Antigen-Antibody Complexes Enhance the Production of Complement Component C3 by Human Mesangial Cells

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Abstract. Deposition of immune complexes (ICX), with or without complement, occurs in various forms of glomerulonephritis. It has been reported that upregulation of complement C3 mRNA expression is found in kidneys of patients with ICX glomerulonephritis. In vitro studies have indicated that mesangial cells (MC) synthesize C3. Furthermore, MC express FcγRIII receptors. This study investigates whether ICX alter C3 and factor H production by MC. MC were cultured in medium alone or in medium with insoluble heat-aggregated rat IgG (AlgG) or with insoluble ICX. Basal production of C3 and factor H was 10 ± 1 ng/10^6 and 605 ± 15 ng/10^6 cells, respectively. The presence of 400 μg/ml AlgG or ICX resulted in upregulation of C3 production to 999 ± 15 ng/10^6 and 510 ± 1 ng/10^6 cells, respectively, whereas no significant change in factor H production was observed. The upregulation of C3 was inhibitable by cycloheximide, suggesting that de novo protein synthesis was required. By reverse transcription PCR and Northern blot analysis, it was demonstrated that C3 and interleukin-6 mRNA expression was upregulated in MC after incubation with AlgG. No detectable change in factor H mRNA expression was seen. In conclusion, it is shown that incubation of MC with AlgG or ICX not only results in upregulated production of inflammatory mediators such as cytokines, but also leads to an upregulation of C3 synthesis. Therefore, it is hypothesized that ICX deposited within the mesangium may enhance the local production of C3 via interaction with Fc receptors on MC. (J Am Soc Nephrol 8: 1257–1265, 1997)

Immune complex (ICX) glomerulonephritis is characterized by deposition of immunoglobulins in the glomerulus. These deposits often are localized in the mesangial area of the glomerulus (reviewed in reference 1). The immunoglobulins found in the mesangium are either of the IgG isotype (2), the IgA isotype (3), or the IgM isotype (4). The deposition of immunoglobulins in the mesangium may lead to activation of mesangial cells (MC) and production of a number of important immunological mediators, such as interleukin (IL)-1, prostaglandins, reactive oxygen radicals, and monocyte chemoattractant protein-1 (MCP-1) (5–8). It is assumed that activation of MC is mediated via cross-linking of Fc receptors (FcR) on the cell membrane. The presence of both FcγR (9–11) and FcαR (12) on human MC has been shown. Cross-linking of the FcγR on MC via aggregated IgG leads to activation of MC (11,13,14) and a subsequent enhancement of IL-6 synthesis by MC.

In addition to the deposition of immunoglobulins in different forms of glomerulonephritis, deposition of complement components is also found in the glomerulus (reviewed in reference 15). Complement components are of importance in the prevention of precipitation of ICX, but they also play a role in solubilization of deposited ICX (reviewed in reference 16). Furthermore, activation of complement results in the generation of C5a and recruitment of inflammatory cells into the mesangium. The complement components found in the mesangial deposits may be derived from the circulation, and thus synthesized by hepatocytes in the liver, or they are produced locally by intrinsic renal cells. Both in situ and in vivo studies have shown that local synthesis of complement components occurs in the kidney. In situ it was demonstrated that mRNA for C4 is detectable in tubuli of kidneys from healthy subjects (17), whereas mRNA for C3 is found only in the cortex of kidneys from patients with, for instance, mesangioproliferative glomerulonephritis II, systemic lupus erythematosus, or ICX glomerulonephritis (18, 19). Furthermore, we have described recently that complement components are expressed at specific stages of glomerular development and that mRNA expression for several complement components is found in fetal kidneys (20). In vitro studies have indicated that several renal cell types are able to produce complement components and that cytokines influence the amount of complement synthesized (21–24). For MC, it has been shown that C3 production is induced after stimulation with IL-1 and interferon (IFN)-γ (25–27), whereas factor H production already occurs under basal conditions and is regulated by IFN-γ (26).

