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ORIGINAL ARTICLE

Kidney transplantation in patients suffering from hereditary complete complement C4 deficiency

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Summary

Hereditary complete C4 deficiency (C4def) is a very rare condition that predisposes to immune complex disease and end-stage renal failure. Whether such patients should undergo renal transplantation is debated. The clinical outcome of five transplantations in three C4def patients is described. The first patient lost one allograft after 6 years because of chronic allograft rejection. Back on dialysis, he suffered from meningitis caused by Neisseria menigitidis and Aspergillus. One year after a second transplantation under alemtuzumab induction, he developed fulminant Kaposi's sarcoma and died. His sister is now 6 years post-transplantation without complications. The third patient lost his first graft after 3 years because of chronic allograft nephropathy and recurrence of glomerulonephritis. He has now been living with a second graft for over 9 years. He suffered from pneumonia, a generalized varicella infection and Hemophilis parainfluenzae bronchitis. Patients with complete C4def are at increased risk for infection after kidney transplantation. Under certain precautions and with judicious use of immunosuppression, good long-term results are achievable.

Introduction

The fourth component of complement exists in two isoforms, C4A and C4B, which differ in their physicochemical properties. The genes for C4A and C4B are located on the short arm of chromosome 6 within the major histocompatibility complex (MHC) class III region. Hereditary complete deficiency of C4 is an exceptionally rare disease. A recent review lists 26 cases described in the medical literature [1]. Most of the patients suffer from systemic lupus erythematosus or other immune complex diseases. C4 is of critical importance in the clearance of immune complexes [2]. C4 deficiency (C4def) may also impair complement-dependent phagocytosis of apoptotic blebs and thereby allow the formation of antinuclear antibodies [3]. Complement is also involved in tolerance induction and deletion of autoreactive B lymphocytes [4]. Systemic lupus erythematosus (SLE) associated with C4def can cause glomerulonephritis leading to renal failure. Whether such patients are candidates for renal transplantation is a matter of debate. First, there is concern that such patients, who are inherently immunodeficient, may develop severe infectious complications when receiving immunosuppressive therapy. Second, these patients may also be prone to recurrence of primary glomerular disease in a renal allograft. We here describe our clinical experience in three patients with complete C4def, who developed end-stage renal disease and underwent in total five kidney transplantations.

Patient histories

The clinical histories of the three patients were reported in part previously [5,6]. Patients 1 and 2 are siblings.

Table 1. Synopsis of relevant clinical date of all five transplantations in three patients.

	Patient 1		Patient 2	Patient 3	
Primary disease	SLE, MPGN		SLE, MPGN	Henoch Schoenlein purpura nephritis	
Gender, age	Male, died at age 30		Female, 37	Male, 46	
Age at onset of ESRD	16		26	23	
	1. Transplant	2. Transplant		1. Transplant	2. Transplant
Age at Tx	18	29	31	24	36
HLA mismatches A/B/DR	2/1/1	2/2/1	1/2/1	2/1/2	2/2/2
Immunosuppression	Cyclosporin A	Alemtuzumab	Daclizumab	Cyclosporin A	Tacrolimus, MMF
	Azathioprin	Steroid bolus	Tacrolimus	Azathioprin	
	Switch to tacrolimus and MMF	Tacrolimus	MMF		
Acute rejection	1	0	1	1	0
Allograft histology	Chronic allograft nephropathy	Normal	n.a.	Chronic allograft nephropathy and glomerulopathy, mesangial glomerulonephritis	Chronic allograft nephropathy and glomerulopathy, CNI-induced arteriolar hyalinosis
Infections	Neisseria meningitidis and Aspergillus fumigatus meningitis on hemodialysis	Kaposi's sarkoma	None	Pneumonia, Herpes simplex stomatitis	Pneumonia, Varicella, Haemophilus parainfluenzae bronchitis
CMV serology	R−, D−	R−, D+	R+, D+	n.a.	R+, D+
HHV8 PCR	n.a.	Positive	Negative	n.a.	Negative
BK virus diagnostics	n.a.	SV40 negative	PCR urine positive PCR serum negative	n.a.	PCR urine positive PCR serum negative SV40 negative
Graft life	6 years	1 year	6 years+	3½ years	9 years+

MPGN, membranoproliferative glomerulonephritis; n.a., not available.

