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Immunohistochemical demonstration of membrane cofactor protein (MCP) of complement in normal and diseased kidney tissues

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SUMMARY

The immunohistochemically stained membrane cofactor protein of complement (MCP/CD46), one of the complement regulatory proteins, was up-regulated in some diseased kidney tissues. MCP in diseased kidneys was strongly concentrated along the glomerular capillary walls as well as in the mesangial regions, while MCP in normal kidneys was weakly detected in all glomerular structural cells and in the epithelial cells of tubules. Since the enhanced staining was noted in those areas where depositions of C3b/C3c occurred, ongoing complement reaction might be responsible for the up-regulation of MCP expression. MCP expression may be up-regulated by complement fragments generated during complement activation in glomerulonephritis. Furthermore, anti-MCP staining was stronger in intensity in patients with moderate to massive proteinuria, indicating that up-regulation of MCP expression could be directly correlated to the kidney damage.

Keywords membrane cofactor protein complement glomerulonephritis proteinuria

INTRODUCTION

Complement components have been detected in diseased glomeruli [1,2], and it is suggested that complement may play a role in the development of glomerulonephritis. Membrane inhibitors of complement usually protect cells from homologous complement to avoid adverse effect of complement [3,4]. Therefore, it is conceivable that such a membrane regulator might be impaired at the site where homologous complement components deposit. Alternatively, at the site of complement activation, membrane inhibitors might be up-regulated to protect against further tissue damage. Membrane cofactor protein of complement (MCP/CD46), a glycoprotein with mol. wt of 58 kD and 63 kD, is one of the intrinsic inhibitors of complement activation at the C3 step [5–7]. MCP has cofactor activity and binds to C3b and C4b, rendering them sensitive to cleavage by factor I, which results in inactivation of the molecules [5–7]. Decay accelerating factor (DAF), another intrinsic regulatory protein acting at the C3 step, interacts with C3b/C4b and possesses decay-accelerating activity, but not cofactor activity [8–10]. Recently, DAF has been shown to be present in human kidneys [11] and to protect glomerular cells from damage incurred by immune complex- and complement-mediated glomerulonephritis [12]. In addition, it has been reported that 20-kD homologous restriction factor (HRF20/CD59), a membrane protein which inhibits formation of the membrane attack complex of complement on homologous cells [13], has been detected on glomerular cells [14]. However, the distribution of MCP in kidney tissues has not been investigated.

Immunohistochemical studies demonstrated that increased MCP staining is observed at the site of C3b deposition in the diseased glomeruli. Furthermore, there was a remarkable relationship between increased expression of glomerular MCP and proteinuria in patients with various types of glomerulonephritis.

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MATERIALS AND METHODS

Kidney specimens

Normal human kidney tissues were obtained from normal portions of kidney from four nephrectomies undertaken for renal tumours. For diseased tissues, 105 renal biopsy specimens were selected from the files of the Second Department of Internal Medicine, Nihon University School of Medicine. For all biopsy specimens, light, electron and immunofluorescence microscopic studies were performed.

Antibodies

Immunoreagents used in this study were two anti-C3 MoAbs (C-5G, which reacts with C3b and C3c, but not with native C3

Table 1. Summary of the immunohistochemical study and clinical characteristics in 105 cases with various types of glomerulonephritis

Diagnosis	Number of cases	MCP			C3b/C3c			C3dg			Age (years)	Urine protein excretion (g/day)	Creatinine clearance (ml/min)
		1+	2+	3+	−	1+	2+	1+	2+	3+			
MCNS	18	18			18			15	3		25·1±10·1	9·92±9·65	96·4±10·8
Membranous GN	16	7	3	6	7	4	5		5	11	42·8±10·1	3·57±2·30	87·4±16·5
MPGN	6		1	5		3	3			6	35·5±18·3	4·76±2·90	80·0±5·5
IgA nephropathy	32	16	12	4	16	6	10	4	9	19	29·6±8·9	1·62±1·18	83·6±11·9
mesPGN	7	5	2		5	2		3	4		30·4±13·6	0·98±0·43	91·1±13·6
Lupus nephritis	21	9	4	8	9	4	8		7	14	29·1±10·9	2·75±3·10	86·6±15·5
PSAGN	4		1	3		1	3			4	35·0±12·9	1·38±1·06	88·3±13·8
FGS	1	1			1			1			31	3·14	64·1

Grade of staining intensity: −, negative; 1+, moderate; 2+ strong; 3+, very strong.