Because it is known that triggering of FcγR on MC results in activation of MC and the subsequent production of a number of mediators, we analyzed the effect of IgG-containing complexes on the production of complement C3 and factor H by MC in vitro. C3 production was studied because C3 plays a key role in the classical and alternative pathway of complement (28), and factor H production was chosen because it is a
regulator of the amplification convertase of complement (29). The results indicate that ICX enhance C3 production by MC, whereas factor H synthesis is not significantly influenced.

Materials and Methods

Reagents

Trypsin, fungizone, 2,2'-amino-bis-3-ethylbenzthiazoline-6-sulfonic acid, collagenase type IA, FITC-phalloidin, rabbit anti-smooth muscle actin (Sigma Chemical, St. Louis, MO), Dulbecco's modified Eagle's medium (DMEM) (Seromed; Biochrom, Berlin, Germany), fetal calf serum (FCS) (Hyclone Laboratories, Logan, UT), digoxigenin N-hydroxysuccinimide (DIG-NHS), blocking reagent and sh-F(ab') anti-DIG horseradish peroxidase (Boehringer Mannheim, Mannheim, Germany), bicinechonic acid protein reagent (Pierce, Rockford, IL), 0.45-μm filter (Schleicher & Schuell, Dassel, Germany), and diethylaminoethyl Sephacel (Pharmacia LKB, Woerden, The Netherlands) were purchased as indicated. Affinity-purified rabbit IgG antihuman C3 and rabbit IgG antihuman factor H were prepared as described previously (30). RNAzol (Cinna/Biotecx, Houston, TX), Moloney murine leukemia virus reverse transcriptase, oligo(dT) 12-18 primer (Gibco BRL, Gaithersburg, MD), dNTP (Pharmacia LKB, Uppsala, Sweden), Taq polymerase (Perkin-Elmer, Norwalk, CT), Hybond N+ (Amersham, Arlington Heights, IL), and DIG-11-dUTP (Boehringer Mannheim) were used for RNA isolation, reverse transcription (RT)-PCR, and Southern blotting. Reinforced nitrocellulose (Schleicher & Schuell), a Bio-dot Apparatus (Bio-Rad Laboratories, Bochninger Mannheim) were used for RNA isolation, reverse transcription (RT)-PCR, and Southern blotting. Reinforced nitrocellulose (Schleicher & Schuell), a Bio-dot Apparatus (Bio-Rad Laboratories, Hercules, CA), and [α-32P]dCTP (3000 Ci/mmol) (Amersham) were used for dot blotting and Northern blotting. Human C3 cDNA probe C3.A1 (31) was provided by Dr. B. F. Tack (Research Institute of Scrripps Clinic, La Jolla, CA), factor H cDNA probe R2a (32) was supplied by Dr. R. B. Sim (Medical Research Council, Immunochemistry Unit, University of Oxford, UK), and FcyRIII cDNA probe pGP5 (33) was provided by Dr. M. de Haas (Central Laboratory for Bloodtransfusion, Amsterdam, The Netherlands). IL-6 cDNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA were obtained from the American Type Culture Collection (Rockville, MD).

Isolation and Aggregation of Rat IgG

Rat IgG was purified from normal rat serum following standard procedures (34) and sterilized by 0.45-μm filtration. To obtain aggregated rat IgG (AlgG), purified IgG was heat-aggregated for 4 h at 63°C at a concentration of 10 mg/ml in phosphate-buffered saline (PBS) and centrifuged for 10 min at 3000 rpm. The pellet contained the insoluble aggregates, and the supernatant contained the soluble aggregates. The pellet was resuspended in PBS, and the protein concentration was determined by bicinchoninic acid protein assay reagent. The aggregates were stored in aliquots at −20°C.

