Enhanced expression of complement C5a receptor mRNA in human diseased kidney assessed by in situ hybridization

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Background. Anaphylatoxin C5a mediates inflammatory responses through interaction with a specific C5a receptor (C5aR), the expression of which is thought to be restricted to peripheral blood leukocytes. Although the presence of C5aR on cultured mesangial cells and tubular epithelial cells has recently been documented, the tissue distribution of C5aR in diseased kidney has not yet been determined.

Methods. Immunohistochemistry and nonradioactive in situ hybridization for C5aR were performed in 34 tissue samples of kidneys from patients with various renal diseases, including 4 with minimal change nephrotic syndrome (MCNS), 5 with membranous nephropathy (MN), and 25 with mesangial proliferative glomerulonephritis (mesGN; 15 patients with IgA nephropathy, 5 with non-IgA mesGN, and 5 with lupus nephritis). Normal portions of surgically resected kidney served as the control.

Results. In normal kidneys, C5aR protein was detected in tubular epithelial cells, while C5aR mRNA was detected in a few glomerular cells, tubular epithelial cells, and vascular endothelial and smooth muscle cells. In MCNS, the distribution of C5aR protein and mRNA was similar to that in normal kidneys. In MN and mesGN, C5aR protein and mRNA were detected in mesangial cells, glomerular epithelial and endothelial cells, Bowman's capsule cells, tubular cells, infiltrating cells, and vascular endothelial and smooth muscle cells. The glomerular expression of C5aR mRNA and protein correlated positively with the degree of mesangial hypercellularity and mesangial matrix expansion in mesGN. In the tubulointerstitium, interstitial expression of C5aR mRNA correlated positively with the degree of tubular atrophy and interstitial broadening in mesGN. Furthermore, the interstitial expression of C5aR mRNA correlated positively with the level of serum creatinine.

Conclusions. Our results indicate that renal cells produce C5aR and that activation of C5a/C5aR pathway on renal cells may be involved in tissue injury in mesGN.

Complement is one of the major mediators of inflammation and tissue injury in immune-complex–mediated glomerulonephritis. Complement activation occurs through classic and alternative pathways, resulting in the release of anaphylatoxins or the formation of the terminal complement components C5b-9. While the role of C5b-9 in the development of glomerulonephritis has been defined in various types of human and experimental models of glomerulonephritis [1–3], the relative role of anaphylatoxins remains obscure.

The anaphylatoxin C5a derived from the fifth complement component is composed of 74 amino terminal amino acids of the C5 α chain [4]. C5a is an important mediator of the inflammatory response. On the development of glomerulonephritis, it has been assumed that the primary effects of C5a are mediated locally via direct/indirect induction of cytokine synthesis, enhancement of vascular permeability, attraction of phagocytes, and migration of neutrophils to the inflammatory sites in the kidney [5]. C5a acts by binding to a specific, high-affinity C5a receptor (C5aR), which is a member of the rhodopsin family of G-protein–coupled receptors, and contains seven transmembrane segments [6]. Expression of the C5aR has been demonstrated in bone marrow-derived cell lines [7], including neutrophils, macrophages and monocytes, liver parenchymal cells [8], lung vascular smooth muscles and endothelial cells, bronchial and alveolar epithelial cells [8], and renal cells [8–11]. The expression of C5aR has been recently detected in the kidney of baboon by Northern blot analysis [8]. In cultured human mesangial cells, expression of C5aR has been demonstrated by immunofluorescence studies, and the

presence of C5aR mRNA was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) [9, 10]. These in vitro studies suggested that C5a exerts its damaging effects on renal tissues via the expression of its receptor in these tissues and that activation of this receptor may play a role in the development of glomerulonephritis. More recently, using immunohistochemistry, the expression of C5aR in proximal tubular epithelial cells was detected in normal human kidney [11]. To our knowledge, however, the expression of C5aR and the cellular origin of the receptor in human diseased kidneys have not yet been clarified.

In the present study, we used immunohistochemistry and nonradioactive in situ hybridization to examine C5aR in human normal and diseased kidneys. Our results showed the presence of C5aR as well as C5aR mRNA in the kidneys, and that the level of expression of C5aR mRNA correlated positively with the degree of tissue injury both in glomeruli and interstitium in human diseased kidneys. Our results indicate that renal cells produce C5aR and that the C5a/C5aR pathway may be a candidate for inducing tissue injury. It is also suggested that the effect of complement activation on tissue injury is not only through the formation of C5b-9, but also through the anaphylatoxin C5a in both glomeruli and interstitium in human glomerulonephritis.

METHODS

Kidney tissues

A total of 34 renal biopsy specimens from 34 patients with renal diseases were examined, including nonproliferative [4 patients with minimal-change nephrotic syndrome (MCNS) and 5 patients with membranous nephropathy (MN)] and mesangial proliferative glomerulonephritis [mesGN; 15 patients with IgA nephropathy (IgAN), 5 patients with non-IgA mesGN (non-IgAN), and 5 patients with lupus nephritis (WHO Class IIb (LN)]. None of the patients were treated with steroids or immunosuppressive drugs before renal biopsy. Five tissue specimens from uninvolved areas of adenocarcinomatous kidneys served as normal controls. Each patient gave an informed consent to renal biopsy and the present study. Diagnosis of renal disease was based on clinical, light microscopic, immunofluorescence, and electron microscopic findings. Samples were frozen in O.C.T. compound (Miles Inc., Elkhardt, IN, USA) and were stored at $-80°C$ until use. The following laboratory parameters were determined at the time of biopsy: age, gender, proteinuria, hematuria, and the level of serum creatinine. The severity of hematuria was graded as follows: 0 = 0 to 5 erythrocytes per high-power field; 1 = 5 to 30 erythrocytes per high-power field; 2 = >30 erythrocytes per high-power field;

and grade 3 for macroscopic hematuria. The clinical parameters are summarized in Table 1.

