Contribution of Renal Secreted Complement C3 to the Circulating Pool in Humans

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Contribution of Renal Secreted Complement C3 to the Circulating Pool in Humans


Complement C3 produced within the kidney may be an important mediator of local inflammatory and immunological injury. The overall level of renal C3 production and consequently its contribution to the total circulating C3 level are, however, unknown. This was investigated by using the conversion of C3 from recipient to donor allotype following renal transplantation. The C3 F and S allotypes of 80 consecutive renal donor-recipient pairs (148 individuals) were determined by amplification refractory mutation system analysis. The extent of allotype conversion in C3 F/S mismatched recipients was quantified at different stages after transplantation, using an enzyme-linked immunosorbent assay specific for the HAV 4-1 polymorphism of C3 that is strongly associated with C3F. Twenty-one of the eighty recipients were potentially informative, i.e., were C3 SS recipients of C3 FF or FS donor kidneys. In the early postoperative period, donor-derived C3 (HAV 4-1-positive) was undetectable, increasing to 9.6% of the total circulating C3 at times of acute allograft rejection. When graft dysfunction occurred from causes other than rejection, donor C3 remained undetectable. After stable graft function was attained (3–13 mo after transplantation), donor C3 made up 4.5% of the total circulating C3 pool. Our findings demonstrate that human transplant kidney in the resting state is a significant source of extrahepatic C3. Its heightened local synthesis during rejection episodes suggests a possible pathogenic role for C3 in this immunological process. The Journal of Immunology, 1999, 162: 4336–4341.

The occurrence of two C3 allotypes, one of which can be detected by a mAb, affords a unique opportunity for the study of locally secreted C3 by using the conversion of C3 protein from recipient to donor allotype following organ transplantation mismatched at the C3 F/S locus. Naughton et al. (15) reported a donor-derived C3 contribution of 0.2–5.2% to the total circulating C3 in the immediate posttransplant period in six informative bone marrow transplant recipients (FS marrow into SS patient). Monocytes were demonstrated to be the source of the C3 in this study. In four informative liver transplant recipients (SS liver into FS patient), total extrahepatic C3 contributed to between 5.6–11.4% of the circulating C3 pool. It was thus apparent that there was an uncoupled source of circulating C3 in this study.

Various cell types of the kidney are capable of producing C3 in vitro (16–18). In vivo, immunological injury as a result of interstitial nephritis, immune complex glomerulonephritis, and allograft rejection up-regulates local C3 gene expression (16, 19–21), indicating that locally synthesized C3 may be an important mediator of inflammatory and immunological injury within the kidney. Interpretation of these findings, however, is limited by the lack of data on the actual amount of complement that the kidney is capable of producing in normal and inflamed states. To date, the overall contribution of renal C3 production to the total circulating C3 level under physiologic and pathologic conditions is unknown.

The aim of the present study was to quantify the renal contribution of C3 to the systemic pool by measuring the extent of allotype conversion following renal transplantation in recipients who had informative mismatches at the C3 F/S locus. The change in the renal contribution of C3 to the circulating pool and its functional relevance during periods of allograft dysfunction are also considered.

Materials and Methods

Patients

Ethical approval was granted by the local research ethics committee. Determination of the C3 allotype by ARMS was conducted on 80 consecutive...
renal donor-recipient pairs (148 individuals) who underwent renal transplantation from September 1996 to August 1997 at the Department of Nephrology and Transplantation of Guy’s Hospital for end-stage renal failure. Informative subjects, defined as C3 SS homozygous recipients of C3 FS heterozygous or C3 FF homozygous kidneys, were followed posttransplant with regular serum sampling. All serum samples were stored in aliquots at \( -70^\circ C \) before analysis.

### Preparation of donor and recipient DNA

Genomic DNA was extracted at tissue typing using the salt method (22) from peripheral blood leukocytes. Genomic DNA was stored at \(-20^\circ C\) until further use.