Preparation of IgG ICX

Anti-bovine serum albumin (BSA) antibodies were raised in rabbits by intramuscular immunization with BSA in Freund's complete adjuvant. IgG anti-BSA was isolated from rabbit serum by precipitation with 33% (NH₄)₂SO₄ followed by anion exchange chromatography on diethylaminoethyl Sephacel. IgG-containing fractions were pooled, concentrated, and affinity-purified using a BSA-immunosorbent column. Affinity-purified IgG anti-BSA was eluted with glycine-HCl (pH 2.8) and dialyzed against PBS. For the preparation of ICX, rabbit IgG-anti BSA (2.5 mg/ml) and BSA (0.5 mg/ml) were sterilized by 0.45-μm filtration, mixed in a ratio of 5:1, incubated for 1 h at 37°C and overnight at 4°C, and centrifuged for 10 min at 3000 rpm. The pellet containing the insoluble complexes was resuspended in PBS and, after determination of the protein concentration, stored in aliquots at −20°C.

Glomerular Cell Cultures

MC were isolated as described previously (26). The glomeruli used for the MC cultures were obtained from kidneys that were not suitable for transplantation because of anatomical reasons. Four different MC lines were used in the experiments: MC from a 13-yr-old donor (line 1), a 19-yr-old donor (line 2), a 50-yr-old donor (line 3), and a 62-yr-old donor (line 4). MC were characterized by the following: (1) uniform fluorescence with FITC-phalloidin for actin and with a monoclonal antibody directed against vimentin; and (2) absence of immunofluorescence, using factor VIII and cytokeratin antibodies. In addition, it was shown that all MC stained positive with rabbit anti-smooth muscle actin (9). Isolation and characterization of glomerular visceral epithelial cells (GVEC) were performed as described by van Det et al. (35).

To determine protein synthesis, MC between subcultures 2 and 8 were trypsinized and seeded in 24-well tissue culture plates (10⁶ cells/plate) in DMEM containing 5% FCS. MC were rendered quiescent overnight by culture in DMEM/0.5% FCS and were subsequently cultured for an additional 72 h in DMEM/0.5% FCS with or without stimuli. The total cell number in each well was determined at the end of the experiment, using a Coulter Counter (Coulter Electronics, Mijdrecht, The Netherlands).

For RNA isolation, MC and GVEC were seeded into 250-ml culture flasks and cultured until confluence was reached. MC and GVEC were rendered quiescent overnight by culture in DMEM/0.5% FCS and cultured subsequently in DMEM/0.5% FCS with or without stimuli for 48 h. Culture supernatants were used to determine the production of C3 and factor H by sandwich enzyme-linked immunosorbent assay (ELISA), and total RNA was isolated using RNazol.

Sandwich ELISA

Wells in 96-well microtiter plates (Titertek Flow Laboratories, Zwanenburg, The Netherlands) were coated with affinity-purified rabbit antihuman C3 and rabbit antihuman factor H in 0.1 M carbonate buffer, pH 9.6, for 2 h at 37°C, essentially as reported previously (20). Culture supernatants of MC were incubated in the wells for 1 h at 37°C. Blank values were obtained from wells incubated with medium alone. Subsequently, the wells were washed and incubated with DIG-conjugated, affinity-purified rabbit antihuman C3 and antihuman factor H. The DIG conjugates were prepared following the manufacturer's instructions. Finally, bound antibody was detected using horseradish peroxidase-labeled anti-DIG F(ab') fragments and horseradish peroxidase substrate 2,2'-amino-bis-3-ethylbenzthiazoline-6-sulfonic acid. The amount of substrate generated was determined using a Titertek Multiskan at an optical density of 415. The concentration ranges were 153 to 9788 pg/ml for the C3 ELISA and 82 to 5225 pg/ml for the factor H ELISA. In the present study, we used aggregates and ICX composed of rat and rabbit IgG, because aggregated human IgG contains sufficient complement to induce positive reading in the sandwich ELISA (36).

RT-PCR

Total cellular RNA, isolated from MC or GVEC, was reverse-transcribed into cDNA by oligo(dT) priming (26). Deoxyligonucleotide primers were constructed from the published cDNA sequences of C3 (31), factor H (32), and β-actin (37). The primers for FcyRIII were described by Radeke et al. (10). All primers were synthesized on
a DNA synthesizer (Cyclone, Millipore, Bedford, MA) by the deoxyxynucleoside phosphoramidite method (38). The sequences of the primers are given in Table 1.

