Renal Cortical Complement C3 Gene Expression in IgA Nephropathy

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Abstract. Glomerular C3 deposits are commonly found in immunoglobulin A (IgA) nephropathy. Renal gene expression and protein synthesis of complement components have been shown in settings of tissue inflammation. In this study, the pathogenetic involvement of locally produced C3 in IgA nephropathy was analyzed. C3 gene expression was analyzed by reverse transcription, polymerase chain reaction, and in situ hybridization techniques. C3 mRNA was detected in 56% of cases, with a significantly higher percentage in patients with moderate-to-severe lesions than in those with mild lesions ($P < 0.01$). By in situ hybridization, C3 transcript was predominantly expressed by tubular cells and some interstitial cells. C3 mRNA was also observed on glomerular parietal epithelial cells. Immunoreactive native C3 was detected on cortical tubuli by an anti-C3c immunoalkaline-phosphatase technique. A significant correlation was found between renal C3 transcription and glomerulosclerosis, intracapillary proliferation (both $P < 0.005$) and markers of interstitial damage, including tubular atrophy ($P < 0.05$), interstitial infiltration ($P < 0.05$), and fibrosis ($P < 0.005$). Proteinuria ($P < 0.05$), but not serum creatinine, at the time of renal biopsy correlated with C3 mRNA. In conclusion, it was demonstrated that the C3 gene was expressed primarily in proximal tubular cells and occasionally in glomerular crescents, and that its expression correlated with clinical and histologic markers of severity and poor outcome of IgA nephropathy. Thus, a pathogenetic involvement of the local transcription and translation of the C3 gene in IgA nephropathy was suggested. (J Am Soc Nephrol 8: 415–425, 1997)

Glomerular deposits of C3 are often observed in primary immunoglobulin A (IgAN), a form of chronic glomerulonephritis characterized by diffuse glomerular IgA deposition (1). The granular pattern of C3 deposits represents a hallmark of complement (C) activation, which probably occurs either in circulation or locally after formation and/or renal deposition of IgA immune complexes. It has been demonstrated experimentally that activation of C may determine some pathophysiologic alterations associated with glomerulonephritis (2).

Another mechanism that could involve the pathogenetic participation of C in IgAN and could also theoretically account for the generation of C deposits is represented by the local synthesis of C by renal cells. It has been demonstrated that renal cells in culture, such as mesangial, glomerular, and tubular epithelial cells, are able to synthesize C components C3, C4 (3–8), and some regulatory factors (5,9–11). Activation of gene expression and protein synthesis of these C factors is induced by proinflammatory substances such as cytokines or an activated terminal C complex, which may play a pathogenetic role in IgAN (9,12). Studies on renal tissue have also demonstrated both in animal models and human specimens that C is synthesized locally in several kidney diseases (13–19). We previously showed in an experimental model of IgAN that C3 mRNA is detectable in renal cortex, and is induced by passive administration of IgA immune complexes containing a nephritogenic antigen (20). Moreover, we noticed a synergism between IgA immune complexes and the proinflammatory cytokines interleukin-1 (IL-1) and IL-6 in the induction of cortical C3 transcript. Therefore, we extended our studies and investigated this phenomenon in human IgAN, a disease in which inflammatory phenomena are present to a variable extent. Perhaps the histological appearance of the glomerulonephritis resembles a spectrum from very mild lesions to severe and progressive renal damage. Moreover, we sought possible correlations with clinical and histological features of disease severity. This study shows evidence that C3 is produced within renal cortex in IgAN in parallel with clinical and histologic signs of severe and progressive disease.

Materials and Methods

Patients

Twenty-five IgAN patients were enrolled in the study. Diagnosis was made by renal biopsy and standard examination of the cortical tissue by light microscopy and immunofluorescence. In all patients, clinical conditions that configure a secondary IgAN (systemic lupus erythematosus, liver cirrhosis, and Schönlein-Henoch purpura) were excluded. As routinely performed in our institution, the histologic diagnosis was also supplemented with a grading of disease severity according to the five-level classification of K. Lee et al. (21). Conventionally, we indicate G1 to G2 classes as mild and nonprogressive disease, whereas G3 to G5 classes involve moderate-to-severe disease...
with a potential of progression to end-stage renal failure. Six renal specimens, obtained through biopsy performed for diagnostic purposes in patients with microhematuria and no immunohistological alterations at light microscopy examination, were used as negative controls.