Oligonucleotides and labeling

A 30 mer sequence of mRNA was selected for human C5aR. Antisense oligonucleotides for human C5aR corresponded to bases 52 to 81 of human C5aR [12]. The selected sequence was significantly different from other known sequences deposited in the latest release of the gene bank data (GenBank, Release 113, September 1999). The oligonucleotide was synthesized on an automatic DNA synthesizer (model 391, PCR-MATE EP; Applied Biosystems, Foster City, CA, USA) and was labeled using a digoxigenin (DIG) oligonucleotide tailing kit according to the current protocol (Boehringer Mannheim Biochemica, Mannheim, Germany).

In situ hybridization

Nonradioactive in situ hybridization was performed according to a modified method developed in our laboratory [13]. In brief, the specimens were cut to a thickness of 4 μm and were placed on glass slides coated with 3-aminopropyltriethoxysilane (A3684; Sigma, St. Louis, MO, USA). The sections were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and then deproteinized using HCl and proteinase K (Sigma P-4914). After prehybridization, the sections were hybridized with DIG-labeled oligonucleotide probe in the prehybridization buffer. After washing with 0.075% BRIJ (Sigma 430 AG-6) in ×2 and ×0.5 standard saline citrate (SSC) at room temperature, sections were stained immunohistochemically to visualize the hybridized DIG-labeled probe using mouse monoclonal anti-DIG antibody (Boehringer Mannheim Biochemica), horseradish peroxidase (HRP)-conjugated rabbit anti-mouse antibody (Dako P260; Dakopatts, Glostrup, Denmark) and HRP-conjugated swine anti-rabbit antibody (Dako P399) successively. Color was developed by reaction with H_2O_2 and diaminobenzidine tetrahydrochloride. Finally, sections were counterstained with methyl green and were mounted. Cells clearly stained in the cytoplasm or stained with a perinuclear pattern were identified as C5aR mRNA-positive cells. On the other hand, cells with nuclei stained with methyl green alone were considered negative for C5aR mRNA.

Two observers, independently and without the prior knowledge of clinical and laboratory data, examined the tissues histologically and evaluated the expression of C5aR mRNA in each biopsy. For semiquantitative histologic grading of the glomeruli, after color development of immunohistochemistry, sections were stained by periodic acid-Schiff (PAS) reaction, which allowed the identification of histologic changes such as mesangial expansion. PAS staining also allowed identification of the exact location of those cells positive for C5aR mRNA relative

Table 1. Clinical characteristics of patients enrolled in this study

No.	Sex	Age years	Diagnosis	Serum creatinine mg/dL	Urinary protein g/24 h	Grade of hematuria[a]	IF C3[b]	IF IgG[b]
1	M	26	MCNS	0.8	4.7	0	−	±
2	F	15	MCNS	0.7	7.4	0	−	−
3	M	20	MCNS	0.6	6.6	0	−	−
4	F	22	MCNS	0.7	4.4	0	−	−
5	F	60	MN	0.9	1.1	2	3+	2+
6	M	54	MN	0.8	2.5	1	2+	+
7	M	52	MN	0.9	2.2	1	3+	+
8	M	63	MN	0.9	1.4	1	3+	3+
9	M	46	MN	0.9	1.5	1	3+	2+
10	F	21	IgAN	0.6	0.7	3	2+	+
11	M	21	IgAN	0.9	1.4	3	+	+
12	M	47	IgAN	1.0	0.7	2	+	3+
13	F	24	IgAN	0.7	0.1	3	2+	+
14	M	23	IgAN	1.0	1.2	3	+	+
15	F	46	IgAN	0.9	0.2	2	+	3+
16	F	21	IgAN	0.6	0.1	3	+	+
17	F	44	IgAN	0.9	0.7	3	+	+
18	F	39	IgAN	1.0	0.2	0	+	+
19	F	39	IgAN	0.6	0.2	3	+	3+
20	M	35	IgAN	1.0	1.2	3	+	2+
21	M	56	IgAN	1.5	0.4	2	+	+
22	M	51	IgAN	1.5	5.3	2	±	+
23	M	44	IgAN	1.5	0.9	3	3+	2+
24	F	20	IgAN	0.6	0.4	3	2+	2+
25	F	44	Non-IgAN	3.3	8.1	3	2+	2+
26	F	42	Non-IgAN	1.0	0.4	3	+	+
27	M	46	Non-IgAN	2.0	1.3	3	±	±
28	M	44	Non-IgAN	1.5	0.5	3	±	±
29	M	35	Non-IgAN	1.5	0.3	3	+	±
30	M	45	LN	1.7	0.8	2	2+	+
31	F	28	LN	0.8	0.6	3	3+	+
32	M	54	LN	0.8	2.5	1	3+	3+
33	F	48	LN	0.8	1.5	0	+	+
34	F	33	LN	0.5	1.5	3	2+	2+

Abbreviations are: M, male; F, female; IF; immunofluorescence microscopy; MCNS, minimal change nephrotic syndrome; MN, membranous nephropathy; IgAN, IgA nephropathy; LN; lupus nephritis.