Data represent means±s.d. MCNS, minimal-change nephrotic syndrome; GN, glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; mesPGN, mesangial proliferative glomerulonephritis; PSAGN, post-streptococcal acute glomerulonephritis; FGS, focal glomerular sclerosis.

nor with C3dg; and G-3E, which reacts only with C3dg) [15], which were generous gifts from Dr T. Fujita (Fukushima Medical College, Fukushima, Japan); and two anti-MCP MoAbs (TRA-2-10 and GB24) [16]. Goat anti-mouse IgG was purchased from Sigma Chemical Co. (St Louis, MO) and was biotinylated as described elsewhere [17].

Immunohistochemical procedures

For immunohistochemistry, frozen blocks cut into 2-μm sections were fixed for 10 min in cold acetone at 4°C, air-dried, and washed for over 30 min in PBS at room temperature. The fixed sections were immersed for 30 min in methanol containing 0·3% hydrogen peroxide (v/v) for quenching of endogenous peroxidase activity. After washing in PBS, the sections were incubated for 20 min with normal goat serum at room temperature, and then incubated at room temperature for 30 min with the first antibodies diluted in PBS containing 1% bovine serum albumin (BSA), before rewashing with PBS. The sections were incubated for 30 min at room temperature with biotinylated goat anti-mouse IgG. After washing in PBS, the sections were incubated for 40 min with the avidin-biotin-peroxidase complex (ABC kit, Vector Laboratories, Burlingame, CA), washed for 60 min in PBS, and developed for 5 min with 0·5 mg/ml of diaminobenzidine in 0·05 M Tris buffer, pH 7·5, containing 0·0?% hydrogen peroxide (v/v). After washing for 10 min in tap water, the tissues were counterstained with haematoxylin, mounted, and observed under a conventional light microscope. Negative controls were incubated with an irrelevant IgG (a mouse anti-rat membrane inhibitor MoAb [18]) as a primary antibody. Staining intensity was estimated on a − to 3+ scale.

Evaluation of clinical data of subjects with glomerulonephritis

The cases were divided into two groups based on the staining intensity of MCP in the glomeruli: a group with elevated MCP staining (2+ or 3+) and another with ordinary staining (1+). Comparisons of urine protein excretion at the time of renal biopsy between the two groups were made using Student's *t*-test for each type of glomerulonephritis.

RESULTS

Distribution of MCP and C3 cleavage fragments in normal kidneys

The distribution and staining intensity of MCP obtained with the two MoAbs, each of which recognized a different epitope, yielded the same results. In normal kidney tissues, MCP was detected in arteriolar walls and tubular membranes of interstitium, and in Bowman's capsules, mesangial regions and basement membranes of glomeruli (Fig. 1a). Its staining intensity in the glomerular capillary walls was weaker than that in the mesangial areas (Fig. 1b). Strong MCP staining was observed in the vascular pole of glomeruli, the region of the juxtaglomerular apparatus (JGA) (Fig. 1c).

In all sections examined, C3dg deposits were observed in the mesangial areas, along the glomerular capillary walls and Bowman's capsules, and in the renal interstitial vessels and some tubules, whereas no C3b/C3c antigen was detected (Fig. 1d, e). No staining was observed in the same tissue stained with an irrelevant MoAb (Fig. 1f).