Another sister also suffers from SLE and developed cerebral vasculitis [7]. Patient 3 comes from a different family. His brother, also C4def, is healthy.

All the patients described here are homozygous for the HLA A30 B18 DR7 haplotype. RFLP analysis revealed that this haplotype had two short mutant C4B genes (C4BQ0) and no C4A gene. Nine new nucleotide changes, none of which lay in exon areas, were localized in the introns 19, 20, 28, 30, and 31 of the C4B gene. The mutation in intron 28 ($G \rightarrow A$ substitution in position 8127) is predicted to cause an RNA splicing defect [8]. C4 was always undetectable in patients' serum before and after transplantation, whereas C3 levels were normal. Testing of the three complement pathways (Wielisa COMPL300, Wieslab, Lund, Sweden) showed no activity of the classical and lectin pathway, but normal function of the alternative pathway. Tests for antinuclear antibodies (ANA) were positive only in patient 2 with all subtypes being negative.

All renal allografts came from deceased donors. A synopsis of relevant clinical data for all transplantations is provided in Table 1.

Patient 1

This boy presented with SLE-typical symptoms at the age of five, developed serious membranoproliferative glomerulonephritis and, despite various immunosuppressive therapies including cyclophosphamide, developed end-stage renal disease at the age of 16. He received his first kidney transplant at the age of 18. Initial immunosuppression consisted of cyclosporin A, azathioprine and steroids. A biopsy-proven acute rejection was successfully reversed by steroid bolus treatment. Initial serum creatinine was 1.36 mg/dl. Within the following years, serum creatinine increased steadily and heavy proteinuria developed. A renal biopsy showed only two sclerosed glomeruli, interstitial fibrosis and tubular atrophy suggestive of chronic allograft nephropathy. Immunosuppression was switched to tacrolimus and MMF. Six and a half years after grafting, the patient had to return to hemodialysis. After three months on hemodialysis, he developed meningitis caused by Neisseria meningitides and Aspergillus fumigatus, which was effectively treated with ceftriaxon, liposomal amphotericin B for three weeks and oral itraconazole for the next 4 months [9]. In 2005, the patient, then 29 years old, received his second renal graft. Initial imunosuppression consisted of two doses of alemtuzumab 20 mg and one bolus of prednisolone 250 mg, followed by tacrolimus monotherapy. He also received valgancyclovir for a CMV-mismatch and cotrimoxazole. Serum creatinine was 0.9 mg/dl and urinalysis was normal.

One year after grafting, the patient complained of fatigue, abdominal pain and fever. Blood tests showed pancytopenia. Further workup revealed multiple gastrointestinal angiodysplasias and prominent lymph nodes. The histological examination of an axillary node showed Kaposi's sarcoma. Two weeks later, the patient developed respiratory insufficiency. He was transferred to intensive care, and assisted ventilation had to be started. Chest X-ray suggested Aspergillus infection, and antimycotic therapy was started. Spores of C. albicans were found in the oral mucosa and gastric fluid. Immunosuppression was discontinued. He developed diffuse lung hemorrhage and multi-organ failure. The patient died of respiratory failure.

Postmortem histology confirmed lung bleeding and diffuse alveolar damage as well as capillary proliferates and large areas of fibrosis. The gastric wall, epiglottic tissue, several skin lesions and paraaortal connective tissue revealed various stages of capillary proliferation among strands of atypical spindle cells resembling different stages of Kaposi's sarcoma formation (Fig. 1). Examination of the renal transplant did not show any signs of glomerular disease, acute rejection or chronic allograft nephropathy.