### ARMS analysis for determination of C3 allotypes

To determine C3 allotype, ARMS analysis was conducted using a modified version of a previously described method (23). Oligonucleotide primers (30 bases) were chosen such that one primer is complementary to one or the other allovariable allele, and a reverse primer located upstream of the allovariable site giving a product size of approximately 300 bp for the C3 F/S polymorphism. Primer sequences were 5’-CCAACAGGAGTTCAAGTCAGAAAAGGTGC-3’ for C3S, 5’-CCAACAGGAGTTCAAGTCAGAAAGGTGC-3’ for C3F, and 5’-TGTTGACCATTGACCGTCGCCGCCACCGGTA-3’ for the common primer. Paired PCR reactions were performed with both tubes containing the common primer. One tube of each pair contained the C3F primer, while the other contained the C3S primer. The PCR reactions were performed on a Perkin-Elmer DNA Thermal Cycler (Perkin-Elmer, Beaconsfield, U.K.) in a total volume of 25 µl containing approximately 0.2 µg of genomic DNA, 0.4 mM deoxynucleotide triphosphates (Boehringer Mannheim, Lewes, U.K.), 10 mM Tris-HCl (pH 9.0), 50 mM potassium chloride, 0.1% Triton X-100; 3 mM magnesium chloride, 1 µM of each primer, and 0.3 µl of Taq DNA polymerase (Promega, Madison, WI). PCR reaction conditions were 33 cycles, with denaturing at 94°C for 30 s, and a combined annealing and extension step at 73°C for 60 s, followed by a 10-min extension step at 72°C at the end of the 33 cycles. The amplified DNA was visualized after electrophoresis in 1.4% agarose gels containing ethidium bromide and compared with controls of known C3 F/S allotype.

### ELISA for quantification of HAV 4-1-positive serum C3

Baseline C3 HAV 4-1 concentrations were assayed on serum samples obtained at 2 wk after the attainment of a stable graft function posttransplant as reflected by serum creatinine levels. Thereafter, C3 HAV 4-1 concentrations were assayed at times of graft dysfunction as reflected by a rise in serum creatinine which necessitated a graft biopsy. The assay for C3 HAV 4-1 concentrations was performed for 4-1 antigen as the quantitative readout (24, 25). The assay was based on the sandwich ELISA with the polyclonal rabbit anti-human C3 antibody (Dako) diluted 1/5000. After a final wash, the membrane was developed by incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG (Dako) diluted 1/10000 and 1/5000, respectively.

### ELISA for quantification of total serum C3

Total serum C3 concentrations were measured by ELISA. Nunc MaxiSorp immunoplates were coated overnight at 4°C with 100 µl of sheep anti-human C3c (The Binding Site, Birmingham, U.K.) and horseradish peroxidase-conjugated goat anti-sheep IgG (Dako) diluted 1/10,000 and 1/5,000, respectively. The enzyme activity was assessed after incubation with o-phenylene diamine by measuring absorbance at 490 nm (MRX 1.1, Dynatech Laboratories, Chantilly, VA). C3 HAV 4-1 homozygous-positive serum of known C3 concentration (as determined in the total C3 ELISA described below) was added in duplicate to generate a standard curve and was included in every reaction. The limit of sensitivity of this assay was 0.4% C3 HAV 4-1-positive in C3 HAV 4-1 –ve serum.

### Protein electrophoresis and immunoblotting

Serum samples were subjected to high voltage agarose gel electrophoresis according to a previously described method (26). Briefly, 5 µl of serum was applied to a 1% agarose gel and run in barbitone buffer (pH 8.6) at 5°C for 4.5 h at 250 V. At the end of the electrophoresis, the gel was blotted onto a nitrocellulose membrane (Hybond-C Super, Amersham, Little Chalfont, U.K.) and incubated at room temperature for 2 h with mAb HAV 4-1 at a concentration of 200 ng/ml in PBS, 0.05% Tween, and 10% milk powder. After washing in PBS and 0.05% Tween, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Dako) diluted 1/5000. After a final wash, the membrane was developed with diaminobenzidine (FAST DAB tablet set, Sigma, Poole, U.K.) according to the manufacturer’s instructions to visualize binding of the HAV 4-1 Ab.

**Table I. Demographic and clinical characteristics of the 21 informative recipients**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.1 ± 17.1 (range 6–74)</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>12:9</td>
</tr>
<tr>
<td>Cadaveric/ live-related transplants</td>
<td>18:3</td>
</tr>
<tr>
<td>First:second grafts</td>
<td>19:2</td>
</tr>
<tr>
<td>Previously transfused:untransfused</td>
<td>10:11</td>
</tr>
<tr>
<td>Follow-up duration (months)</td>
<td>8.8 ± 3.2</td>
</tr>
<tr>
<td>No. of days posttransplant of first serum</td>
<td>31 ± 27.4</td>
</tr>
<tr>
<td>Sample (2 wk after attaining a stable (range, 16–138; median, 21) creatinine)b</td>
<td></td>
</tr>
</tbody>
</table>