MCP and C3 cleavage fragments in diseased kidney

The range of histopathologic diagnoses of 105 patients' biopsy specimens and distributions of MCP and C3 cleavage fragments, C3b/C3c and C3dg, are summarized in Table 1. In some cases (6/6 membranoproliferative glomerulonephritis (MPGN), 16/32 IgA nephropathy, 12/21 lupus nephritis, 9/16 membranous nephropathy (MN), 2/7 mesangial proliferative glomerulonephritis (mesPGN) and 4/4 post-streptococcal acute glomerulonephritis (PSAGN)), MCP staining was significantly

Fig. 1. (see next page) Immunohistochemical demonstration of MCP in normal kidney tissue. (a) A section demonstrates the presence of MCP in Bowman's capsules, mesangial regions and basement membranes of the glomeruli and in tubular membranes of the interstitium (×200). (b) The staining intensity of MCP in the glomerular capillary walls is weaker than that in the mesangium and Bowman's capsule (×400). (c) MCP is strongly detected in the region of the juxtaglomerular apparatus (×400). In normal kidney tissue, C3dg deposits are seen (d), but C3b/C3c antigen cannot be detected (e). (d, e, ×200). (f) A section was stained with MoAb against rat membrane inhibitor as a negative control. No staining is observed (×200).