Amplification of cDNA by PCR was performed by a modified procedure of Saiki (39). Ten microliters of cDNA (from a 1 to 100 diluted cDNA-stock for complement factors and FcyRIII and from a procedure of Saiki (39). Ten microliters of cDNA (from a 1 to 1000 diluted cDNA-stock for β-actin), 50 pmol of each primer, and 0.5 U Taq DNA polymerase were added to a final volume of 100 μl (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.0 mM MgCl2, 2 ng/ml BSA, and 0.25 mM of each dNTP). The mixture was heated at 95°C for 5 min followed by 33 cycles, each consisting of 2.5 min at 95°C, 1.5 min at 55°C, and 1.0 min at 72°C. After termination of the last cycle, the samples were chilled at 4°C. Ten microliters of the amplified DNA were electrophoresed on a 1.0% agarose gel and transferred overnight to Hybond N+ with 0.4 M NaOH. After neutralization with 2X SSC, the filters were dried and stored at room temperature until use. Southern blot hybridization with DIG-11-dUTP nick-translated C3, factor H, and β-actin probes was performed as described previously (20). Southern blots with FcyRIII PCR products were hybridized under high stringent conditions with [α-32P]dCTP by random priming labeling (42). The probes used were a 1300-bp BglII-BamHI fragment derived from clone pGPS, a DNA synthesizer (Cyclone, Millipore, Bedford, MA) by the deoxyxynucleoside phosphoramidite method (38). The sequences of the primers are given in Table 1.

**Northern Blotting**

Total cellular RNA was isolated using RNAzol. Twenty micrograms of total RNA were separated on a formaldehyde containing 1% agarose gel and blotted onto nitrocellulose filters. Gel electrophoresis, RNA transfer, and high stringent hybridization were performed as described previously (40). The CDNA probes were radiolabeled with [α-32P]dCTP by random priming labeling (42). The probes used were a 1500-bp PstI C3 cDNA fragment derived from clone C3A1, a 1500-bp ClaI-BamHI factor H cDNA fragment derived from clone R2a, and a 1450-bp BgIII-BamHI IL-6 cDNA fragment. Finally, the filters were hybridized with a 1300-bp EcoRI GAPDH cDNA fragment to quantify the amount of RNA loaded per lane.

**Statistical Analyses**

Linear regression analysis was performed for the data in Figure 1, A and B. The data in Figures 2 and 4 were analyzed by ANOVA. P values of 0.05 were used to determine significance.

**Results**

**Stimulation of MC with AlG and Rabbit IgG ICX**

It was shown previously, that human MC produce C3 and factor H and that this production is regulated by cytokines (25–27). In the study presented here, we investigated whether stimulation of MC with AlG and ICX leads to a change in complement C3 and factor H production. MC (line 3) were incubated for 72 h with increasing concentrations of insoluble AlG, and the supernatants were assayed for C3 and factor H. MC cultured in medium alone produced 10 ± 1 ng C3/10⁶ cells and 605 ± 15 ng factor H/10⁶ cells, respectively (Figure 1A).

Incubation of MC with increasing concentrations AlG resulted in a dose-dependent enhancement of C3 synthesis up to 999 ± 15 ng/10⁶ cells (P < 0.001), whereas a nonsignificant (P > 0.1), 1.1-fold increase in the amount of factor H was observed. Next, MC (line 1) were incubated for 72 h with increasing concentrations of insoluble ICX. The basal C3 production of 17 ± 4 ng/10⁶ cells was enhanced up to 470 ± 118 ng/10⁶ cells (P < 0.001) (Figure 1B), whereas for factor H, no significant change in the basal production of 319 ± 52 ng/10⁶ cells was seen (P > 0.5).