Patient 2

The older sister of patient 1, now 36 years of age, suffered from SLE with membranoproliferative glomerulonephritis since early childhood. At age 26, she had to start hemodialysis and, 5 years later, she received a renal allograft. Initial immunosuppression consisted of cyclosporin A, steroids, MMF and daclizumab. She received steroid bolus treatment for a clinically suspected acute rejection episode. Her further course after transplantation was uncomplicated. Current immunosuppression consists of tacrolimus (trough level 3–5 ng/ml) and 1 g MMF daily. Her actual serum creatinine 6 years after transplantation is 0.7 mg/dl and urinalysis is normal. She did not experience either infective complications or recurrence of disease.

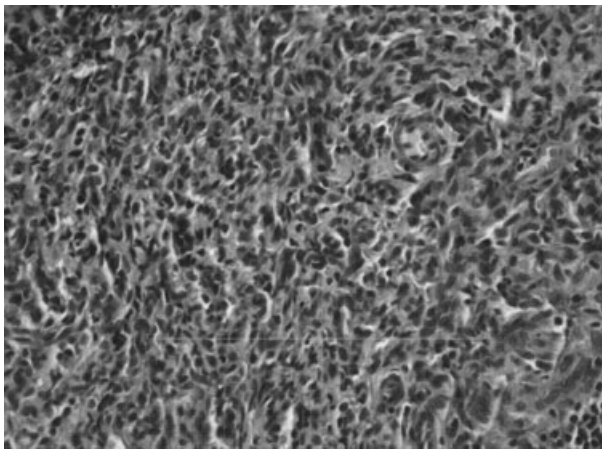

Figure 1 Kaposi's sarcoma of a lymph node showing proliferation of spindle cells separated by slit-like spaces containing erythrocytes. Cytoplasmic and extracellular hyaline globules were positive for periodic acid Schiff-staining (Hematoxylin-eosin staining, ×400 magnification).

Patient 3

This patient, 45-year-old man developed Henoch-Schoenlein purpura at age 17. Seven years later, he had to undergo hemodialysis. After 1 year, he received a cadaveric renal allograft. Immunosuppression consisted of cyclosporin A and steroids. An acute rejection episode responded to steroid bolus treatment. After 2 years proteinuria and microscopic hematuria were noted. A transplant biopsy revealed recurrence of primary disease with mesangial deposits of IgG, IgA, IgM, and C3 as well as chronic allograft nephropathy. Azathioprine was added and steroid dosage was increased. The patient developed pneumonia and severe herpes virus stomatitis. Three years after transplantation, hemodialysis had to be restarted.

After 8 years on dialysis he received a second allograft in 1997. His initial immunosuppression consisted of cyclosporin A, azathioprine and steroids. A biopsy-proven acute rejection was treated with a steroid bolus. Tacrolimus was given instead of cyclosporin A. He also developed pneumonia in the postoperative period, which was successfully treated with empiric antibiotic therapy. Serum creatinine was around 2.4 mg/dl. In 2003 MMF was given instead of azathioprine and tacrolimus was decreased, accompanied by an improvement in renal function with a serum creatinine of 1.7 mg/dl. In 2005, the patient experienced a generalized varicella infection with a skin rash, although he had suffered from chickenpox in childhood. One year later, he suffered from severe bronchitis caused by Hemophilus parainfluenzae. Because of a slight increase in serum creatinine to 2.1 mg/dl and development of proteinuria (0.4–0.6 g per 24 h), a transplant biopsy was performed in 2006. Histological evaluation showed severe calcineurin inibitor-associated arteriolopathy. Banff 2 sclerosing allograft nephropathy with moderate interstitial fibrosis and tubular atrophy was noted. Two out of six glomeruli were sclerosed. The other glomeruli revealed mild mesangial expansion with double contours of the basement membranes. Immunofluorescence showed segmental granular deposits of IgG, IgA, IgM, C3, and C1q along the basement membrane. Interestingly, there was weak positivity of C4def in a granular pattern along the glomerular capillaries (Fig. 2). Peritubular capillaries were negative. Tubular epithelial