The patient population studied was characterized by a mean age of 27.2 ± 10.7 (range, 7 to 61) and a male-to-female ratio of 17 to 8. Informed consent to participate in the study was obtained from each patient.

**Specimens**

Renal biopsies were performed using Colt needles (Sterilab, Milan, Italy). Two cortical fragments were obtained from each patient. Immunofluorescence and light microscopy were performed routinely to establish the histologic diagnosis. A portion of the cortical fragment was utilized for RNA extraction and in situ hybridization.

**RNA Extraction**

The cortical fragments of IgAN patient biopsies suitable for RNA extraction or from control subjects were immediately frozen in a 4 M guanidinium isothiocyanate–based denaturing solution and stored at −80°C. Tissue was homogenized with an ultraturrax. RNA extraction was carried out by single-step acid phenol-chloroform extraction according to the methods of Chomczynski and Sacchi (22).

**Polymerase Chain Reaction Analysis**

DNA was first reverse-transcribed into cDNA with the RNA-PCR kit (Perkin-Elmer, Norwalk, CT), which uses a murine Moloney leukemia virus (MMLV) reverse transcriptase. Random hexamers were used as universal primers for RNA. The reaction mixture in polymerase chain reaction (PCR) buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3) included, in a final volume of 20 µL: total RNA (approximately 1 µg), 100 U MMLV, dNTP 1 mM each, RNase inhibitor 20 U, and MgCl₂ 5 mM. Incubation was performed at 42°C for 30 min. After completion of the reaction, samples were heated at 99°C for 5 min to inactivate the reverse transcriptase. The cDNA generated were subsequently used as templates for the PCR. An aliquot of the reverse-transcription mixture (1.5 µL) was added with 200 µM dNTP, 0.4 µM each primer, and AmpliTaq DNA polymerase 1.25 U (Perkin-Elmer Cetus), to a final volume of 50 µL. PCR amplification was carried out for a total of 30 cycles in a thermal cycler (Perkin-Elmer): each cycle composed of the following three steps: denaturing, 60 s at 95°C; annealing, 90 s at 60°C; primer extension, 60 s at 72°C. The number of cycles was chosen to achieve, in the linear range of amplification product–amount of mRNA, a high sensitivity of the method and prevention of the formation of nonspecific amplification products.

To minimize interassay variations, all amplifications were carried out at the same time.

**Primers**

Sequences for human C3 specific primers were kindly donated by Dr. Abdalla Rifai (Providence, RI). Upstream and downstream primers were, respectively, CTTGATGCTGCTCAATGGC and CTTGAGATGGTACACAGTACC. The two primers encompassed a 554-base pair (bp) fragment of the cDNA encoding for the α-chain region of C3, which spanned Exon 33 to Exon 39 of the genome sequence (23). We used GAPDH as a housekeeping gene. To this purpose, two primers were generated: upstream, ATTCGGTTGCTAT-ACCAGGA; and downstream, TGGTATCTGGAGGACTCATGAC, which defined a 450-bp fragment.

**Analysis of Amplified Products**

After PCR amplification, 7 µL of the sample were separated onto 1.5% agarose in Tris-borate-EDTA (TBE) buffer. To increase the specificity of message detection, nucleic acids were blotted onto nylon filters (Nytran N.V., Mortsel, Belgium) and hybridized with a human C3-specific probe. The clone pHLC3.11, which contains a 4.2-kilobase (kb) insert, was obtained from American Type Culture Collection (Bethesda, MD). The insert was removed by Clai-SalI double-digestion, which yielded two fragments of 2.4 and 1.8 kb. The smallest fragment was labeled by a nonisotopic random primer labeling system (Amersham, Little Chalfont, UK) and used for hybridization. Nylon filters were finally developed by the ECL method (Amersham) and exposed to x-ray films for 2 min. From each blot, a second film was obtained after an overnight exposure, to exclude eventual low-level messages.

Autoradiography bands were quantitated by a computer-based morphometric analysis system composed of an Arcus II scanner (Agfa-Gevaert N.V., Mortsel, Belgium) connected to a PowerPC computer (Macintosh, Cupertino, CA). Imported data were analyzed quantitatively by OptiLab Pro 2.6.1 software (Graftek, Villanterio, PV, Italy), which operated a color-based pitch densitometry. Results are expressed as C3/GAPDH intensity ratio.