**Table II. Causes of end-stage renal failure in the 21 informative transplant recipients**

<table>
<thead>
<tr>
<th>Causes of Renal Failure</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflux nephropathy</td>
<td>4</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>2</td>
</tr>
<tr>
<td>Dysplastic/hypoplastic kidneys</td>
<td>2</td>
</tr>
<tr>
<td>Posterior urethral valves</td>
<td>1</td>
</tr>
<tr>
<td>Ig A nephropathy</td>
<td>2</td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis</td>
<td>2</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>1</td>
</tr>
<tr>
<td>Mesangiocapillary glomerulonephritis</td>
<td>1</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>1</td>
</tr>
<tr>
<td>Urate nephropathy</td>
<td>1</td>
</tr>
<tr>
<td>Hypertensive nephropathy</td>
<td>1</td>
</tr>
<tr>
<td>Renal artery stenosis</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table III. Causes of impaired graft function at the time of sampling**

<table>
<thead>
<tr>
<th>Causes of impaired graft function</th>
<th>No. of Patient Episodes</th>
<th>No. of Serum Samples Available for ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular rejectionc</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Acute tubular necrosisd</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cytomegalovirus disease</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cyclosporin toxicitye</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vascular thrombosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>22</td>
</tr>
</tbody>
</table>

*Proven histologically on transplant biopsies.
tion (25). In addition, as little as 200 ng, instead of 1 ng, of genomic DNA used for amplification was sufficient for satisfactory amplification. The amount of genomic DNA used for amplification was 200 ng. Both panels show the typical result of experiments performed on three separate occasions.

**Results**

**ARMS technique**

Contrary to previously reported protocols (23) that used relatively high concentrations of magnesium chloride (6.7 mM) and deoxynucleotide triphosphates (1.25 mM) in the PCR reaction, we found the optimal concentrations of MgCl₂ and dNTPs to be 3.0 and 0.4 mM, respectively (Fig. 1). Optimization of the reaction conditions will influence product yield and accuracy of amplification (25). In addition, as little as 200 ng, instead of 1 μg, of genomic DNA was sufficient for satisfactory amplification.

**Informative transplant recipients**

Twenty-one potentially informative C3 SS recipients were identified on ARMS analysis. Their demographic data and causes of end-stage renal failure are listed in Tables I and II, respectively. Among the 80 donor-recipient pairs, the gene frequency of C3F and C3S was 54 of 296 (18.2%) and 242 of 296 (81.8%), respectively. Two subjects received kidneys from C3 FF homozygous donors, while 19 received kidneys from C3 FS heterozygotes. None of the patients were receiving immunosuppressive treatment before transplantation, nor had any patient been transplanted within the previous 3 yr. All patients received triple immunosuppressive therapy with cyclosporin, azathioprine, and prednisolone as induction and subsequent maintenance immunosuppression. Two patients were subsequently switched to a tacrolimus-based regimen because of recurrent rejection. One patient developed acute graft thrombosis at 1 mo and had a graft nephrectomy. One patient died of central nervous system lymphoma 8 mo after the transplant. The other 19 patients remain well with functioning grafts. There was no graft loss as a direct consequence of rejection.

Detection of C3 HAV 4-1 in the early posttransplant period

Total C3 and HAV 4-1-positive C3 were measured in parallel by double ligand ELISA on every serum sample. The total C3 levels of the 21 subjects ranged from 78–102 mg/dl (mean, 93.8) and were similar to those in healthy subjects (range, 80–120 mg/dl; mean, 96.6). The level of C3 HAV 4-1 as a marker of donor-derived C3 in the posttransplant serum was expressed as a percentage of the total C3. At baseline (2 wk after attaining a stable plasma creatinine level), only two patients had detectable levels of C3 HAV 4-1 in their serum, which contributed to 0.7 and 1% of the total circulating C3. Both of these patients had received kidneys from donors who were C3 FF homozygotes, while the remaining 19 patients with undetectable C3 HAV 4-1 in their baseline posttransplant serum had all received C3 FS heterozygous grafts (Fig. 2).