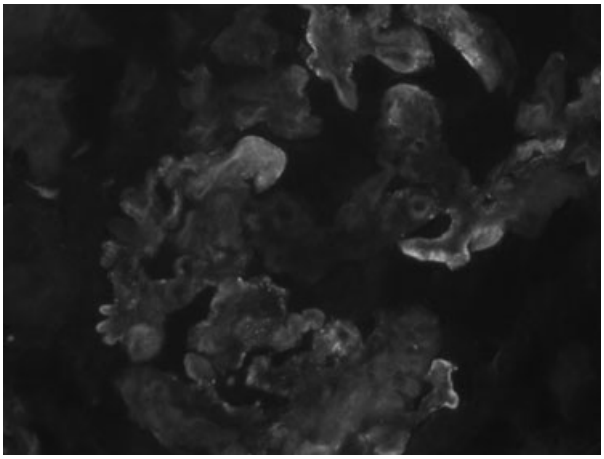

Figure 2 Immunofluorescence of the renal biopsy from patient 3 shows weak C4def deposition along the glomerular capillary wall (×400 magnification).

cells were focally positive for HLA DR. Staining for SV40 antigen was negative. Currently, the patient is treated with tacrolimus trough levels of 3–5 ng/ml and MMF 1 g per day.

Discussion

Our experience with patients suffering from complete C4def indicates that renal transplantation is possible and successful, but that there is an increased risk of infectious complications. We are aware that any conclusions drawn in this report are based on a very limited number of transplantations. Nevertheless, this is the only experience with transplantation in C4def patients reported so far and we feel that this information might be helpful in the management of such patients as well as patients with other classical pathway component deficiencies such as C2 or C1q deficiency.

Regarding the recurrence of primary glomerular disease, this risk seems to be minor. We found mesangial deposits of IgA and proliferation of mesangial cells in the first graft from patient 3, whose primary renal disease was Henoch-Schoenlein purpura nephritis. However, chronic allograft nephropathy was also present, and we feel that both factors equally contributed to loss of graft function. Histological examination of his second allograft and also of the second graft of patient 1 did not reveal any signs of recurrence of disease. In addition, urinalysis in patient 2 is normal, which makes recurrence of membranoproliferative glomerulonephritis in her allograft very unlikely.

More worrisome is the increased risk of infection in these patients, especially for herpes viruses and encapsulated bacteria. Complement plays a major role in the host response to viruses [10,11]. It is necessary for mounting a sufficient humoral and cellular immune response. Viruses may also be attacked by antibody-dependent complement activation. Enveloped viruses such as herpes viruses directly activate complement via all three pathways, the classical, lectin and the alternative pathway. Herpes viruses have developed specific strategies to evade complement attack [12]. For example, the Kaposica viral protein of Kaposi's sarcoma-associated herpes virus serves as a cofactor for factor I in the inactivation of surface-bound C3b and C4b molecules and induces accelerated decay of C3 convertases [13,14]. It is very likely that C4def was largely responsible for the fulminant and lethal course of Kaposi's sarcoma in patient 1. We do not know whether the patient carried HHV8 prior to transplantation or whether he acquired the virus at transplantation. We therefore suggest that both recipient and donor be screened for HHV8 infection before transplantation and that a kidney from an HHV8-positive donor should not be transplanted to a C4def patient. Another unique observation is the development of a second generalized varicella infection in patient 3. He had undergone this infection as a child, but had not developed persistent immunity, as shown by negative antibody titers. The rather mild infection was limited to the skin and responded to oral antiviral therapy.

The classical pathway of complement also plays a critical role in the defense against encapsulated bacteria. Patients with C4B deficiency are prone to infections with Neisseria meningitidis, Hemophilus influenzae and Streptococcus pneumoniae [15]. It is therefore likely that Neisseria meningitis in patient 1 and Hemophilus bronchitis in patient 3 were consequences of complement deficiency. The causative agents of the two pneumonias in patient 3 were not identified. Patients with C4def should receive meningococcal, pneumococcal and Hemophilus influenzae vaccines prior to transplantation.