**In Situ Hybridization**

Frozen 4-µm-thick sections were cut from renal biopsies, collected onto polylysine-coated slides, dried briefly onto a hot plate (80°C), and fixed for 20 min in 4% paraformaldehyde. Sections were then washed in phosphate-buffered saline, dehydrated in graded ethanol, and used for hybridization as previously described (24,25). Each specimen was hybridized with either an antisense or a sense RNA probe. Autoradiography was performed by dipping the dehydrated slides into Ilford G5 nuclear emulsion (Ilford, Mobberley Cheshire, UK), and subsequent development was performed with Kodak D19 developer (Kodak, Hemel Hampstead, UK). Finally, slides were counterstained with hematoxylin.

The C3 probe used for in situ hybridization was generated from a pHLC3.11 clone. After double-digestion of the entire plasmid with SalI-Sall restriction endonucleases, a 403-bp fragment was selected to be subcloned into a pGEM3zf plasmid (Promega Biotech, Zurich, Switzerland). After linearization with either SalI or HindIII restriction endonuclease, SP6 and T7 RNA polymerase (Boehringer Mannheim, Mannheim, Germany) were used to generate run-off transcripts of either the antisense (complementary to mRNA) or sense (anticomplementary, negative control) 35S-labeled strands, respectively.

**Immunohistochemistry for C3**

To detect native C3 synthesized locally within renal tissue, a modification of a previous method, based on a monoclonal anti-C3c (18), was applied. Frozen renal sections were fixed in paraformaldehyde. The primary antibody was a mouse monoclonal anti-human C3c (Quidel, San Diego, CA) used at a dilution of 1:400. This antibody is presumed to detect native C3 predominantly, because the protein included within immune deposits, deriving from fixation of circulating C3, should be primarily in the form of C3d (26).

After reaction with a secondary rabbit anti-mouse antibody, development of the immune reaction was performed with the alkaline phosphatase–anti-alkaline phosphatase complex (APAAP) method (Dako, Milan, Italy), using new fuchsin as substrate, as previously described (24,25).
**Statistical Analyses**

Statistical comparison of cortical C3 gene expression by PCR analysis and clinical and histologic features of disease severity was performed by means of linear regression analysis, χ² test, or unpaired t test as indicated in the Results section.

**Results**

Analysis of C3 gene expression on total renal cortex of IgAN patients by PCR showed the presence of the specific transcript in 14 biopsies (56%), whereas only one specimen of six (16.6%) of the control group showed a weak positive transcript (χ² = 4.01, P < 0.05). The intensity of the message was normalized against the housekeeping GAPDH gene expression. The C3 gene expression was rather variable among IgAN patients (Figure 1, A and B) and control subjects (Figure 1C). In the group of patients with mild lesions (G1 to G2), five cases were positive (5 of 12; 41.7%), whereas the percentage of positive cases among the moderate-to-severe lesions group (G3 to G5) was significantly higher (69.2%; χ² = 6.82, P < 0.01).

Glomerular deposits of immunoglobulins and C3 were semiquantitatively evaluated by immunofluorescence and assigned a score of 0 to 3. Immune deposits of C3 were detected almost exclusively within glomeruli in 83% of cases. The pattern of distribution paralleled the deposition of IgA in mesangial area. No correlation between C3 cortical gene expression and the magnitude of immune deposits was found. Fine granular parietal deposits were also demonstrable in six cases, independent of the C3 gene detection. In three cases, arteriolar subintimal deposition of C3 was also noted. However, no specimen showed tubular staining with the anti-C3 fluoresceinated antibody.

The C3 gene expression did not correlate with glomerular IgG, IgM, or fibrinogen deposits.

Individual features of the severity of the histologic damage were determined by the routine examination of renal biopsies and are summarized in Table 1. Statistical analysis by χ² test was performed, comparing the percentages of a specific feature observed in the two groups of patients showing either cortical C3 mRNA expression or not. As reported in Table 2, C3 gene expression was more frequent in the presence of histologic markers of glomerular damage, such as glomerular sclerosis, affecting more than 25% of examined glomeruli and endocapillary proliferation. Conversely, no correlation was found between C3 mRNA and the crescents present in more than 10% of the glomeruli. A significant correlation was also noted between cortical C3 gene expression and markers of interstitial damage, including tubular atrophy, interstitial mononuclear cell infiltration, and fibrosis. Finally, the concomitance of arteriolar hyalinosis with the typical glomerular lesions did not significantly correlate with C3 gene expression.