[a] The severity of hematuria was graded as described in the **Methods** section

[b] Semiquantitative assessment of staining was: −, no staining; intensity of staining was graded from ± to 3+

to the glomerular basement membrane and mesangial area. The cells were identified as mesangial cells within the PAS-positive mesangial matrix and glomerular epithelial cells on the capillary basement membrane.

Since the degree of glomerular injury is heterogeneous among glomeruli during the progression of glomerulonephritis, it is possible that the level of expression of C5aR mRNA may relate to the degree of glomerular injury in each glomerulus. Hence, we evaluated the degree of glomerular injury as well as expression of each C5aR mRNA in each glomerulus rather than averaging the injury in each section. In this study, five to seven glomeruli, each with an equatorial plane cross-section, were analyzed in each section. Mesangial hypercellularity and mesangial matrix expansion were graded separately using a 0 to 3+ scale (absent or minimal, mild, moderate, and marked) according to methods described previously [14]. In the tubulointerstitium, we examined three to five fields of the cortical interstitium in each section under a low magnification (×100) and analyzed three representative interstitial fields, as described previously [15]. In each field, tubulointerstitial change, tubular atrophy, and interstitial broadening were graded separately using a 0 to 3+ scale as described previously in this article.

After determining the degree of glomerular and tubulointerstitial injury, in the same glomeruli and interstitial fields, semiquantitation of the expression of C5aR mRNA was performed. The numbers of immunoreactive cells in renal tissues were scored separately relative to the total number of glomerular cells, mesangial cells and glomerular epithelial cells, tubules, and interstitium according to the following scheme, as described previously [16], with some modification: 0 = no positive cells; 1 = scattered single positive cells in a focal distribution; 2 = diffusely scattered positive cells; and 3 = numerous positive cells, frequently in clusters.

To evaluate the specificity of the signals for C5aR mRNA, we performed three control experiments, including pretreatment of RNase, a study with a sense probe and a competitive study, as described previously [17, 18].
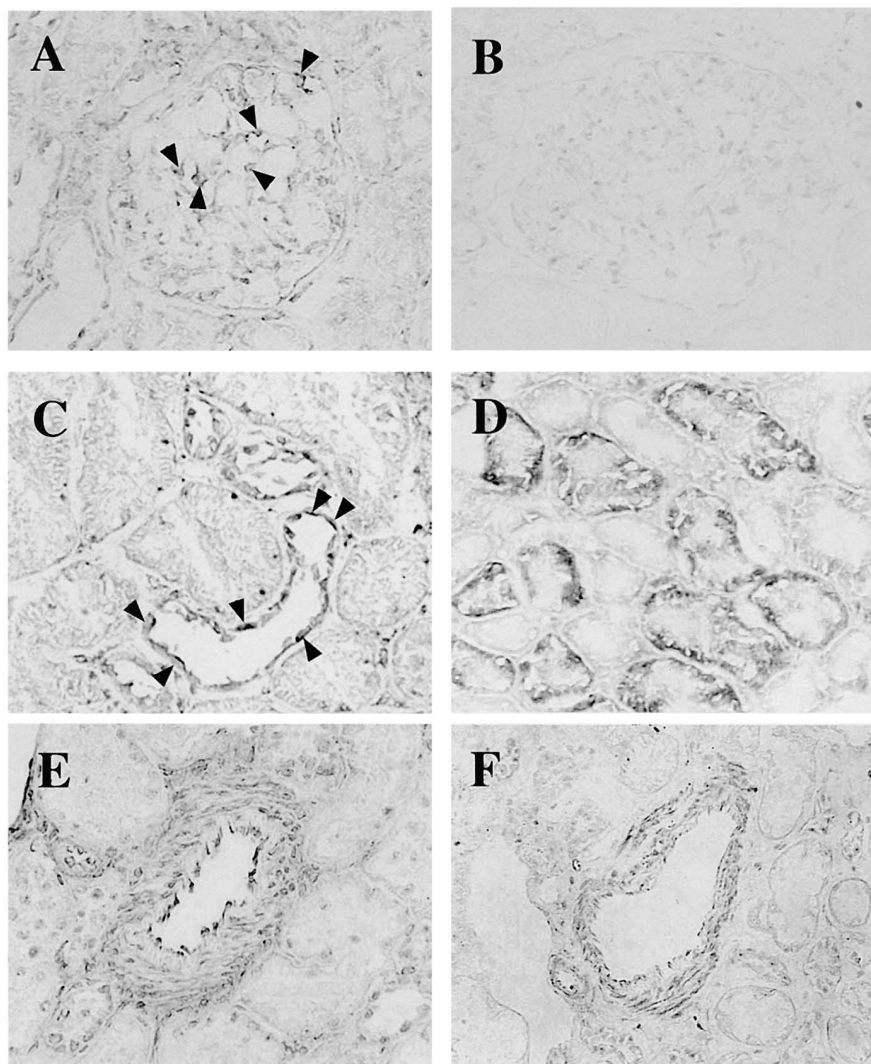
Fig. 1. In situ hybridization study for C5aR mRNA (*A, C,* and *E*) and immunohistochemistry for C5aR protein (*B, D,* and *F*) in renal sections of control kidney. Note the weak C5aR mRNA signals, particularly in glomerular epithelial cells and cells of Bowman's capsule (A, arrowheads). Note also the lack of C5aR protein in the glomeruli of the control kidney (B). C5aR mRNA is expressed in tubular epithelial cells (C, arrowheads), vascular endothelial cells, and smooth muscle cells (E). C5aR protein is also present in tubular epithelial cells (D), vascular endothelial cells, and smooth muscle cells (F). Magnification ×200.