When MC (line 2) were cultured with 200 μg/ml AlG, a time-dependent increase in the C3 synthesis was observed up to 263 ng/10⁶ cells after 96 h of culture, compared with the C3 production by nonstimulated cells of 44 ± 6.7 ng/10⁶ cells (P < 0.001) (Figure 2A). After incubation with AlG, there was no significant change in the factor H production of 164 ± 13 ng/10⁶ cells after 96 h of culture compared with MC cultured in medium alone. Exposure of MC to 200 μg/ml ICX also resulted in a time-dependent increase of the C3 production up to 389 ± 27 ng/10⁶ cells after 96 h of culture compared with the synthesis of 29 ± 1 ng/10⁶ cells by nonstimulated cells (P < 0.001) (Figure 2B). There was a nonsignificant, 1.1-fold increase in factor H production by MC cultured with ICX compared with MC cultured in medium alone.

The effect of AlG and ICX in a concentration of 200 μg/ml was tested in various experiments on four different cell lines (Table 2). Both AlG and ICX enhanced C3 production significantly (P < 0.05), and the fold increase varied between 3 and 20. Whereas the amount of C3 synthesized by the individual cell lines was highly variable, there were no great intracellular differences. As a control, MC were incubated for 72 h with BSA (200 μg/ml) and rabbit α-BSA (200 μg/ml) alone. No significant differences in the C3 synthesis were observed between cells incubated with medium alone or cells incubated with BSA or rabbit α-BSA (results not shown).

**Effect of Polymyxin B on the C3 Production by MC**

To exclude a possible effect of endotoxins on complement production, MC (line 4) were stimulated with AlG in the presence or absence of polymyxin B. MC were incubated for 72 h in medium alone, in medium with AlG (200 μg/ml), or AlG with polymyxin B (1 μg/ml) and in medium with lipo-

**Table 1. Summary of primer sequences**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Coding Sequence</th>
<th>Bases</th>
<th>Anti-Coding Sequence</th>
<th>Bases</th>
<th>Amplicon Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>TGGATGACAAGGTCACCCCT</td>
<td>4627 to 4646</td>
<td>GACACATTGCTCTCGTGA</td>
<td>5015 to 5034</td>
<td>408</td>
</tr>
<tr>
<td>Factor H</td>
<td>CAGAGACACCTCTGTGTGA</td>
<td>3202 to 3221</td>
<td>TGCAACGTTGAGTACTCA</td>
<td>3741 to 3760</td>
<td>559</td>
</tr>
<tr>
<td>FcyRIII</td>
<td>ATGTGGCAGGTCCTCTCCC-AACTG</td>
<td>34 to 58</td>
<td>GGTGATGTTCACAGTCTTG-AAGACAC</td>
<td>573 to 600</td>
<td>566</td>
</tr>
<tr>
<td>β-actin</td>
<td>CTACAATGAGCTCGGTG</td>
<td>311 to 330</td>
<td>AAGGAAGGCTGGAAGAGTGC</td>
<td>818 to 838</td>
<td>527</td>
</tr>
</tbody>
</table>
polysaccharide (LPS) (10 μg/ml) or LPS with polymyxin B. Again, C3 production was enhanced after incubation with AlgG (Figure 3). The LPS-induced C3 synthesis was blocked by polymyxin B, whereas the AlgG-induced C3 production was not affected by polymyxin B.

For the Upregulation of C3 Synthesis, Both de novo Protein Synthesis and Increased mRNA Expression Are Required

To investigate whether de novo protein synthesis is necessary for the enhanced C3 synthesis, MC (line 2) were cultured for 72 h in medium alone or in medium supplemented with ICX (200 μg/ml) or ICX together with cycloheximide (1 μg/ml). Incubation of MC with ICX resulted in a significant upregulation of C3 synthesis (P < 0.05) (Figure 4). When the MC were cultured in the presence of ICX and cycloheximide, C3 production was reduced compared with the ICX-stimulated C3 production (P < 0.05), suggesting that de novo protein synthesis of C3 is required.