Up to the present time, the 21 informative subjects had experienced 31 episodes of impaired graft function, the causes of which are grouped and summarized in Table III. At times of acute cellular rejection, C3 HAV 4-1 contributed to between 1.5–8% (mean, 4.8%) of the total circulating C3. As all donors in this group were C3 FS heterozygotes, it follows that the transplant kidney contributed to between 3–16% (mean, 9.6%) of the total circulating C3 during this time, assuming the same degree of expression with each C3 allele. Four patients had acute tubular necrosis (ATN), of which only one had detectable C3 HAV 4-1 during this event. This patient was one of the two who had received a C3 FF homozygous kidney, and the level of C3 HAV 4-1 detected during ATN was 1% of the total circulating C3, which was no different from his baseline value. There were two episodes each of urinary tract infection and systemic CMV disease, and one each of cyclosporin toxicity and transplant renal artery occlusion, all associated with a significant rise in serum creatinine. None of these episodes resulted in detectable C3 HAV 4-1 in the corresponding serum samples (Fig. 2).

Detection of C3 HAV 4-1 in the stable (posttransplant) phase

To evaluate the graft’s ability to synthesize C3 in the steady state, C3 HAV 4-1 was assayed again in 19 of these subjects, 3–13 mo after transplantation (median, 9 mo) when they had stable graft function with serum creatinine <2.0 mg/dl (180 μmol/l). At this stage, C3 HAV 4-1 was detected in 17 subjects and ranged between 1–5% of the total circulating C3. The overall mean (±SD) for the 19 patients was 2.46 ± 1.71%. Correcting for the fact that two patients had received C3 FF kidneys and the rest received C3 FS kidneys, it can be calculated that the donor kidney contributed to an average of 4.5% of the total circulating C3. There was no correlation with the level in the early phase, the number of previous rejection episodes, or serum creatinine. Because acute rejection led to a distinct elevation of donor-derived C3, subjects were segregated into two groups according to whether they had rejected. In the rejecting group, there was heighted renal C3 production during the acute episode followed by a decline to the steady state level. In the nonrejecting group, C3 HAV 4-1 was detectable only during the stable phase, at levels comparable to those in the rejecting group at that stage (Fig. 4).
Detection of C3 HAV 4-1 by immunoblotting following electrophoresis

Complement was separated from other serum proteins by subjecting serum samples to prolonged high voltage agarose gel electrophoresis, followed by protein transfer onto a nitrocellulose membrane, which was then incubated with mAb HAV 4-1 for detection of C3 HAV 4-1 protein. Known C3 FF and C3 SS sera were included simultaneously in each run as positive and negative controls, respectively. Detection of C3 HAV 4-1 correlated with its levels on ELISA. In the early postoperative phase, C3 HAV 4-1 was demonstrated in 11 of the 12 rejecting serum samples with C3 HAV 4-1 levels 1.5% of the total C3, whereas those remaining serum with C3 HAV 4-1 levels <1.5% total C3 were negative. Immunoblotting of the stable (posttransplant) serum showed a similar correlation with ELISA results (Fig. 5).

Discussion

This study is the first to quantify the synthesis of C3 by the human kidney in vivo. The ARMS technique was used in F/S allotyping for its accurateness and rapidity. Renal transplant recipients with informative C3 allotype mismatches at the F/S locus were studied after transplantation. C3 HAV 4-1 was used as a marker of donor-derived C3F. In the immediate postoperative phase, defined arbitrarily as the time when serum creatinine had fallen to baseline levels for 2 wk, only two patients had detectable C3 HAV 4-1 at 0.7 and 1% of their total C3, as determined by ELISA. Since both patients had received C3 FF homozygous kidneys, it can be inferred that the other 19 patients who had received C3 FS heterozygous kidneys were also producing C3 at this stage, but the level of C3 HAV 4-1 was below the detection limit of our assay. This is consistent with the ELISA sensitivity of 0.4% C3 HAV 4-1-positive in C3 HAV 4-1-negative serum.

As the HAV 4-1 C3 allotype could represent a novel epitope for the HAV 4-1-negative recipients, it is possible that some or all of these recipients could have developed Abs against C3 HAV 4-1. The fact that C3 HAV 4-1 persisted in the serum of 19 subjects for at least 3–13 mo (median, 9 mo) may provide evidence against the relevance of anti-C3 HAV 4-1 Ab in this context. Moreover, the circulating levels of C3 HAV 4-1 increased with time, which suggests that if Abs against the novel epitope were present, they did not substantially alter the detection of C3 HAV 4-1 in the recipient serum. It is possible that the presence of Ab against the HAV 4-1 epitope could interfere with the assay for C3 HAV 4-1; however, this would tend to underestimate the level of C3 HAV 4-1 detected, as it would compete for the same epitope as the plated Ab in the ELISA assay.