Immunohistochemistry

Sections from frozen kidney tissues were reacted with a monoclonal antibody against CD88 (Serotec, Oxford, UK) to identify human C5aR. A monoclonal antibody against CD 68 (M718 Dako; Dakopatts) was also used to identify any infiltrating monocytes and macrophages. After reacting with the first antibodies, the same sections were reacted with HRP-conjugated rabbit anti-mouse immunoglobulin and HRP-conjugated swine anti-rabbit immunoglobulin, as described in the procedure of in situ hybridization. Mouse IgG1 was used as a negative control (Dako X931; Dakopatts) instead of monoclonal antibodies. The number of CD88-positive cells in renal tissues was scored as described for the in situ hybridization procedure.

Statistical analysis

Data were expressed as mean ± SD. Differences between different groups were tested for statistical signifi-

cance using one-way analysis of variance (ANOVA) with Scheffe's *F* test. Correlations between two variables were tested for significance using the Spearman's rank correlation test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

C5aR mRNA and C5aR protein in normal kidneys

Glomeruli. In situ hybridization showed the presence of a weak but significant signal for C5aR mRNA in mesangial cells, cells of Bowman's capsule, and glomerular epithelial cells (Fig. 1A). However, no positive immunostaining for C5aR protein was observed in the glomerulus (Fig. 1B).

Interstitium. C5aR mRNA was observed in some tubular epithelial cells (Fig. 1C), vascular endothelial (Fig. 1E), and smooth muscle cells (Fig. 1E) in the interstitium of the normal control tissue. Consistently, immunohisto-
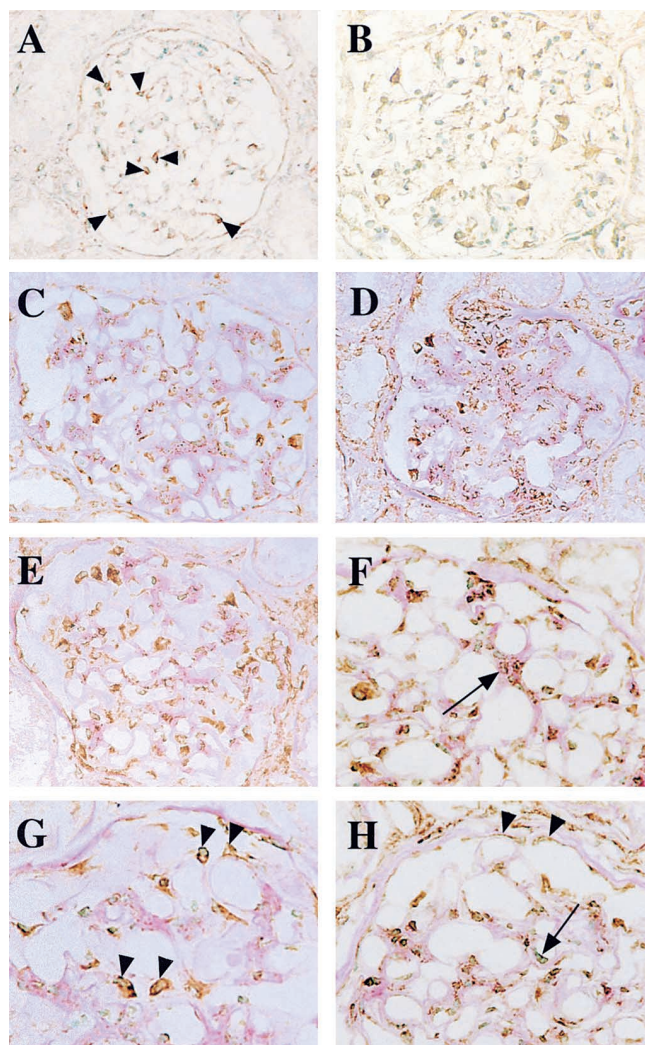
Fig. 2. In situ hybridization study for C5aR mRNA in sections of patients with minimal change nephrotic syndrome (MCNS; ***A*)**, **membranous nephropathy (MN;** ***B*)**, **IgA nephropathy (IgAN;** ***C*, mild lesion;** ***D*, severe lesion), and lupus nephropathy (LN;** ***E*).** In situ hybridization followed by PAS staining in IgA nephropathy (*F–H*). Note the expression of C5aR mRNA in mesangial cells (F, arrow), glomerular epithelial cells (G, arrowheads), cells of Bowman's capsule (H, arrowheads), and glomerular endothelial cells (H, arrow). Magnification A–E, ×200; F–H, ×400.