Because incubation of MC with AlgG results in an upregulation of C3 production, we also investigated whether steady-state levels of mRNA for C3 and factor H were affected. MC (line 1) were incubated in medium alone, in medium with AlgG (200 μg/ml), or with AlgG and polymyxin B (1 μg/ml). After 72 h of culture, supernatants were harvested and total cellular RNA was isolated and used to synthesize cDNA for investigation of mRNA expression by RT-PCR analysis. An upregulation of C3 mRNA by MC was observed after incubation with AlgG or AlgG with polymyxin B (Figure 5), whereas the factor H mRNA expression was not influenced by AlgG. As a control, β-actin mRNA expression was assessed. The effect of AlgG on the expression of C3 and factor H mRNA by GVEC was also investigated as a renal cell control experiment. GVEC were cultured in medium with or without AlgG (200 μg/ml), and after 72 h of culture, supernatants were harvested and total cellular RNA was isolated to generate cDNA. The basal C3 protein synthesis by GVEC was not changed after incubation with AlgG (results not shown). RT-PCR analysis showed that steady-state levels of C3 and factor H mRNA in GVEC were also not influenced after incubation with AlgG (Figure 5).

To obtain a more quantitative assessment of mRNA expression, total RNA from MC (line 4) and GVEC, incubated in medium alone or in medium with AlgG (200 μg/ml), was used to perform Northern blot analysis. RNA was separated on an agarose gel and transferred to a nitrocellulose filter. The filter was subsequently hybridized with C3-, IL-6-, and GAPDH-cDNA probes. The C3 cDNA probe detected a 5.2-kb band, and the IL-6 cDNA probe detected a 1.2-kb band. The GAPDH cDNA probe detected a 1.3-kb band and was used as a control. For MC, an induction of both C3 and IL-6 mRNA expression was observed after incubation with AlgG (200 μg/ml) (Figure 6A). The steady-state levels of C3 and IL-6 mRNA expression in GVEC were not changed after incubation with AlgG. The expression of GAPDH mRNA was used to standardize the amounts of RNA that were loaded on the gel. By density measurements, a clear signal over background was detected for C3 and IL-6 mRNA in MC cultured in medium alone on the original autoradiograms. The ratios of C3 and IL-6 over GAPDH were calculated for all conditions. It was found that the ratio of C3 and IL-6 over GAPDH in MC exposed to AlgG was seven- and 35-fold higher, respectively, compared with the nonstimulated cells.

Figure 1. Production of C3 and factor H by human mesangial cells (MC) after incubation with increasing concentrations of heat-aggregated rat IgG (AlgG) or immune complexes (ICX). MC were incubated with increasing concentrations of AlgG (Panel A) and ICX (Panel B), and after 72 h of incubation the supernatants were harvested and assessed for C3 and factor H by sandwich enzyme-linked immunosorbent assay (ELISA). The results are expressed as mean ± SD of experiments in triplicate culture. The results of one out of three experiments, with cell lines derived from different donors, are shown.
Expression of FcγRIII mRNA in MC and GVEC

Earlier studies conducted by our laboratory and others (10) have demonstrated the presence of FcγRIII on human MC by fluorescence-activated cell sorter analysis (Becton Dickinson) and in vivo binding experiments (9). To investigate whether the MC used in our experiments express mRNA for FcγRIII, RT-PCR analysis was performed on mRNA, isolated from MC (line 1) incubated for 48 h in medium alone or in medium with IFN-γ (1000 U/ml). In this experiment, the 566-bp FcγRIII-PCR product of MC cDNA was not visible after ethidium bromide staining of the agarose gel, loaded with 20 µl of PCR product, whereas the FcγRIII-PCR product of monocyte cDNA or whole kidney cortex cDNA was clearly visible (results not shown). FcγRIII-PCR product of MC, however, could be identified when the Southern blot was hybridized with the FcγRIII cDNA probe (Figure 7). RT-PCR on MC cDNA with β-actin primers showed that comparable amounts of cDNA were used.