Statistical analysis was also performed using the pathophysiologic indexes of glomerulonephritis at the time of renal biopsy. The entire patient population was divided into two groups on the basis of whether the C3 message expression in renal cortex was positive or not. Mean values of serum creatinine and proteinuria were compared by t test in the two groups. Although mean creatinine values were not statistically different, proteinuria was significantly higher (P < 0.05) in patients with C3 gene expression (Figure 2).

To validate and localize the C3 transcript expression in the renal cortices of patients with IgAN, biopsy specimens were analyzed by in situ hybridization with a C3-specific probe obtained from a well-characterized clone (pHLC3.11). Before being used on renal tissue, the probe was tested on HepG2 cells, which constitutively express C3 mRNA at a high level. On such a positive control, antisense and sense probes generated a strong signal and a reasonable background, respectively, with a high signal-to-noise ratio (Figure 3, A and B). Kidney biopsies from patients with G2 lesions that demonstrated a
**Table 1.** Summary of the histologic features associated with mesangial hypercellularity and matrix increase observed in IgAN patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Patient</th>
<th>Histologic Grading</th>
<th>C3/GAPDH Ratio</th>
<th>Glomerular Sclerosis (%)</th>
<th>Endocapillary Proliferation</th>
<th>Crescent (%)</th>
<th>Tubular Atrophy</th>
<th>Arteriolar Hyalinosis</th>
<th>Interstitial Infiltration</th>
<th>Interstitial Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P.M.</td>
<td>G1</td>
<td>0</td>
<td>11.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>C.D.</td>
<td>G2</td>
<td>0.26</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Z.A.</td>
<td>G2</td>
<td>0</td>
<td>13.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>F.I.</td>
<td>G2</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>10</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>D.F.G.</td>
<td>G2</td>
<td>0</td>
<td>8.3</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>C.G.</td>
<td>G2</td>
<td>0</td>
<td>5</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>D.M.P.</td>
<td>G2</td>
<td>2.07</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>P.R.</td>
<td>G2</td>
<td>3.23</td>
<td>16.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>P.G.</td>
<td>G2</td>
<td>0</td>
<td>2.2</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>S.C.</td>
<td>G2</td>
<td>1.20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>D.B.S.</td>
<td>G2</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>C.L.</td>
<td>G2</td>
<td>0.34</td>
<td>9.1</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>S.N.</td>
<td>G3</td>
<td>0.16</td>
<td>25</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>C.V.</td>
<td>G3</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>16.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>M.F.</td>
<td>G3</td>
<td>0</td>
<td>46.7</td>
<td>+</td>
<td>6.7</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>F.O.</td>
<td>G3</td>
<td>0.70</td>
<td>42.9</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>C.N.</td>
<td>G3</td>
<td>1.28</td>
<td>33.3</td>
<td>+</td>
<td>20</td>
<td>+</td>
<td>++</td>
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<td>+</td>
</tr>
<tr>
<td>18</td>
<td>S.L.</td>
<td>G3</td>
<td>0.58</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>L.L.</td>
<td>G4</td>
<td>1.63</td>
<td>46.1</td>
<td>+</td>
<td>30.8</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>B.F.</td>
<td>G4</td>
<td>1.66</td>
<td>47.4</td>
<td>+</td>
<td>26.3</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>21</td>
<td>D.M.</td>
<td>G4</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>22</td>
<td>O.F.</td>
<td>G4</td>
<td>0.36</td>
<td>66.7</td>
<td>+</td>
<td>44.4</td>
<td>++</td>
<td>0</td>
<td>1</td>
<td>++</td>
</tr>
<tr>
<td>23</td>
<td>L.D.</td>
<td>G4</td>
<td>1.70</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>L.N.</td>
<td>G5</td>
<td>0</td>
<td>84.6</td>
<td>0</td>
<td>15.4</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>M.D.</td>
<td>G5</td>
<td>1.34</td>
<td>70</td>
<td>++</td>
<td>20</td>
<td>+++</td>
<td>0</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

* Presence and intensity of the C3 specific message in renal cortex is expressed by C3/GAPDH intensity ratio. For individual histopathologic elements, each specimen was assigned from absent (0) to +++. 