We have previously demonstrated transcription of C3 mRNA of donor origin in renal cortical biopsies taken during transient allograft dysfunction, and that the predominant site of C3 protein produced within the graft kidney was the tubular epithelial cell (21), as supported by allotype-specific staining of sections of renal cortex (21). However, it was unclear whether this locally synthesized C3 protein ever reached the systemic circulation to a detectable level and, if so, to what extent? The present study indicated that the donor kidney contributed to between 2–16% of the total circulating C3 during rejection episodes. This significant degree of spill-over.

**FIGURE 2.** C3 HAV 4-1 in recipient serum. Quantification of C3 HAV 4-1 as a reflection of donor-derived C3F in the posttransplant serum of the 21 informative recipients at baseline (see text for definition) and during the 22 episodes of allograft dysfunction segregated into six groups by causes as shown. C3 HAV 4-1 levels were determined by double ligand ELISA (sensitivity, 0.4% C3 HAV 4-1-positive in C3 HAV 4-1-negative serum) and expressed as percentage of the total circulating C3 for each individual serum sample. ● Patients who received C3 FF homozygous kidneys. Horizontal lines represent mean values. UTI, culture-positive urinary tract infection; CMV, systemic CMV disease; CyA tox, cyclosporin toxicity; Vascular, graft thrombosis.

**FIGURE 3.** Distribution of C3 HAV 4-1 levels in the stable (posttransplant) phase of 19 subjects. C3 HAV 4-1 levels were determined by ELISA 3–13 mo (median, 9 mo) after transplantation when graft function was stable with serum creatinine <2.0 mg/dl (180 μmol/l). No correlation was found in relation to C3 HAV 4-1 levels or rejection status in the early phase and serum creatinine. Seventeen subjects showed detectable C3 HAV 4-1 levels, while two had levels below the detection threshold of the assay. Overall mean ± SD, 2.46 ± 1.71%.
up-regulation of IL-2 and IFN-γ MHC class I/II mismatched renal transplantation, which showed in keeping with our unpublished observation in a rat model of functional outcome of the transplanted kidney (26).

ischemic injury and also by the association of donor C3F with poor over in nonimmunological events such as ATN, drug toxicity, or inflammation. This is further supported by the absence of this spill-over rejection. This implies that C3 may have a pathogenic role in renal synthesis potentiates both cellular and humoral immunity leading to both T and B cells (1). It is possible therefore that local C3 synthesis may contribute to other complement-dependent processes, including the mediation of ischemia reperfusion injury (28), immune-mediated tissue injury (29), and the solubilization and clearance of immune complexes deposited in the kidney (30). However, the exact functional role of local C3 synthesis during such immunological events as allograft rejection has yet to be defined.

Circulating donor-specific complement has not previously been demonstrated beyond 3 mo after renal or bone marrow transplantation (15, 21). Our findings here indicate that the kidney is able to produce a significant amount of C3 under normal conditions. Three to thirteen months after transplantation, the graft kidney contributed to 4.5% of the circulating C3 pool regardless of the rejection status in the early postoperative period, suggesting that the kidney has a genuine function of C3 synthesis, akin to the observation of delayed erythropoietin production after renal transplantation. Although cells of myeloid origin (3–5, 7), vascular endothelial cells (18), the epithelium of the gastrointestinal tract (31, 32), and the kidneys (16, 18, 21) have all been cited to be candidate sources of extrahepatic C3, it is apparent from this study that the kidney is responsible for a major portion of the “missing” pool of extrahepatic C3.

Other potential sources of donor-derived C3 in addition to the tubular epithelial cell need to be considered. A previous kidney transplant is one possibility. Two patients in this series were receiving second grafts at 7 and 18 yr after the first graft. The fact that the first grafts were nonfunctional and that donor C3 was detected in patients receiving their first graft makes them unlikely to be a viable source of C3 synthesis. Dendritic cells or passenger leukocytes from the donor marrow and transferred with the donor kidney (33) are another possibility. However, this is not supported by in situ hybridization studies, which demonstrated the absence of C3 mRNA expression by macrophage-like cells within human renal biopsies, even when they were in abundance in the interstitium (34). Furthermore, the expression of donor C3 mRNA up to 2 mo after transplantation (21) argues against dendritic cell chimerism, as these cells become undetectable immunohistologically in the acute graft rejection. Local C3 synthesis may contribute to other complement-dependent processes, including the mediation of ischemia reperfusion injury (28), immune-mediated tissue injury (29), and the solubilization and clearance of immune complexes deposited in the kidney (30). However, the exact functional role of local C3 synthesis during such immunological events as allograft rejection has yet to be defined.