Discussion

Activation of MC by ICX results in an increased synthesis of complement component C3. Incubation of MC with AlgG or with ICX composed of rabbit IgG and antigen, as models for ICX, resulted in a dose-dependent upregulation of the C3 production by MC (Figures 1 and 2). The effect of AlgG and ICX on C3 production by MC was time-dependent. The difference in onset of C3 production by AlgG and ICX in this experiment was not found in time-course experiments with the other MC lines. The basal factor H synthesis by MC was not affected by AlgG or ICX. Although great variance in the amounts of C3 synthesized by the various MC lines occurred...
Table 2. Effect of AlG or ICX on the C3 synthesis by various MC lines*

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Donor Age</th>
<th>Stimulus</th>
<th>C3 Synthesis</th>
<th>Fold Increase</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Basal</td>
<td>Stimulated</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>ICX</td>
<td>29 ± 4</td>
<td>592 ± 88</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>AlG</td>
<td>34 ± 3</td>
<td>89 ± 35</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>AlG</td>
<td>4.5 ± 0.4</td>
<td>46 ± 15</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>ICX</td>
<td>27 ± 4</td>
<td>116 ± 16</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>ICX</td>
<td>23 ± 10</td>
<td>130 ± 23</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>AlG</td>
<td>58 ± 5</td>
<td>730 ± 158</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>ICX</td>
<td>2.0 ± 0.6</td>
<td>27 ± 10</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>AlG</td>
<td>2.5 ± 0.7</td>
<td>32.7 ± 5</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>AlG</td>
<td>3.8 ± 1.0</td>
<td>39.3 ± 2.0</td>
<td>10</td>
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</tbody>
</table>

* MC were cultured in medium alone or incubated with 200 μg/ml AlG or ICX, and after 72 h of incubation the supernatants were harvested and assessed for C3 by sandwich enzyme-linked immunosorbent assay. The results are expressed as mean ± SD (ng/10⁶ cells) of experiments in triplicate culture. AlG, heat-aggregated rat IgG; ICX, immune complex; MC, mesangial cell.

Figure 3. Effect of polymyxin B on C3 synthesis by human MC after incubation with AlG. MC were incubated for 72 h in medium alone, in medium with AlG (200 μg/ml), in medium with AlG and polymyxin B (polyB; 1 μg/ml), in medium with lipopolysaccharide (LPS; 10 μg/ml), and in medium with LPS and polymyxin B. After 72 h of incubation, culture supernatants were harvested and assessed for C3 by sandwich ELISA. The results are expressed as mean ± SD of experiments in triplicate culture. The results of one out of three experiments, with cell lines derived from different donors, are shown.

(Table 1), both AlG and ICX significantly increased the C3 synthesis by all of the MC lines used in this study. Upregulation of C3 production by MC after incubation with ICX was dependent on de novo protein synthesis (Figure 4). Furthermore, stimulation of MC by AlG resulted in an increase in the steady-state mRNA levels of C3 (Figures 5 and 6). Stimulation of MC with ICX also resulted in an increase in the C3 mRNA levels (result not shown). Factor H mRNA levels were not changed after incubation with AlG or ICX.

To investigate whether the effect of AlG stimulation on complement production was cell-type-specific, we also cultured GVEC with AlG. Both C3 and factor H steady-state mRNA levels were not changed after incubation with AlG (Figures 5 and 6). IL-6 mRNA expression by GVEC also was not affected after incubation with AlG. Thus, the stimulation of complement C3 production by ICX seems to be specific for MC.