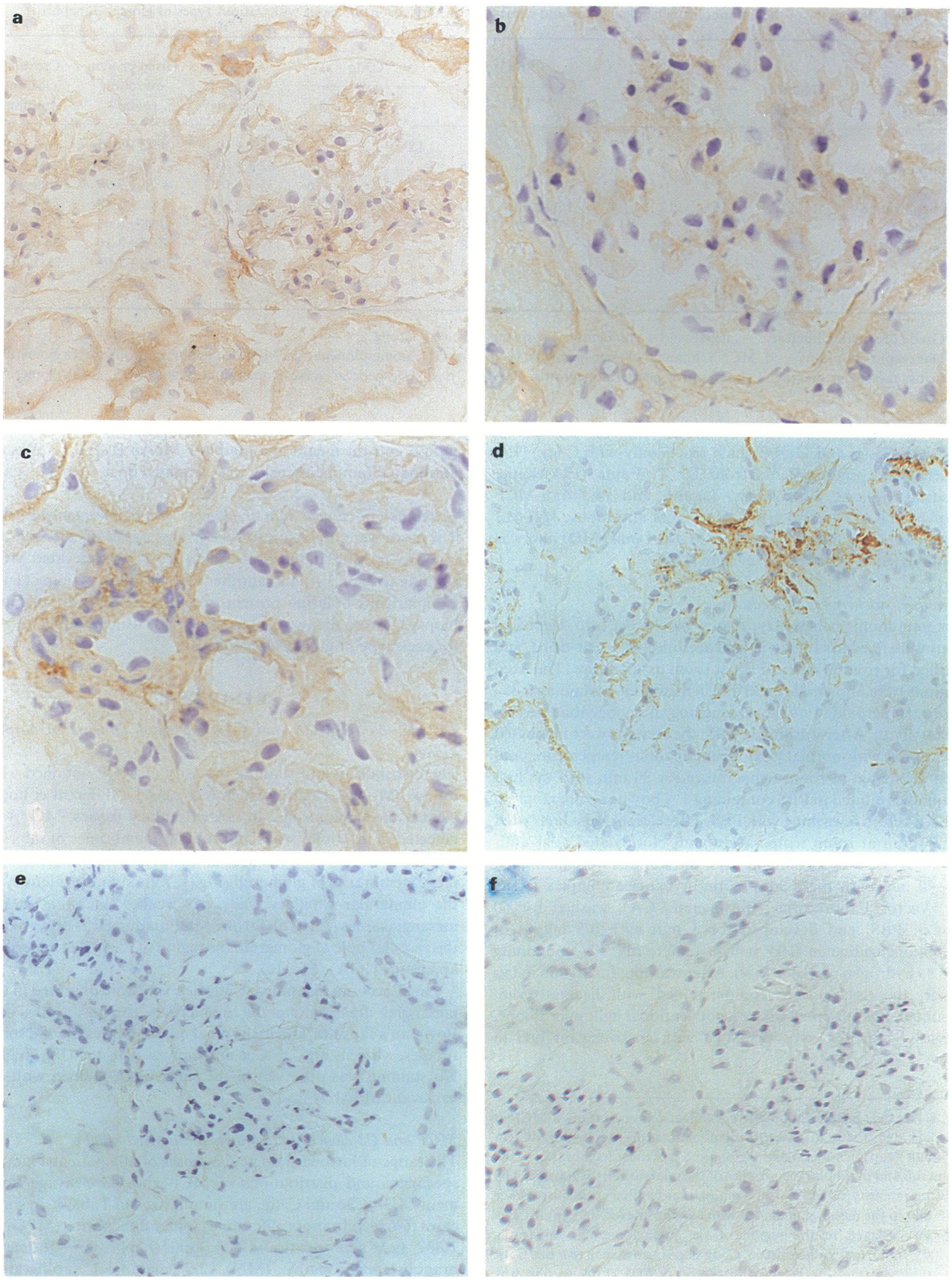
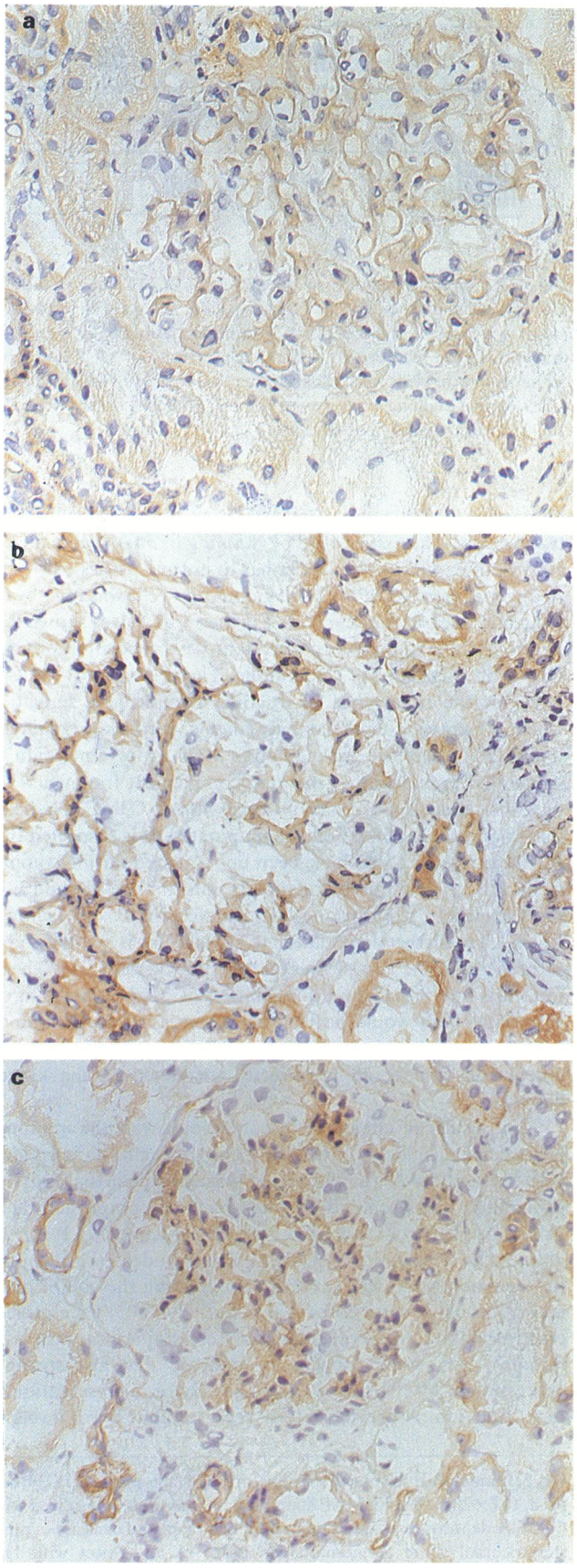


