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Polyomavirus nephropathy in renal transplantation: a clinico-pathological study

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Abstract Polyomavirus (PV) nephropathy is a rare cause of graft dysfunction, but it may accompany acute rejection (AR), resulting in complications with respect to its diagnosis and treatment. To examine the validity of tubulitis and inflammatory phenotype in the diagnosis of concurrent AR, we reviewed the renal histology of ten biopsy samples from nine patients with PV nephropathy, and the immunohistochemistry from eight samples. Tubulitis was present in seven patients and was associated with AR in six. The degrees of tubulitis and interstitial inflammation were higher in biopsy samples with AR than in those without, but the degree of tubulitis was not related to the degree of interstitial inflammation. Virally infected cells were rare in the samples with no, or mild, tubulitis, but did not increase with the degree of interstitial inflammation. Immuno-phenotyping of inflammatory cells did not show any T-cell dominance in AR: T cells were dominant over B cells in three of six samples with AR and both samples without AR. Although the degrees of tubulitis and interstitial inflammation were higher in the AR subjects, the presence of tubulitis or inflammatory phenotype was not helpful in the diagnosis of concurrent AR. Further studies will be required to find a better marker for coexisting AR in patients with PV nephropathy and to establish strategies for treatment.

Keywords Renal transplantation · Polyomavirus · Immunohistochemistry · Acute rejection · Inflammatory cells · Interstitial fibrosis

Introduction

Polyomavirus (PV) nephropathy rarely causes renal allograft dysfunction. Since the late 1990s there has been an increase in the prevalence of PV nephropathy associated with heavy immunosuppression using tacrolimus and/or mycophenolate mofetil (MMF) [10]. PV nephropathy is characterized by the presence of virally infected tubular cells that show intra-nuclear paracrystalline inclusions of approximately 30–50 nm in diameter, which are frequently detached from the tubular basement membrane and obstruct the tubular lumen. In contrast to a consistent tubular injury, interstitial inflammation varies, ranging from a negligible to an extensive association with tubulitis [7], and the site of inflammation is not always concordant with that of the tubulopathy [11].

PV nephropathy can be complicated by acute rejection (AR), which gives rise to confusion in its diagnosis and treatment. Until now, there have been few reports on the diagnostic criteria of concurrent AR. Contrary to the T-cell dominance in AR infiltrates, B lymphocyte-rich

[1], plasma cell-rich infiltrates, and tubular invasion by plasma cells have been reported as supportive evidence for PV nephropathy [6, 22]. Nickeleit et al. [12] reported HLA-DR upregulation in tubules as an adjunctive marker for the diagnosis of concurrent AR. However, plasma cells can also be seen in AR [4], and the expression of HLA-DR can be upregulated in renal ischemia in the absence of AR [19, 20]. With regard to treatment, steroid pulse therapy has been reported to have been helpful in the restoration of renal function in only a fraction of patients with concurrent AR [9, 11, 17].

Another problem with PV nephropathy is its progression to chronic graft dysfunction and subsequent graft loss. Interstitial fibrosis develops as the inflammation subsides, and viruses are no longer detectable in the fibrotic stage [6]. Factors other than the persistence of the virus may play a role in the progression of the disease. However, little is known about the clinical or histological features that favor its progression [2]. To increase our experience with this rare complication, we studied the histological features and interstitial inflammatory phenotypes in nine cases of PV nephropathy and correlated these with their clinical courses.

Patients and methods

Patients

PV nephropathy was diagnosed in ten biopsy samples obtained between 1997 and 2001 from nine patients, including three outside consultation cases. With the exception of one consultation case, all cases of PV nephropathy at our department were correctly diagnosed. One case (patient 1) had been diagnosed as having AR and had been treated with increased immunosuppression prior to consultation. A renal allograft biopsy was performed in all patients due to an acute rise in serum creatinine. The diagnosis of PV nephropathy was made after an average of 12.9 months (range: 4–44 months, median: 8 months) after transplantation. PV was demonstrated either by positive staining against SV40 large T antigen cross-reactive to human BK and JC strains or by electron microscopy. A summary of the clinical data is shown in Table 1. Seven patients received cyclosporine-based immunosuppression, with MMF in the case of four patients, and two received tacrolimus. Three patients had previously had clinical episodes of AR without biopsy confirmation: one episode in two patients, and two in one patient, which had been treated with a steroid pulse.

Histology and immunohistochemistry

All biopsy samples were fixed in formalin and studied by light microscopy. The sections were stained with hematoxylin & eosin (H&E), periodic acid-Schiff (PAS), acid fuchsin orange G or trichrome, and methenamine silver. Immunofluorescence and electron microscopy were carried out on eight and four samples, respectively. We reviewed four, or more, PAS-stained sections in each biopsy sample to evaluate interstitial inflammation, tubulitis, and vasculitis. The degree of tubulitis in the non-cytopathic tubules and interstitial inflammation were divided into three grades—mild, moderate, or severe—according to the Banff scheme [21]. Inflammation that occupied less than 5% of the cortical interstitium was considered to be negative. AR was defined as the presence of interstitial inflammation, edema, and tubulitis, in non-atrophic and non-cytopathic tubules, and/or intimal arteritis. Phenotyping of inflammatory cells was performed on eight biopsy samples from seven patients. Formalin-fixed, paraffin-embedded 3-μm sections were stained with antibodies directed against B cells (CD20, Dako, Glostrup, Denmark, 1:100) and T cells (CD45RO, Dako, 1:100) and monocytes/macrophages (CD68, KP1 clone, Dako, 1:75). Analysis of cytotoxic cells and the expression of tubular HLA-DR were performed on five biopsy samples from four patients, with antibodies directed against CD8 (Dako, 1:50), granzyme B