Several authors have shown the presence of FcγRIII on MC, both at the protein and the mRNA level (9–11). In concordance with these findings, we also demonstrate the expression of FcγRIII mRNA in MC used in the present study (Figure 7). Numerous studies have shown that incubation of MC with aggregated IgG results in the stimulation of MC and the production of, for instance, IL-6 (10,11,13,14), IL-1 (5,43), monocytic chemoattractant protein-1 (MCP-1) (8), superoxide (7), platelet-activating factor, and prostaglandins (6). In the present study, we demonstrate that incubation of MC with AlG results in an increased IL-6 protein synthesis (result not shown) and mRNA expression (Figure 6). The induction of C3 production by MC incubated with AlG is probably mediated via interaction of the Fc-part of the immunoglobulins and the FcγR on MC. This is suggested by the absence of a response, i.e., an increase in C3 production, after incubation of MC with antibody alone or with (aggregated) F(ab')² fragments (results not shown). To trigger FcγRIII directly, MC were cultured in the presence of monoclonal antibodies against FcγRIII, and the supernatants were assessed for C3. No significant upregulation in C3 production was seen (results not shown). It is possible that other anti-FcγR monoclonal antibodies might be able to trigger FcγR and thereby induce upregulation of C3; however, more detailed studies are required to elucidate this question. In this study, we used rat and rabbit immunoglobulin to cross-link
human Fc receptors on MC. Human IgG preparations could not be used because of cross-reactivity in the C3 ELISA. Therefore, AlgG from rat origin was used. In cell ELISA studies, it was found that both human and rat AlgG react with human MC in a dose-dependent manner to the same extent.

Deposition of immunoglobulins and complement components is found in kidneys from patients with glomerulonephritis. The question is whether these complement proteins are derived from the circulation and thus deposited before renal localization of ICX or whether these complement components are synthesized locally by renal cells and subsequently deposited on the immunoglobulins. Increasing evidence is available regarding the synthesis of complement within the kidney. Elevated C3 mRNA levels are found in kidneys from patients with various kidney diseases by in situ hybridization and RT-PCR analysis (18,19). Furthermore, the synthesis of various complement components by in vitro cultures of MC, GVEC, and proximal tubular epithelial cells is regulated by cytokines (21–25,27,40). In a previous study, we reported on C3 and factor H synthesis by MC in vitro and the effect of IL-1 and IFN-γ on this production. In this article, we show that AlgG and ICX are able to induce C3 synthesis by MC. At the moment it is not clear whether MC in vivo also respond to ICX-mediated stimulation with increased C3 production. However, Abe et al. recently showed by in situ hybridization that mRNA expression for C3 in glomeruli of kidneys from patients with IgA nephropathy is increased (44). In glomeruli of kidneys from normal individuals, no message for C3 was detectable.

What are the implications of these findings for the in vivo handling of ICX? Complement component C3 is involved in both the inhibition of precipitation of ICX and in the solubilization of deposited ICX (reviewed in reference 16). It is known...
that more ICX disorders occur in (partially) complement-deficient individuals than in normal subjects (45), suggesting a role for complement in the handling of ICX. Components of the classical pathway of complement prevent the formation of large insoluble complexes (46). When ICX are precipitated in, for instance, the mesangium, complement-mediated solubilization may lead to removal of the complexes from the mesangium (47,48). For the solubilization of the complexes, an intact amplification pathway is required. Local synthesis of C3 by MC after interaction between the FcγR on MC and the Fc-part of IgG may have beneficial effects on the removal of IgG ICX. Besides C3, factor B, factor D, and properdin are required for the activation of the alternative pathway of complement. Presently, we are investigating whether ICX stimulation of MC in vitro leads to increased synthesis of the other alternative pathway components. The finding that factor H synthesis by MC (and GVEC) is not changed after ICX stimulation is also of importance for the handling of ICX deposited in the kidney. Factor H regulates the activation of the alternative pathway of complement (29), and a local increase in factor H synthesis would lead to a decrease in activation of the alternative pathway and thereby to an impaired removal of ICX. Thus, locally produced complement not only may contribute to local inflammation, but it may also play a protective role by increasing the removal of locally deposited ICX from the glomerular mesangium.

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References


Figure 7. Expression of mRNA for FcγRIII in human MC. Total RNA was isolated from MC incubated with medium or medium with interferon-γ (1000 U/ml). After 48 h of culture, total RNA was isolated and RT-PCR analysis was performed on 1 μg of total RNA. PCR products were blotted to Hybond N+ and detected with a specific probe for FcγRIII. The β-actin PCR product was visualized by ethidium bromide staining of the gel. The results of one out of three experiments, with cell lines derived from different donors, are shown.