Fig. 1. (see previous page for caption)



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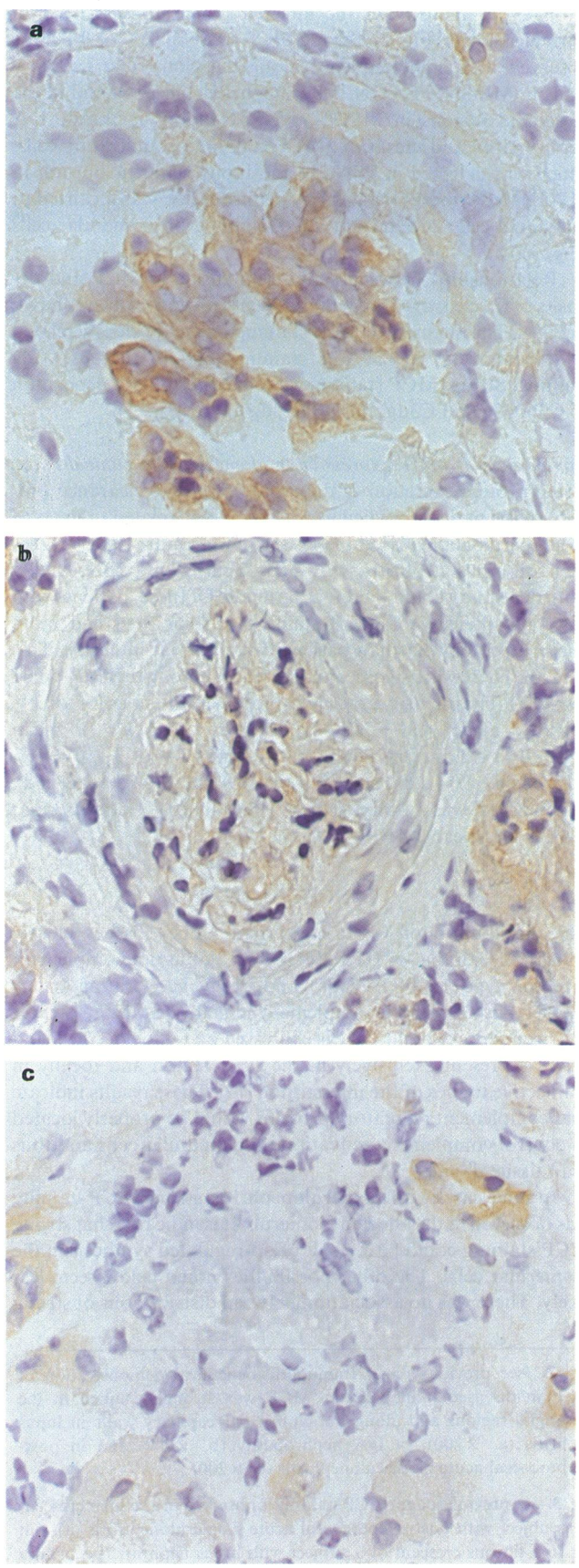


Fig. 3. (see next page for caption)

stronger in intensity in the affected glomeruli than that in normal subjects (Fig. 2). Strong MCP staining (2+ or 3+) was observed with high frequency along the glomerular capillary walls in MPGN, PSAGN, lupus nephritis and MN, and in the mesangial regions in MPGN, PSAGN, lupus nephritis, IgA nephropathy and mesPGN. MCP staining of infiltrating cells, which was detected in PSAGN and lupus nephritis but not in MPGN, MN, mesPGN and IgA nephropathy, was localized to individual cells and did not affect staining of glomerular cells (Fig. 3).