negative C3 message during PCR analysis showed a pattern with the antisense probe that did not differ from the sense background control or the normal specimen (Figure 4, A through D). Biopsies from patients with G4 to G5 IgAN, remarkable tubulointerstitial damage with tubular atrophy and extensive mononuclear infiltration, and a rapid decline in renal function revealed C3 gene transcription prominently expressed in proximal tubules, especially in those showing atrophy (Figure 4, E and F). Interestingly, glomeruli with cellular or fibrocellular crescents were also positive on peripheral cells, which could be identified as parietal epithelial cells (Figure 5). Conversely, obsolescent glomeruli were negative for the C3 mRNA.

To increase the sensitivity of the C3 protein detection, with the goal of demonstrating that translation of C3 occurred in the areas of C3 mRNA detection, we performed immunohistologic staining, previously validated onto HepG2 cells (Figure 6, A and B), with a monoclonal anti-C3c antibody of two specimens with high message expressions. Both of these cases showed C3c immunoreactivity primarily in the tubulointerstitial compartment (Figure 7A), with some tubules demonstrating a specific apical concentration of the protein (Figure 7B).

**Table 2.** Statistical analysis comparing histopathologic features of all patients studied and positivity of the C3 mRNA

<table>
<thead>
<tr>
<th>Histopathologic Feature</th>
<th>χ² Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular sclerosis</td>
<td>8.83</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>(≥25% glomeruli)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocapillary proliferation</td>
<td>7.70</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Crescents (&gt;10% glomeruli)</td>
<td>3.00</td>
<td>NS</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>4.97</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Arteriolar hyalinosis</td>
<td>0.82</td>
<td>NS</td>
</tr>
<tr>
<td>Interstitial infiltration</td>
<td>5.46</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>8.83</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

* Patients were divided into two groups according to the C3 mRNA cortical expression by PCR. The statistically significant association of each individual feature with the C3-transcript positivity was assessed by χ² analysis. 

b NS, not statistically significant.
Discussion

The production of C components within the renal cortex may play a pathogenetic role in settings of tissue inflammation, as occurs in glomerulonephritis. In the article presented here, we show evidence that C3 is transcribed and translated in renal cortical cells in human IgAN. The results were gained by application of two different molecular biology techniques—which are highly specific if probed properly—and the immunohistologic demonstration of the protein with a monoclonal anti-C3c. C3 gene expression correlated with histologic elements of disease severity. C3 mRNA and protein were localized primarily in the tubulointerstitial tissue and also in some glomeruli.

The participation of C as a mediator of immune inflammation has been consistently proposed as a chief component of the pathogenesis of different types of glomerulonephritis (27-29). In the classic view, C factors are activated through either the classic or alternate pathway in conditions such as glomerular deposition of circulating immune complexes or formation of immune complexes in situ. In the first case, C is primarily activated in circulation, whereas in the second case, it is activated locally in the kidney tissues. This study, along with previous reports (17,18), sheds light on the possibility that local synthesis of C factors may represent an additional mechanism by which C participates in the pathogenesis of glomerulonephritis.

According to a preliminary report by Sacks et al. (17), approximately half of the biopsy specimens studied showed a positive C3 transcript by PCR analysis. By this technique, and under the conditions used, we could establish a clear-cut separation between cases that showed C3 mRNA and those that were negative. In addition, we did not find significant correlation between the intensity of C3 expression (as indicated by C3/GAPDH ratio) and the overall histologic severity of disease (data not shown). Because our PCR methodology was designed not to be quantitative, the specimens studied were divided into two groups (positive and negative for C3 transcript) and, in turn, statistical analysis was carried out by comparison of percentages. By this analysis, a correlation was found between C3 mRNA expression and the important elements of severity of glomerulonephritis. Features of glomerular injury that correlated with C3 mRNA expression, such as glomerulosclerosis in more than 25% of glomeruli and intracapillary proliferation, have been associated with an unfavorable outcome in terms of renal survival (30). Interestingly, the participation of tubulointerstitial damage was strictly correlated with the C3 gene expression. It has been demonstrated that the tubulointerstitial damage and lymphocyte infiltration may account for a worse outcome of the disease in IgAN (31-33). From these observations, although more extensive follow-up data are required to draw statistical conclusions, it can be suggested that the renal cortical expression of C3 is an additional pathogenetic factor that parallels an unfavorable outcome of IgAN. As a matter of fact, three patients with G4 to G5 lesions and a strong C3 mRNA expression showed an accelerated progression toward...
end-stage renal failure, and required renal transplantation (data not shown).

