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Deposition of C4-binding Protein and β_1H Globulin in Kidneys of Patients with IgA Nephropathy

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A study on the deposition of complement control proteins in renal tissues from patients with IgA nephropathy is described. Renal biopsy specimens were obtained from patients with IgA nephropathy and other glomerular diseases. These biopsy samples were stained with antisera to human C4-binding protein and β_1H globulin by indirect immunofluorescent staining. It was shown that the complement system is activated in IgA nephropathy via the both alternative and classical pathways.

(Key Words: C4-bp, β_1H, Immunofluorescence, IgA Nephropathy)

INTRODUCTION

IgA nephropathy is characterized by a mesangial deposition of IgA with less intense deposition of IgG, IgM and C3 in patients without any systemic diseases (1). Although the complement system activated in IgA nephropathy is mainly via the alternative pathway (3, 8, 10), early complement components (C_1q and C4) were deposited to some degree in the majority of such patients. The aim of the present study was to elucidate whether early complement components are ubiquitously observed in glomeruli from patients with IgA nephropathy. Control proteins of complements, i.e. C4-binding protein and β_1H globulin, were employed to detect such early components. It is expected that these control proteins combine with the complement components in renal biopsy specimens and thus detect those components more sensitively than routine immunofluorescent techniques using fluorescein-conjugated anti human complement antisera. The results from this study indicated that the complement system activated in IgA nephropathy is via both the alternative and classical pathways.

MATERIALS AND METHODS

Patients; Renal biopsy specimens were obtained from 22 patients with IgA nephropathy. Routine microscopic, immunofluorescent and electron microscopic analyses were performed for the diagnosis of IgA nephropathy. Patients whose biopsy specimens stained predominantly for IgA in mesangial areas were included in the study after the exclusion of patients with SLE, anaphylactoid purpura or other systemic diseases. Twenty patients with other types of chronic glomerulonephritis were also examined. Among the

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20 patients, there were seven patients with chronic proliferative glomerulonephritis (PGN), five patients with membranous nephropathy (MN), four patients with membranoproliferative glomerulonephritis (MPGN), and four patients with benign recurrent hematuria (BRH).

Antisera; Fluorescein-labelled antisera to anti human IgG, IgM, IgA (heavy chain specific), C_1q, C4 and C3 were obtained from the Behringwerke AG (Marburg-Lahn, West Germany) (F/P molar ratios ranged fom 1.8 to 2.9). Fluorescein-labelled antisera to anti-human C5 was obtained from the Medical and Biological Laboratories (MBL) (Tokyo, Japan) (F/P molar ratio was 1.9). Fluorescein-labelled antisera to anti human properdin was obtained from the Kent Laboratories (Redmond, USA) (F/P molar ratio was 3.5). Indirect immunofluorescent studies were performed using rabbit antisera to human C3 activator (B) and C9 obtained from the Behringwerke AG. Anti β_1H globulin (β_1H) and anti C4-binding protein (C4-bp) were prepared by injecting rabbits with the purified proteins suspended in complete Freund' adjuvant as previously described (4, 5). FITC-labelled goat anti rabbit IgG (7S immunoglobulins) sera were obtained from the Meloy Laboratories, USA (F/P molar ratio was 2.7). These antisera were absorbed three times with mouse liver acetone powder. Specificities of these antisera were determined by immunodiffusion and immunoelectrophoresis. FITC-labelled goat anti-rabbit IgG sera were absorbed with normal human serum (blood type: AB) at 4°C overnight. Dilution of antisera was 1:10 in PBS unless mentioned otherwise.

Immunofluorescent studies: Renal biopsy specimens were embedded and rapidly frozen in acetone dry ice, sectioned to 2 to 3μ with a rotary microtome in a cryostat at about −25°C, and air-dried. Immediately before staining, cryostat sections were washed three times in phosphate buffered isotonic saline (PBS, pH 7.2) for 15 min. Cryostat sections of the renal biopsy specimens were stained with these fluorescein-labelled antisera in a moist chamber at 4°C overnight. The sections were incubated with antisera to human C3A(B), C9, β_1H globulin and C4-bp in a moist chamber at 4°C overnight. The sections were washed with PBS and then stained with FITC-labelled goat anti rabbit IgG sera at room temperature for two hours. Other sections were stained wih FITC-labelled goat anti rabbit IgG sera alone to determine whether there was non-specific fluorescence or cross-reactions between these sera and human serum components. The sections were washed with PBS and then covered with buffered glycerol and a cover slip, and examined with a Zeiss Orthoflux microscope (Model 9902; Carl Zeiss, Inc., New York, N.Y.). The intensity of the fluorescence was graded as none (−), trace (±), 1 (+), 2 (+) and 3(+).