Relationships between staining intensity of MCP and deposits of C3 cleavage fragments in identical glomeruli are shown in Fig. 4. The intensity of MCP staining was more closely related to the intensity of C3b/C3c staining than to that of C3dg staining. Strong MCP staining was concordant with deposition of C3b/C3c and C3dg, but not C3dg alone (Fig. 5).

Comparison of MCP expression in glomeruli with clinical data

Urine protein excretion (g/day) and creatinine clearance (ml/min) of the patients studied were shown to be related to the type of glomerulonephritis (Table 1). There were more than 10 cases each of lupus nephritis, IgA nephropathy and MN, and a statistical study could therefore be made. Of these, patients with a strong intensity of MCP staining (2+ or 3+) had more massive proteinuria than patients with normal intensity (1+) (Fig. 6). There were significant differences among them ($P < 0{\cdot}01$ by t-test). The intensity grading in lupus nephritis was 2+ or 3+ ($n=12$), mean $4{\cdot}37 \pm 3{\cdot}27$ s.d. *versus* 1+ ($n=9$), mean $0{\cdot}61 \pm 0{\cdot}41$ s.d.; in IgA nephropathy it was 2+ or 3+ ($n=16$), mean $2{\cdot}46 \pm 1{\cdot}14$ s.d. *versus* 1+ ($n=16$), mean $0{\cdot}77 \pm 0{\cdot}28$ s.d.; and in MN it was 2+ or 3+ ($n=9$), mean $5{\cdot}23 \pm 1{\cdot}61$ s.d. *versus* 1+ ($n=7$), mean $1{\cdot}41 \pm 0{\cdot}59$ s.d.

DISCUSSION

The distribution of MCP in normal kidney tissues closely coincided with that of HRF20/CD59 [13], but not complement receptor type 1 (CR1/CD35) and decay accelerating factor (DAF) that also inhibit complement activation at the C3 step. It has been reported that CR1 is expressed on podocytes [19], and DAF is present exclusively in the JGA region and focally in connective tissues of the interstitium [12]. These results indicate that complement regulatory proteins would be properly located to control complement activation on glomerular cells and thus limit tissue damage.

In some cases with glomerulonephritis, strong MCP staining was observed in the affected glomeruli. It is indicated that strong MCP staining could be a result of up-regulated synthesis in the glomerular cells. The other possibilities noted below seem less likely. There was no association between distributions of strong glomerular MCP staining and localization of glomerular infiltrating cells. The extent of MCP staining in interstitium and in JGA of diseased tissues was similar to normal tissues. Also, the stronger expression of MCP on glomerular cells was not always related to release from the JGA. Furthermore, it was shown that strong MCP staining was concordant with deposition of C3b/C3c and C3dg, but not C3dg alone. The presence of C3b/C3c indicates the occurrence of ongoing complement activation which generates complement fragments, including anaphylatoxins [20]. Therefore, up-regulating MCP expression might be stimulated by nascently generated complement fragments. On the other hand, depositions of C3dg without C3b/C3c indicate that complement activation has terminated. It is suspected that MCP expression could have been up-regulated during complement activation on glomerular cells, and that cell-bound C3b was cleaved to iC3b by the up-regulated MCP and factor I, which led to further degradation to C3dg which is no longer able to perpetuate the complement activating cascade [20]. Therefore, it is likely that the up-regulation of MCP expression on glomerular cells would protect them from autologous complement attack by limiting C3b and/or C4b deposition in glomerulonephritis, and could be directly correlated to the kidney damage.

The results in clinical data correspond with the immunohistochemical findings, since increasing proteinuria correlates with the activity of the disease. It was suspected that massive proteinuria had been caused when ongoing complement activation in glomeruli had overcome up-regulated MCP on the glomerular cells, and that proteinuria might decrease when the up-regulated MCP was capable of regulating the complement activation. Since most cases in this study (98/105) were considered to exhibit normal renal function (creatinine clearance $\geqslant 80$ ml/min), this would appear to indicate that the stronger expression of MCP observed was a key factor in preservation of renal function. A previous study reported that lower levels of