Table 2. Expression of C5aR mRNA in normal and diseased kidneys

Diagnosis	C5aR mRNA score/glomerulus[a]
IgAN	2.40 ± 0.65[b]
Non-IgAN	2.12 ± 0.78[b]
LN	2.72 ± 0.46[b]
MN	1.52 ± 0.51[b]
MCNS	0.67 ± 0.31
Control kidney	0.44 ± 0.45

Data are mean ± SD. Abbreviations are in Table 1.

[a] In each glomerulus, the degree of C5aR mRNA was scored according to the method of Niemir et al [16], with a minor modification. Five to seven glomeruli in each section were analyzed. Cells containing clearly stained cytoplasm were identified as C5aR mRNA and protein-positive cells. Differences between groups were tested for statistical significance using one-way ANOVA with Scheffe's *F* test.
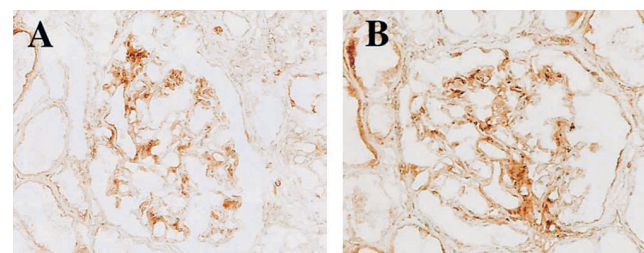
[b] $P < 0.01$ vs. control kidney

Fig. 3. Immunohistochemistry for C5aR protein in glomeruli of patients with IgA nephropathy (*A*) and LN (*B*). C5aR is present in mesangial cells, glomerular epithelial cells, glomerular endothelial cells, and cells of Bowman's capsule. Magnification ×200.

chemical study showed the presence of C5aR protein in tubular epithelial cells (Fig. 1D) and vascular endothelial (Fig. 1F) and smooth muscle cells (Fig. 1F).

C5aR mRNA and C5aR protein in diseased kidneys

Glomeruli. In MCNS, a weak but significant staining for C5aR mRNA was present in the glomeruli (Fig. 2A), but C5aR protein was not observed similar to the control sections (data not shown). However, in MN and mesGN, including IgAN, non-IgAN, and LN, a large number of cells were positive for C5aR mRNA (Fig. 2B–E). The levels of C5aR mRNA expression in MN and mesGN were higher than in the control (Table 2). Overexpression of C5aR mRNA was observed mainly in mesangial cells within the PAS-positive mesangial matrix in mesGN. Marked expression was noted in areas of mesangial proliferation (Fig. 2C–F). The degree of expression of C5aR mRNA in mesangial cells was higher than that in glomerular epithelial cells in mesGN (Table 3). In MN, mainly glomerular epithelial cells were positive for C5aR mRNA (Fig. 2B). The degree of C5aR mRNA expression in glomerular epithelial cells was higher than in mesangial cells (Table 3). Some glomerular epithelial cells on the capillary basement membrane, cells of Bowman's capsule, and glomerular endothelial cells were also positive for C5aR mRNA (Fig. 2G, H). Immunostaining for C5aR was observed mainly in mesangial cells in IgAN (Fig. 3A), non-IgAN, and LN (Fig. 3B). Some glomerular epithelial cells, cells of Bowman's capsule, and glomerular endothelial cells were positive for C5aR protein (Fig. 3). The level of C5aR mRNA expression correlated significantly with the degree of mesangial hypercellularity and mesangial matrix expansion in mesGN (Table 4). Furthermore, glomerular expression of C5aR protein correlated with the degree of mesangial hypercellularity and mesangial matrix expansion (Table 4).

Interstitium. In MCNS, the signal for C5aR mRNA was

Table 3. Expression of C5aR mRNA in mesangial cells and glomerular epithelial cells in mesGN and MN

Diagnosis	C5aR mRNA expression in mesangial cell/glomerulus[a]	C5aR mRNA expression in epithelial cell/glomerulus[a]
IgAN	2.83 ± 0.83[b]	1.33 ± 0.59
Non-IgAN	2.50 ± 0.51[b]	1.28 ± 0.46
LN	2.89 ± 0.32	2.67 ± 0.23
MN	1.05 ± 0.25	2.22 ± 0.43[b]

Data are mean ± SD. Abbreviations are in Table 1.

[a] In each glomerulus, the degree of C5aR mRNA in mesangial and glomerular epithelial cells was scored separately according to the method of Niemir et al [16], with a minor modification: 0 = no positive cells; 1 = scattered single positive cells; 2 = diffusely scattered positive cells; and 3 = numerous positive cells. Five to seven glomeruli in each section were analyzed. Cells containing clearly stained cytoplasm were identified as C5aR mRNA-positive cells. The cells were identified as mesangial cells within the PAS-positive mesangial matrix and glomerular epithelial cells on the capillary basement membrane. Difference between the C5aR mRNA score in mesangial cells and in glomerular epithelial cells was tested for statistical significance using one-way analysis of variance with Scheffe's F test.

[b] In IgAN and non-IgAN, the C5aR mRNA expression in mesangial cells is higher than in glomerular epithelial cells; in contrast, in MN, the C5aR mRNA score in glomerular epithelial cells is higher than in mesangial cells ($P < 0.05$)

Table 4. Correlation between C5aR mRNA and protein expression and degree of tissue injury in patients with mesangial proliferative glomerulonephritis (mesGN)

	C5aR mRNA[c]	C5aR protein[c]
Mesangial hypercellularity[a]	0.724[d]	0.791[d]
Mesangial matrix expansion[a]	0.837[d]	0.632[e]
Tubular atrophy[b]	0.865[d]	0.410
Interstitial broadening[b]	0.889[d]	0.327

[a] The degrees of mesangial hypercellularity and mesangial matrix expansion were graded from 0 to 3 according to the method of Yoshioka et al [14]; five to seven glomeruli in each section were analyzed.