Another threat to patients after renal transplantation is polyomavirus nephritis. Virus PCR was positive in urine and negative in blood in patients 2 and 3, 6 and 9 years after transplantation. In addition, the transplant biopsy from patient 3 and the postmortem examination of the graft from patient 1 did not show any sign of polyomavirus nephritis and were negative for SV40 immunostaining. Whether complement attack is important in the defense against BK virus has not been investigated. Complement very likely plays a role in the humoral immune response to the virus. Impaired antibody production against polyomavirus has been described in mice deficient of complement receptor 2 on B lymphocytes [16]. The significance, however, of neutralizing antibodies against polyomavirus is at present unclear [17]. The prolonged shedding of virus in the urine suggests that C4def patients have some difficulty clearing the virus, and we would

recommend regular testing for virus DNA in blood and urine of these individuals.

What is the optimal immunosuppressive regimen in these patients? It is probably wise to avoid anti-lymphocyte antibodies such as ATG or alemtuzumab, because these antibodies may cause over-immunosuppression with deleterious consequences, as exemplified in patient 1. Alemtuzumab caused significant lymphopenia in patient 1, which indicates that lymphocyte depletion by the antibody is not complement-dependent. Our positive experience with MMF in treating the primary disease of patients with C4def favors mycophenoloc acid as an immunosuppressant to prevent recurrence of disease [7,18]. Indeed, we did not find any evidence of such a recurrence in the four grafts under MMF treatment, whereas recurrence was found in the graft without MMF. In addition, we recommend a combination with a calcineurin inhibitor with low target blood levels in the maintenance phase. Patients 2 and 3, now with good long-term transplant function and without serious infectious complications, are treated with tacrolimus (target levels 3–5 ng/ml) and 1 g MMF daily. In view of the role of complement in the defence against herpes viruses, we also recommend generous CMV prophylaxis with valgancyclovir also in CMV-positive recipients.

A surprising observation was the presence of C4def staining, albeit weaker than in normal kidneys, on glomerular capillary walls in the biopsy of patient 3. C4 in this instance could be derived only from the renal allograft. Proximal tubular epithelial cells and glomerular mesangial cells are able to produce C4 upon activation with cytokines such as interferon γ [19,20]. Renal upregulation of C4 transcription by a factor of hundred to thousand has also been observed in a mouse model of allograft rejection [21].

Can C4def have an impact on renal transplantation independent of infectious complications or recurrence of disease, for example could it influence the occurrence of rejection? Acute vascular antibody-mediated rejection is characterized by C4def deposits in peritubular capillaries. We did not observe vascular rejection in any of our patients, but our results by no means prove a protective role of C4def. Transplanting kidneys from C3-deficient mice into normal mice is associated with marked prolongation of graft survival [22]. This finding implies an important role of locally produced C3 in acute allograft rejection. C3 may either play a direct role in T-cell activation or stimulate the antigen-presenting capacity of macrophages and dendritic cells. On the contrary, in a mouse model of renal transplantation using C4def mice either as donors or as recipients, no influence on frequency or severity of acute allograft rejection was observed [21]. We found an acute cellular rejection in four out of five transplantations in C4def patients, which indicates that C4def does not protect against acute rejection.

In summary, our observations with renal transplantation in patients suffering from complete C4def suggest that these patients are prone to infectious complications, but that under certain precautions and judicious use of immunosuppression, good long-term results are achievable. Patients with complete C4def should not be denied renal transplantation.

Authorship

CF: performed pathological examinations, collected data. WM: cared for patients at transplant surgery. CS: performed pathological examinations, wrote part of the manuscript. DH: performed histology of kidney biopsies. FN: cared for patients. JS: performed tests of complement activity. KL: cared for patients, wrote manuscript.

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