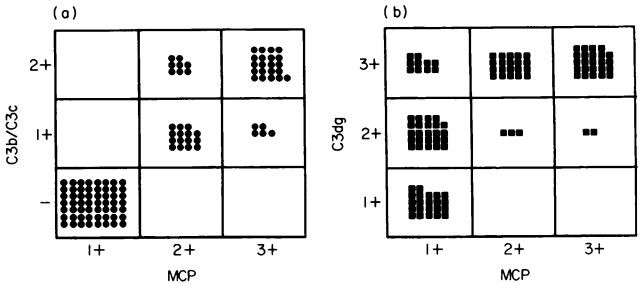
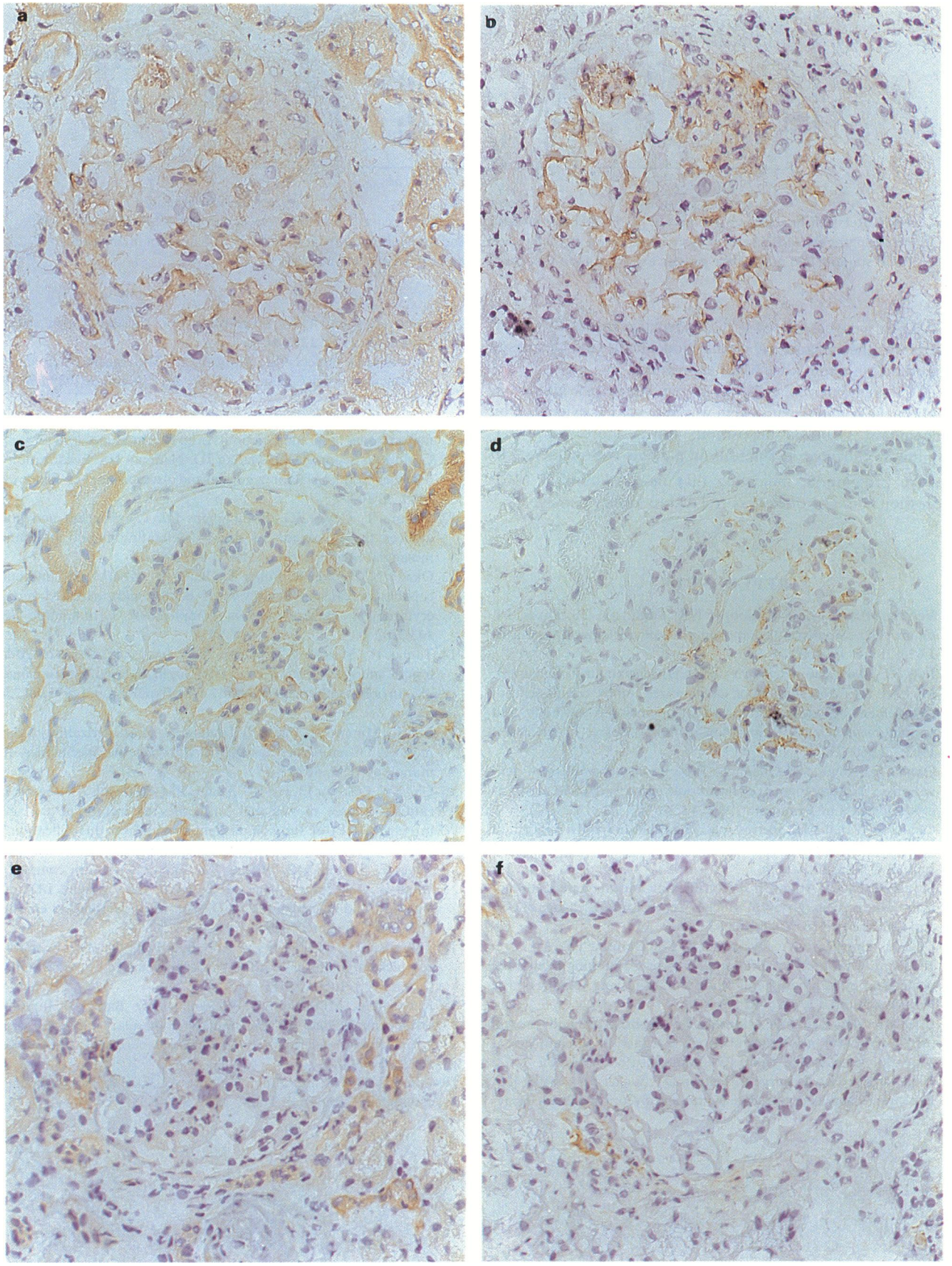