Studies conducted on renal cells in culture by our group and others have demonstrated that C factors can be induced under stimulation with proinflammatory substances in glomerular mesangial and epithelial cells and proximal tubular cells (3–8). Moreover, to discriminate whether the C3 cortical gene expression was only an accompanying phenomenon—for example, one accompanying interstitial mononuclear cell infiltration—we conducted experiments to localize the specific mes-
Figure 4. Photomicrographs representative of the in situ hybridization pattern with the antisense C3 probe. Bright- (A, C, E) and dark- (B, D, F) field of a control specimen (A, B), a patient with IgAN G2 (C, D), and a patient with IgAN G4 and prominent tubulointerstitial damage (E, F) are shown. In the patient with IgAN G2 (C, D), a weak signal comparable with the normal control (A, B) or the same specimen hybridized with the sense probe (not shown) is detectable both in glomerulus and tubulointerstitial compartment. By contrast, a significantly increased grain density is demonstrable in the IgAN G4 specimen (E, F), which localized predominantly on dilated and atrophic tubuli containing hyalin casts. (Original magnification, ×200.)
Figure 5. Photomicrographs of renal biopsy sections from a patient with IgAN G5 and severe interstitial lesions and glomerular crescents, analyzed by in situ hybridization with the antisense C3 probe. Besides the prominent tubular localization (A, B), the C3 message was also demonstrable on glomeruli showing cellular crescents (C, D). At higher magnification (E, F), the source of the transcript could be identified as glomerular parietal epithelial cells. Bright- (A, C, E) and dark- (B, D, F) field photomicrographs are shown. (Original magnification: A, B, ×100; C, D, ×200; E, F, ×400.)
sage by *in situ* hybridization. The C3 mRNA was expressed predominantly by tubular cells; to some extent, the transcript was also detected in interstitial cells and glomeruli. Therefore, we could conclude that the gene expression was linked to intrinsic renal cells and not only to infiltrating mononuclear cells. These data are in accordance with those of a previous study by Welch *et al.* (18) on different nephropathies, which showed a predominant signal in tubular cells. Glomerular inflammatory phenomena may also activate glomerular cells to express C3 mRNA in IgAN. The data are relevant and in parallel with the observation that IL-1 has been shown to participate pathogenetically in experimental and human IgAN (34). The same factor is also the most relevant and potent inductor of C3 mRNA in mesangial and glomerular epithelial cells in culture (5,7).

The finding of an absence of correlation between renal C3 gene expression and C3 immune deposits by immunofluorescence assay could be explained by several considerations. First, although the polyclonal antiserum used for immunofluorescence also reacts with C3c, it specifically detects the C3d region of the protein, which represents the major form of C3 fixed in immune deposits (26). It should, however, disclose the native C3, even though the methodology might not be sensitive enough to detect low concentrations. We therefore used an immunohistologic staining method that was based on an immunohistochemical phosphatase technique which used a monoclonal anti-C3c antibody to detect the native C3 specifically (18).

With this method, we were able to demonstrate that an immunoreactive native C3 paralleled the C3 gene expression, as evidenced by *in situ* hybridization in severe IgAN (Figure 7).
The mechanisms by which the local synthesis of C3 may play a pathogenetic role can only be matters of speculation. Activation of C3 and binding of the split product on mesangial cells may trigger intracellular signals that initiate cell activation and increased expression of regulatory proteins, such as decay-accelerating factor (11). On proximal tubular epithelial cells, the synthesized C3 may participate into the assembly of soluble C5b-9, which is detected at high levels in urine in proteinuric states (35). Moreover, it has been demonstrated that the apical portion of tubular cells does not express regulatory soluble C5b-9, which is detected at high levels in urine in IgAN patients.

In conclusion, this study describes the local transcription and translation of C3 in the renal cortices of IgAN patients. C3 synthesis was localized primarily in proximal tubules and, to a lesser extent, in glomeruli. This phenomenon was associated with clinical and histologic features of disease severity and could have pathogenic relevance in the inflammatory events associated with human IgAN.

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References

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