RESULTS

(1) IgA nephropathy

The results from the immunofluorescent studies on patients with IgA nephropathy and other glomerular diseases are summarized in Tables 1 and 2. IgA was the predominant class of immunoglobulin noted in the glomeruli of all patients with IgA nephropathy (Fig. 1). Four types of IgA nephropathy were classified with respect to the classes of one or more immuno-

globulins deposited in the glomeruli, i.e. deposits of IgA alone (three cases), IgA and IgG (three cases), IgA and IgM (11 cases), and IgA, IgG and IgM (five cases).

Table 1 Incidence of the positive fluorescence of immunoglobulins, complement components and their control proteins in various primary glomerulonephritis cases

	Number of cases	Positive fluorescence											
		IgA	IgG	IgM	C_1q	C4	C3	C5	C9	P	B	C4-bp	β_1H
IgA nephropathy	22	22	8	16	9	6	22	19	14	9	4	8	20
PGN	7	1	2	1	1	0	2	1	0	1	0	2	0
Membranous nephropathy	5	2	5	4	4	3	5	1	3	1	2	5	5
MPGN	4	3	4	2	3	3	4	4	2	2	1	3	4
BRH	4	0	0	0	0	0	1	0	0	0	0	0	0

Table 2 Incidence of the positive fluorescence of complement components and their control proteins in glomeruli from patients with IgA nephropathy

	IgA alone	IgA + IgG	IgA + IgM	IgA + IgG + IgM
Number of cases	3	3	11	5
C_1q	0	1	5	3
C4	1	0	4	1
C3	3	3	11	5
C5	2	2	10	5
C9	3	2	4	5
P	0	0	6	3
B	1	1	0	2
C4-bp	2	1	4	1
β_1H	2	3	11	4

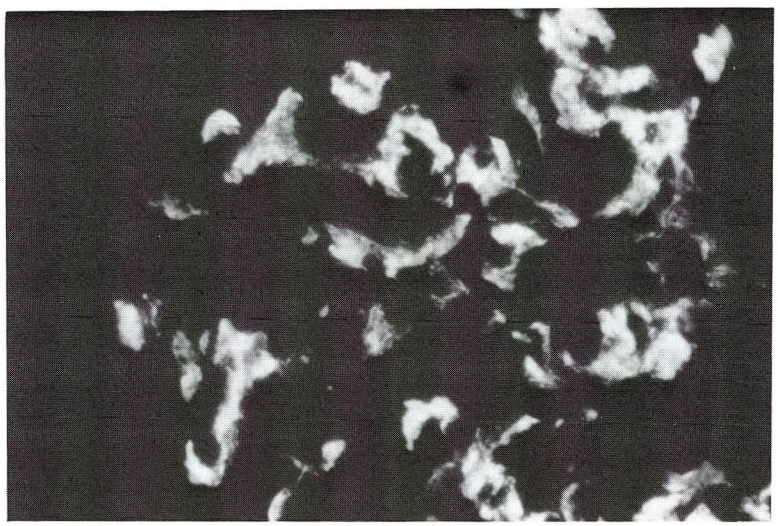

Fig. 1 Diffuse mesangial deposition of IgA (×400)

C_1q was observed in nine out of 22 cases. Although C_1q was not observed in the five cases with deposition of only IgA, it was observed in nine out of 19 cases with combined deposits of immunoglobulins. C4 was observed in six out of 22 cases. Although C4 was observed in one out of three cases with deposition of only IgA, it was observed in five out of 19 cases with combined deposits of immunoglobulins. Prominent deposition of C3 was observed in all cases tested. C5 was observed in 19 out of 22 cases. C9 was observed in 14 out of 22 cases. Properdin was observed in nine out of 21 cases. Factor B was observed in four out of 21 cases. The distribution patterns of C5, C9, properdin and B were almost identical to those of IgA and C3.

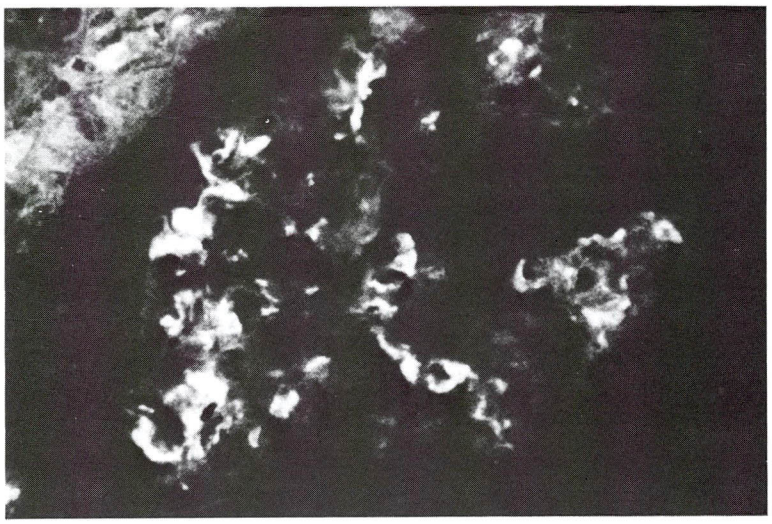

Fig. 2 C4-bp deposition in mesangial areas, segmentally involving capillary walls (× 400)

