphocyte populations in the peripheral blood from patients with IgA nephropathy. Nephron 1982; 31:354-7.
10. Nomoto Y, Sakai H. Cold-reacting antinuclear factor in sera from patients with IgA nephropathy. J Lab Clin Med 1979; 94:76-87.
11. Struckmann J, Manthorpe R, Bendixen G. Anti-ENA antibody in serum determined by ELISA-technique. Allergy 1981; 36: 397-403.
12. Boonpucknavig S, Lohachitranond C, Nimmanitya S. The pattern and nature of lymphocyte population response in dengue hemorrhagic fever. Am J Trop Med Hyg 1979; 28:885-9.
13. Bianco C, Patrick R, Nussenzweig V. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. J Exp Med 1970; 132: 702-20.
14. Ruangjirachuporn W, Boonpucknavig S, Nimmanitya S. Circulating immune complexes in serum from patients with dengue hemorrhagic fever. Clin Exp Immunol 1979; 36:46-53.
15. Gluckman JC, Beaufils H, Sanchez F. Inhibition of complement-dependent lymphocyte rosette formation by sera of patients with chronic glomerulonephritis. Clin Exp Immunol 1976; 26:247-52.
16. Brandslund I, Siersted HC, Jensenius JC, Svehag SE. Detection and quantitation of immune complexes with a rapid polyethylene glycol precipitation complement consumption method (PEG-CC). Methods Enzymol 1981; 74:551-71.
17. Boonpucknavig V, Boonpucknavig S. Glomerulonephritis: pathogenesis, pathology and incidence in Thailand. Ramathibodi Med J 1982; 5:16-35.
18. Kurt Lee S-M, Rao VM, Franklin WA, et al. IgA nephropathy: Morphologic predictors of progressive renal disease. Human Pathol 1982; 13:314-22.
19. Gluckman JC, Jacob N, Beaufils H, et al. Clinical significance of circulating immune complexes detection in chronic glomerulonephritis. Nephron 1978; 22:138-45.
20. Cairns SA, London RA, Mallick NP. Circulating immune complexes in idiopathic glomerular disease. Kidney Int 1982; 21: 507-12.
21. Hall RP, Stachura I, Cason J, Whiteside TL, Lawley TJ. IgA-containing circulating immune complexes in patients with IgA nephropathy. Am J Med 1983; 74:56-63.
22. Coppo R, Basolo B, Martina G, et al. Circulating immune complexes containing IgA, IgG and IgM in patients with primary IgA nephropathy and with Henoch-Schönlein nephritis. Correlation with clinical and histologic signs of activity. Clin Nephrol 1982; 18:230-39.
23. Tsai CC, Giangiacomo J, Zuckner J. Dermal IgA deposits in Henoch-Schönlein purpura and Berger's nephritis. Lancet 1975; 1:342-3.
24. Baart de la Faille-Kuyper EH, Kater L, Kuijten RH, et al. Occurrence of vascular IgA deposits in clinically normal skin of patients with renal disease. Kidney Int 1976; 9:424-9.
25. Tomino Y, Nomoto Y, Endoh M, Sakai H. Deposition of IgA-dominant immune-complexes in muscular vessels from patients with IgA nephropathy. Acta Pathol Jpn 1981; 31:361-5.
26. Berger J, Yaneva H, Nabarra B, Barbanel C. Recurrence of mesangial deposition of IgA after renal transplantation. Kidney Int 1975; 7:232-41.
27. Tomino Y, Endoh M, Nomoto Y, Sakai H. Double immunofluorescence studies of immunoglobulins, complement components and their control proteins in patients with IgA nephropathy. Acta Pathol Jpn 1982; 32:251-6.
28. Nomoto Y, Sakai H, Endoh M, Tomino Y. Scleritis and IgA nephropathy. Arch Intern Med 1980; 140:783-5.
29. Marie-Christinebene GF, Duheille J. IgA nephropathy: characterization of the polymeric nature of mesangial deposits by in vitro binding of free secretory component. Clin Exp Immunol 1982; 47:527-34.
30. Komatsu N, Nagura H, Watanabe K, Nomoto Y, Kobayashi K. Mesangial deposition of J chain-linked polymeric IgA in IgA nephropathy. Nephron 1983; 33:61-4.
31. Boonpucknavig S, Udomsangpetch R. Autoantibodies in viral infection. J Clin Lab Immunol 1983; 10:171-2.
32. Boonpucknavig V, Bhamarapravati N, Boonpucknavig S, Futrakul P, Tanpaichitr P. Glomerular changes in dengue hemorrhagic fever. Arch Pathol Lab Med 1976; 100:206-12.
33. Kashiwabara H, Shishido H, Tomura S, Tuchida H, Miyajima T. Strong association between IgA nephropathy and HLA-DR4 antigen. Kidney Int 1982; 22:377-82.
34. Montoliu J, Darnell A, Torras A, Ercilla G, Valleo M, Revert L. Familial IgA nephropathy: Report of two cases and brief review of the literature. Arch Intern Med 1980; 140:1374-5.