[b] The degrees of tubular atrophy and interstitial broadening were graded separately from 0 to 3 according to the method of Furusu et al [15]; three to five interstitial fields in each section were analyzed.

[c] In each glomerulus and tubulointerstitial field, the degrees of C5aR mRNA and protein staining were scored according to the method of Niemir et al [16] with a minor modification. Cells containing clearly stained cytoplasm were identified as C5aR mRNA and protein-positive cells. Tabular entries are Spearman's correlation coefficients.

[d] $P < 0.01$, [e] $P < 0.05$

detected in tubular epithelial cells, vascular endothelial cells, and vascular smooth muscle cells. C5aR protein was detected in tubular epithelial cells (data not shown). In MN and mesGN, C5aR mRNA was expressed in tubular epithelial cells (Fig. 4A), vascular endothelial cells (Fig. 4B), and vascular smooth muscle cells (Fig. 4B). The signal was particularly strong in the cells of atrophic tubules (Fig. 4C). In areas of severe interstitial expansion, some infiltrating cells were also positive for C5aR mRNA (Fig. 4D). A proportion of these cells was stained for C5aR protein (Fig. 5). The number of C5aR mRNA-positive tubules correlated significantly with the degree of tubular atrophy in mesGN (Table 4). The number of interstitial infiltrating cells positive for C5aR mRNA also correlated with the degree of interstitial broadening (Table 4). However, the extent of staining for C5aR protein in tubules and infiltrating cells did not correlate with the tubulointerstitial changes (Table 4).

Relationship between the expression of C5aR and C3 deposition

In mesGN, the expression of C5aR mRNA and protein did not correlate with the degree of C3 deposition in glomeruli.

Relationships between the expression of C5aR and clinical parameters

We also examined the relationships between proportions of C5aR mRNA-positive cells among total glomerular cells, mesangial cells, glomerular epithelial cells, tubules, and interstitium and protein expression in these cells, and various clinical parameters in patients with mesGN and MN. In mesGN, the degree of expression of C5aR mRNA in the interstitium correlated with the

level of serum creatinine ($P < 0.05$). However, there was no correlation between the expression of C5aR mRNA and the extent of C5aR protein expression and any other clinical parameters in mesGN. In MN, none of the clinical parameters correlated with the level of C5aR mRNA expression among total glomerular cells, mesangial cells, glomerular epithelial cells, and in tubulointerstitial area or with C5aR protein expression.

Evaluation of the specificity of immunohistochemistry and in situ hybridization

No staining was detected in any specimens when mouse IgG1 was used as a negative control instead of monoclonal antibodies in the immunohistochemical studies (data not shown). The specificity of the signal for C5aR mRNA was evaluated by three control experiments. When a 100-fold excess of unlabeled antisense oligonucleotide was added to the standard hybridization mixture containing DIG-labeled C5aR antisense, the signal was completely abolished (Fig. 6). An in situ hybridization study was also performed with a DIG-labeled C5aR sense probe and pretreatment of tissue with RNase. No signal for C5aR mRNA was detected in these control experiments (data not shown).

DISCUSSION

Our study identified the type of cells that produce C5aR mRNA in human kidney. These include mesangial cells, glomerular epithelial and endothelial cells, cells of Bowman's capsule, tubular epithelial cells, vascular endothelial and smooth muscle cells, and infiltrating cells. These results also confirm the presence of C5aR protein in human kidney. Furthermore, we show that the level of C5aR mRNA expression increase with the progression of renal tissue injury in human mesGN.

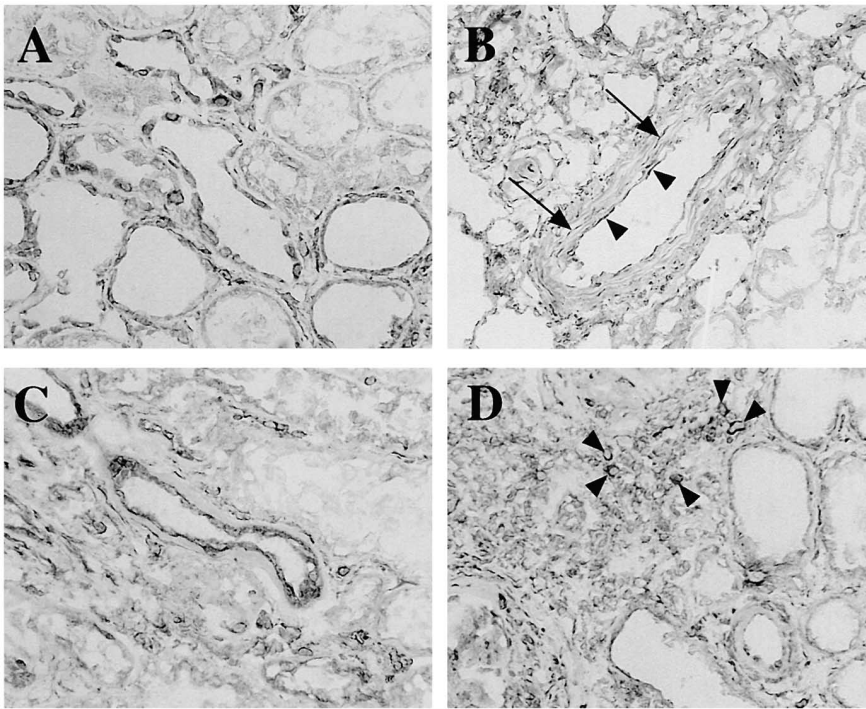Since C5aR is expressed on the surface of circulating