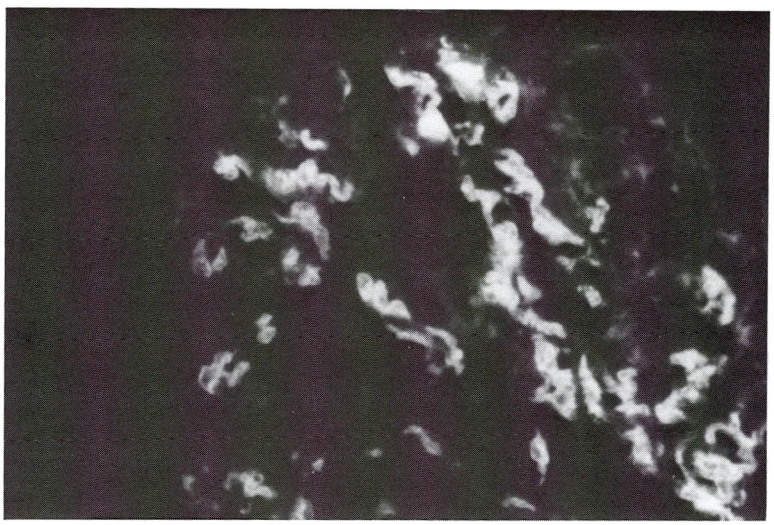

Fig. 3 β_1H globulin deposition in mesangial areas (× 400)

Deposition of C4-bp was observed in eight out of 22 cases (Fig. 2). Although C4-bp was observed in two out of three cases with deposition of only IgA, it was observed in six out of 18 cases with combined deposits of immunoglobulins. C4-bp was mainly detected in the glomerular mesangium and/or glomerular capillary walls. Deposition of β_1H globulin was observed in 20 out of 21 cases (Fig. 3). Although β_1H was observed in two out of three cases with deposition of only IgA, it was observed in all 18 cases with combined deposits of the immunoglobulins tested. The distribution pattern of β_1H was almost identical to that of C3. There were no significant correlations between the glomerular deposition of C4-bp and β_1H and the histopathological changes in IgA nephropathy.

(2) Other types of primary glomerulonephritis

C4-bp and β_1H were observed in all five patients with membranous nephropathy tested. In four patients with MPGN, C4-bp was observed in three cases. β_1H was observed in all cases tested. Although C4-bp was observed in two out of seven cases with PGN, β_1H was not observed in any of the cases tested. C4-bp and β_1H were not observed in any patients with BRH (Table 1).

DISCUSSION

IgA nephropathy is characterized by a mesangial deposition of IgA in renal biopsy specimens by immunofluorescent staining (1). Although IgA nephropathy is usually presumed to be a type of immune complex-mediated glomerulonephritis, the pathogenesis of this disorder, including the mechanism of complement activation, is still obscure. Since the deposition of IgA in IgA nephropathy is frequently associated with IgG and/or IgM (13), it is difficult to determine whether the complement system is activated by IgA or other classes of immunoglobulins. IgA has been considered to fix complement mainly through the alternative pathway (6), and to some extent, by the classical pathway (7). It has been reported that the complement system of IgA nephropathy was activated mainly at C3 via the alternative pathway by IgA (3, 8,10). Recently, we have reported the *in situ* activation of the alternative pathway in renal biopsy specimens from patients with IgA nephropathy (11).

The results obtained from this study showed that glomerular deposition of early complement components (C_1q and C4) was observed in only one case with glomerular deposition of only IgA although late complement components (C3, C5 and C9) were observed in a pattern similar to that of IgA deposition. Glomerular deposition of properdin and factor B was demonstrated in some cases with IgA nephropathy. β_1H globulin was invariably observed with C3 deposited in glomeruli in IgA nephropathy. It has been reported that β_1H globulin bound to C3, presumably C3b, during activation of the complement system in immunologically induced renal diseases (2). Deposition of C_1q and C4 was observed in most cases with combined deposits of IgA and IgM. Although C4-bp deposition was in close correlation with that of C4, the deposition of C4-bp was more intense than that of C4 in some cases. C4-bp, but not C4, was observed in some patients with IgA nephropathy and other

types of primary glomerulonephritis. Fujita (4, 5) and Scharfstein (9) reported that C4-bp had a specific binding affinity for the activated form of C4 (C4b). Moreover, the presence of C4-bp in glomeruli appeared to be a more sensitive indicator of classical pathway activation than the presence of C4 (9).

It is concluded that the results from the present study indicated that the complement system activation in IgA nephropathy is via both the alternative and classical pathways. This assumption was supported by our previous reports (10, 11, 12). Although the predominant pathway of complement activated in patients with IgA nephropathy is an alternative pathway, it is suggested that IgG and IgM antibodies coexisting with the IgA-immune-complex may be responsible for the activation of a classical complement pathway in these patients.

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