Our findings of heightened renal C3 synthesis during rejection is in keeping with our unpublished observation in a rat model of MHC class I/II mismatched renal transplantation, which showed up-regulation of IL-2 and IFN-γ followed by C3 mRNA expression during acute rejection (J. R. Pratt, M. Miyazaki, and S. H. Sacks, unpublished observation). This together with our present findings are entirely consistent with in vitro evidence of local regulation of C3 biosynthesis by cytokines in human proximal tubular epithelial cells (17). Moreover, inhibition of complement in the rat model significantly reduced tubulointerstitial inflammatory injury and lengthened graft survival (27).

C3 has been shown to enhance the Ag-dependent activation of both T and B cells (1). It is possible therefore that local C3 synthesis potentiates both cellular and humoral immunity leading to renal secreted C3 into the systemic pool suggests that locally synthesized C3 may reach high concentrations in the kidney during rejection. This implies that C3 may have a pathogenic role in renal inflammation. This is further supported by the absence of this spill-over in nonimmunological events such as ATN, drug toxicity, or ischemic injury and also by the association of donor C3F with poor functional outcome of the transplanted kidney (26).

The rejecting group showed a distinct peak coinciding with acute graft rejection, which was absent in the other group with impaired graft function from causes other than rejection in the early postoperative phase. In the stable, posttransplant (recovery) phase, both groups showed similar levels of donor-derived C3. Bars represent SDs.

FIGURE 4. C3 HAV 4-1 variation by rejection status. Donor-derived C3 in the rejecting (*, n = 12; †, n = 8; upper panel) and nonrejecting (*, n = 10; †, n = 11; lower panel) groups during the various phases post-transplant. The rejecting group showed a distinct peak coinciding with acute graft rejection, which was absent in the other group with impaired graft function from causes other than rejection in the early postoperative phase. In the stable, posttransplant (recovery) phase, both groups showed similar levels of donor-derived C3. Bars represent SDs.

FIGURE 5. Demonstration of donor-derived C3 in serum by immunoblotting. Five microliters of serum was subjected to high voltage agarose gel electrophoresis, blotted onto a nitrocellulose membrane, and developed with mAb HAV 4-1. FF, C3 FF serum as a positive control; SS, C3 SS serum as a negative control; Baseline, typical serum taken from a subject during the early phase after transplantation; Rejection, typical serum taken from a subject during allograft rejection, confirming the presence of C3 HAV 4-1 protein; 9 mo post TP, recovery phase serum taken 9 mo posttransplant from the same individual shown above (Baseline), demonstrating the phenomenon of C3 alloreaction.
graft 21 days after transplantation (35). Finally, monocytes derived from blood transfusions may theoretically populate the recipient and produce C3. This is refuted by the virtual disappearance of donor monocyte C3 secretion by 6 wk following bone marrow transplantation (15) and the presence of donor-derived C3 in the recovery phase in eight of our patients who had never been transfused. The observation that donor C3 from transfused blood product would only persist in the recipient for up to 48 h after transfusion (15) is a further argument against C3 synthesis by persistence of transfused monocytes.

In conclusion, we have demonstrated that the kidney contributes in a much more significant and sustained manner to the systemic C3 pool than was previously thought. There is increasing indication that the tubular epithelium is the main site of renal C3 production. We have also presented evidence of heightened C3 biosynthesis alongside C3 mRNA transcription during acute allograft rejection (21), indicating a possible pathogenic role for local C3 synthesis in the inflamed kidney. Once again, the potential implications of local complement synthesis for strategies directed against intrarenal immune-mediated and inflammatory tissue injury cannot be overemphasized.

Acknowledgments

We thank Dr. C. Koch (Serum Institute, Copenhagen, Denmark) for the kind gift of the mAb HAV 4-1, and Prof. P. Lachmann (Microbial Immunology, University of Cambridge, Cambridge, U.K.) for the provision of aliquots of his family’s genomic DNA as controls of known C3 F/S allotype.

References