Fig. 4. In situ hybridization study for C5aR mRNA in the interstitium of IgA nephropathy. Tubular epithelial cells positive for C5aR mRNA (*A*). C5aR mRNA is expressed on vascular endothelial cells (*B*, arrowheads) and vascular smooth muscle cells (B, arrows). C5aR mRNA is strongly expressed in epithelial cells of atrophic tubules (*C*). Some infiltrating cells are positively stained for C5aR mRNA (*D*, arrowheads). Magnification A and C, ×250; B and D, ×200.
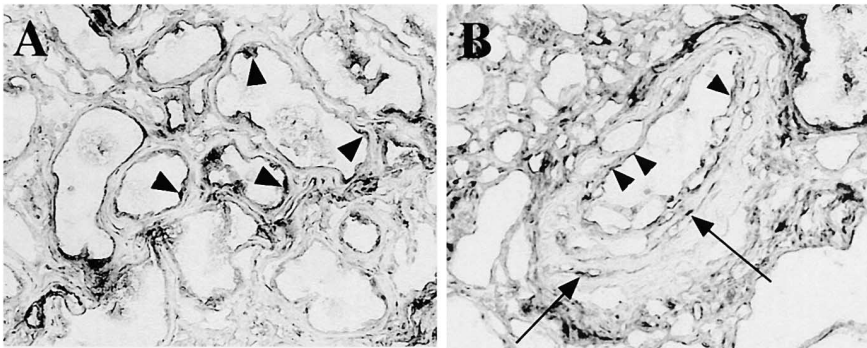

Fig. 5. Immunohistochemistry for C5aR in the interstitium of IgA nephropathy. Note the presence of C5aR protein in tubular epithelial cells (*A*, arrowheads), vascular endothelial cells (*B*, arrowheads), and vascular smooth muscle cells (B, arrows). Magnification ×200.
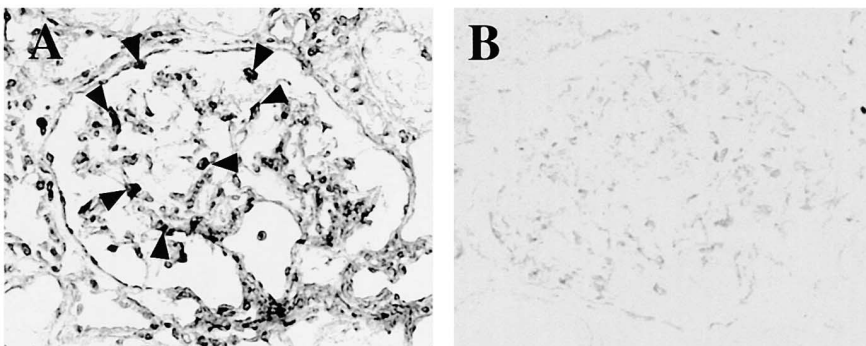

Fig. 6. Competitive study for the specificity of C5aR mRNA signals. C5aR mRNA-positive signals are detected in the glomerulus (*A*, arrowheads). The section hybridized with the C5aR antisense probe in the presence of a 100-fold excess of unlabeled C5aR homologous oligonucleotides. Note the complete disappearance of signals for C5aR mRNA (*B*). Magnification ×200.

peripheral blood cells, including monocytes/macrophages, neutrophils, and leukocytes [19], we examined the contribution of these cells to C5aR mRNA-positive cells found in the glomeruli by searching for CD68 (a marker of monocytes)-positive cells among the C5aR mRNA-positive intraglomerular cells. In fact, immunostaining of monocytes was also detected in glomeruli (data not shown). Moreover, we carefully examined the presence of neutrophils in glomeruli morphologically, and some infiltration of neutrophils was observed. These findings suggest that some infiltrating cells into glomeruli may be relevant to C5aR expression; however, the number of these cells was clearly far less than that of C5aR mRNA-positive glomerular cells. This observation suggests that the large quantity of C5aR in glomeruli is derived primarily from glomerular cells rather than infiltrating monocytes or macrophages.