Fig. 4. Relationship between staining intensity of MCP and that of C3 cleavage fragments ($n=105$). (a) A stronger intensity of anti-MCP staining revealed deposits of C3b/C3c in similar lesions of glomeruli. (b) In contrast, the relationship between the intensity of MCP and C3dg staining is not as clear cut as that between the intensity of MCP and C3b/C3c staining. Staining intensity was graded as follows: −, negative; 1+, slight; 2+, moderate; 3+, strong.

Fig. 2. (see previous page) Immunohistochemical demonstration of MCP in diseased kidney tissue. MCP was strongly stained in the mesangial regions and along the glomerular capillary walls in lupus nephritis (a, ×200), in IgA nephropathy (b, ×200) and in post-streptococcal acute glomerulonephritis (c, ×200).

Fig. 3. (see previous page) MCP staining is observed in a cellular crescent of a subject with post-streptococcal acute glomerulonephritis (a), but not in a fibrous crescent of a subject with lupus nephritis (b), or in a sclerotic lesion of a subject with mesangial proliferative glomerulonephritis (c) (×400).

Fig. 5. Immunohistochemical staining for MCP (a, c, e) and for C3b/C3c (b, d, f) (×200). Strong MCP staining in glomeruli is colocalized with C3b/C3c deposits in a case of lupus nephritis (a, b) and in a case of post-streptococcal acute glomerulonephritis (c, d). A subject with IgA nephropathy has a normal level of intensity of anti-MCP staining (e) in the absence of C3b/C3c deposits in the glomerulus (f).



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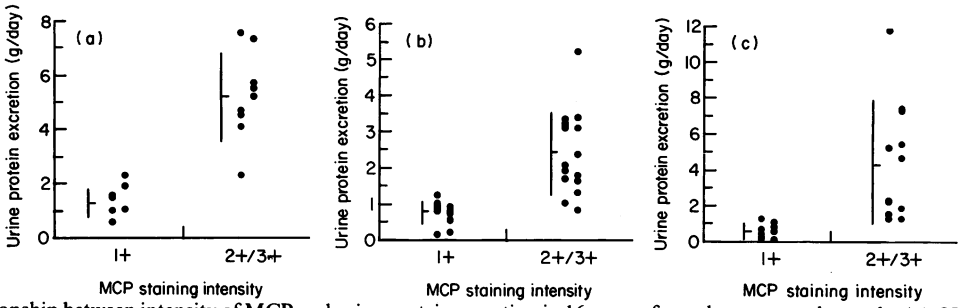

Fig. 6. Relationship between intensity of MCP and urine protein excretion in 16 cases of membranous nephropathy (a), 32 cases of IgA nephropathy (b) and 21 cases of lupus nephritis (c). In each type of glomerulonephritis, almost all subjects with a higher intensity of anti-MCP staining had more urine protein excretion than individuals with normal staining intensity of MCP.

JGA-DAF were associated with abnormal renal function [11]. Therefore, the advance of glomerulonephritis and decreased renal function might alter MCP expression.

MCP on glomerular cells was up-regulated in the glomeruli, in which the complement system was activated as evidenced by concomitant C3b/C3c deposition. Furthermore, anti-MCP staining was stronger in intensity in patients with moderate to massive proteinuria, indicating that up-regulation of MCP expression could be directly correlated to kidney damage.

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