The present study demonstrates that the glomerular expression of C5aR in glomerular cells correlates positively with the degree of mesangial hypercellularity and mesangial matrix expansion in mesGN. Deposition of the terminal complement components, C5b-9, clearly plays a major role in glomerular cell proliferation and the development and progression of glomerular sclerosis [20, 21]. This finding suggests that the interaction between C5a and C5aR on the renal cells also may play a role in this process. In fact, C5a can induce proliferation of cultured human mesangial cells [9] and enhances the release of platelet-derived growth factor [9], which is a known mediator of mesangial proliferation and matrix expansion from mesangial cells. In addition, it is also possible that C5a may alter glomerular blood flow by directly binding to C5aR because C5a causes smooth muscle contraction and mediates changes in both vascular tone and permeability [22]. Since mesangial cells are thought to be derived from vascular smooth muscle cells [23–25], it is possible that the interaction of C5a/C5aR causes mesangial cell contraction. Related to this effect, previous studies demonstrated that a direct injection of C5a into the renal artery and local generation of C5a in passive Heymann nephritis results in significant falls in renal blood flow and glomerular filtration rate [26, 27]. Moreover, Wilmer et al recently demonstrated that C5a causes a progressive increase in the level of transcription factors and up-regulation of mRNA level for the early response genes c-*jun* and c-*fos* on cultured human mesangial cells [10]. Based on these early findings together with the present results, we speculate that C5a may participate in the glomerular injury in mesGN via glomerular expression of its receptor.

The present study also shows the tubular and interstitial expression of C5aR, as the expression of C5aR mRNA in tubules and interstitium correlates positively with the degree of tubulointerstitial injury in mesGN. Since complements are activated in the tubulointerstitial area in humans [2, 28] and animal models [29] of glomerulonephritis, C5a, which is generated during complement activation, may be involved in mediating tubulointerstitial injury via tubulointerstitial expression of its receptor. C5a can stimulate the release of cytokines and chemokines from mesangial cells [9] and inflammatory cells [30, 31]. It is possible that C5a may have a similar effect on tubular epithelial cells. In fact, tubular epithelial cells can produce a variety of cytokines [for example, interleukin-6 (IL-6) [32] and tumor necrosis factor-α (TNF-α) [33]] and chemokines [IL-8 [34], monocyte chemoattractant protein-1 (MCP-1) [35], and RANTES [36]]. In addition, C5a binding to C5aR leads to an up-regulated expression of adhesion molecules in endothelial cells and margination of inflammatory cells on pulmonary vessels during complement activation in vivo [37–39]. This evidence suggests that C5a may up-regulate the chemotaxis and attraction of interstitial infiltrating cells by binding to locally synthesized C5aR and that the interaction of C5a/C5aR in tubulointerstitium may influence the progression of tubulointerstitial injury.

Immunohistochemical staining in the present study failed to demonstrate the expression of C5aR in the glomeruli of normal kidneys and MCNS, although C5a mRNA was expressed in glomerular cells. This different expression pattern suggests that either the level of basal glomerular expression of C5aR protein in normal kidney and MCNS in vivo is below the level of detection by immunohistochemistry or that up-regulation of C5aR expression may occur in mesGN or MN. The mediators responsible for C5aR up-regulation in renal cells are not yet known. Previous studies identified TNF-α as an essential mediator of C5aR expression in mouse brain [40]. Furthermore, interferon-γ (INF-γ), an important immunoregulatory cytokine that is produced in glomerulonephritis [41], also stimulates the synthesis of C5aR in human myeloblastic cells [42]. However, the mediator(s) responsible for inducing C5aR expression in renal cells remains to be elucidated.

Complement activation occurs frequently in human glomerulonephritis, and thus, the increased expression of C5aR in renal cells may be important for the progression of glomerulonephritis. Although our knowledge about the function of C5a/C5aR in diseased kidney is limited, the precise roles of C5a/C5aR in immune-complex diseases in the lung, skin, and peritoneum are well-defined in studies of the the reverse passive arthus reaction using C5aR-deficient mice [43, 44]. In these studies, neutrophil migration was found to be substantially impaired in the lung, skin, and peritoneum, and pulmonary vascular permeability was significantly decreased in C5aR-deficient mice compared with C5aR-sufficient mice. Furthermore, a near-complete protection against histologic damage was identified in the lungs of C5aR-deficient mice [44]. On the other hand, using the C5aR antagonist,

Heller et al demonstrated similar effects for neutrophil accumulation and migration to immune complex diseases in mice, that is, the reverse passive arthus reaction in the lung, skin, and peritoneum [45]. They also showed that administration of C5aR antagonist reduced local and remote tissue injury in intestinal ischemia/reperfusion injury. These findings indicate that C5a is an important mediator that can trigger the inflammatory cascade seen in immune complex diseases and ischemia/reperfusion injury. The findings also suggest that inhibition of C5a/C5aR interaction may be useful in reducing the extent and severity of renal injury in glomerulonephritis.

In conclusion, the present study demonstrates the expression of C5aR and C5aR mRNA in mesangial cells, glomerular epithelial and endothelial cells, and cells of Bowman's capsule in the human diseased kidney. Furthermore, C5aR is also expressed and synthesized in tubular epithelial cells, vascular smooth muscles and endothelial cells, and infiltrating cells in the interstitium. Our findings suggest that the C5a/C5aR interaction may play a pathogenic role in the development and progression of glomerulonephritis, and that such an interaction can be an additional mechanism through which complement activation induces renal injury.

APPENDIX

Abbreviations used in this article are: C5aR, C5a receptor; DIG, digoxigenin; HRP, horseradish peroxidase; IFN-γ, interferon-γ; IgAN, immunoglobulin A nephropathy; IL, interleukin; LN, lupus nephropathy; MCNS, minimal change nephrotic syndrome; mesGN, mesangial proliferative glomerulonephritis; MN, membranous nephropathy; PAS, periodic acid-Schiff reaction; TNF-α, tumor necrosis factor-α.

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