Table 1 Clinical findings (*L* living, *C* cadaver, *H* haploidentical, *I* identical, *U* unknown, *CyA* cyclosporine, *S* steroid, *SP* steroid pulse therapy, *FK* tacrolimus, *AZA* azathioprine, *Inc* increased, *Dec* decreased, *F* failure)

Patient no.	1	2	3	4	5	6	7	8	9
Recipient age (years), gender	24, M	47, M	25, M	26, M	24, M	25, M	32, M	45, F	38, M
Donor type	L	L	L	L	C	L	L	C	L
HLA match	H	I	H	U	U	I	I	U	I
Previous episodes of acute rejection	0	0	0	0	1	2	I	U	0
Immunosuppression									
At time of biopsy	CyA, S, AZA	FK, S	CyA, S, MMF	CyA, S, MMF	CyA, S	CyA, S, MMF	CyA, S, MMF	CyA, S, AZA	FK, S, MMF
After diagnosis	Inc (OKT3, SP, MMF)	Dec (CyA, S)	Dec (CyA, S)	Dec (CyA, S, MMF)	Dec (SP, CyA, S)	Dec (CyA, S)	Dec (CyA, S)	Dec (CyA, S)	Dec (FK, S)
Time to biopsy (months)	8	4	8	11	4	13	44	20	4
Serum creatinine (mg/dl)									
At baseline	1.5	1.8	1.1	2.3	2.1	1.3	1.6	1.1	1.5
At time of biopsy	2.2	2.7	1.9	2.7	2.8	6.2	3	4.7	2.4
At the latest follow-up	F	1.6	1.7	4.5	2.2	F	F	F	1.8
(Duration, months)	(4)	(37)	(34)	(26)	(28)	(13)	(13)	(1)	(4)

(Monosan, Uden, The Netherlands, 1:40), and HLA-DR (Dako, 1:100). To demonstrate PV, we used the polyclonal antibody against SV40 large T antigen (Calbiochem, Cambridge, Mass., 1:200). In brief, xylene was used to remove the paraffin from the sections, which were then rehydrated in graded alcohol. After being treated with 3% H_2O_2 to abolish the endogenous peroxidase activity, they were incubated with primary antibodies at room temperature for 30–60 min, with (CD45RO, CD8, granzyme B, HLA-DR, and SV40) or without (CD20 and CD68) microwave pre-treatment, followed by incubation overnight at 4°C. The sections were then washed with phosphate-buffered saline, and, using the LSAB kit (Dako), we sequentially applied link antibodies and streptavidin-biotin peroxidase. Diaminobenzidine (DAB) and 3-amino-9-ethylcarbazole (AEC) were used to color the reactions. Using an ocular grid (10×10) attached to an Olympus BH-2 microscope we counted the positively stained inflammatory cells in all of the cortical interstitium, including the fibrotic areas, and expressed them as number per mm^2. An average of 1.68 mm^2 was examined per sample (range: 0.28–3.21 mm^2). The ratio between T cells and B cells was presented as the ratio of CD45RO-positive cells to CD20-positive cells, per mm^2. Tubular HLA-DR expression was considered to be positive when a tubule showed any sign of positive cytoplasmic staining.

Results

Tubulointerstitial nephritis

Infected tubular epithelial cells showed enlarged nuclei, with homogenous ground-glass intra-nuclear inclusions, on H&E-stained sections. They were scattered in both the cortex and medulla, but were more prominent at the latter, whether attached to the tubular basement membranes or freely in the lumen. The number of virally infected cells was variable from tubule to tubule, mostly 1–4, but up to 10, per tubular cross-section, but they were rare in samples with no, or mild, tubulitis. Viral inclusions were highlighted brown by use of an antibody against the SV40 large T antigen and DAB as a chromogen, which revealed 35–50 nm para-crystalline structures on electron microscopy. Positive immunohistochemical staining was seen not only in the enlarged tubular nuclei bearing viral inclusions, but also in the apparently normal nuclei of tubules without tubulitis. In patient 6, viral inclusions were also present in parietal epithelial cells.

Tubular invasion by lymphocytes (tubulitis) was present in seven patients and was associated with AR in six (patients 1, 3, 5, 6, 7, and 9). Tubulitis was present in both the cytopathic and non-cytopathic tubules, but was more frequent in the latter. No tubular invasion by plasma cells was observed, not even in plasma cell-rich areas. The degree of tubulitis in non-cytopathic tubules was moderate ("t2") in one patient, but severe ("t3") in five with AR. Intimal arteritis was not present. In patient 8, the degree of tubulitis was "t1", and tubulitis of non-infected tubules was present in the vicinity of the virally infected tubules. Interstitial inflammation was moderate to severe in four of six patients with moderate-to-severe tubulitis, whereas severe interstitial inflammation was present in one of three patients with mild tubulitis. Interstitial inflammation of the cortex was equal to or less intense than that of the medulla, with the exception of patient 9. Only the renal cortical tissue was included in the biopsy sample of patient 2. The inflammatory infiltrate comprised mostly lymphocytes and plasma cells, either equally or with a slight lymphocyte predominance, and was occasionally mixed with neutrophils. In patient 5, a repeat biopsy, performed after methylprednisolone pulse therapy, showed a decrease, yet persistence, of virus-infected cells, with a similar degree of severe tubulitis and interstitial mononuclear infiltrates. Interstitial fibrosis was moderate to severe in three patients (patients 6–8).

Inflammatory immuno-phenotypes

Immuno-phenotyping of the inflammatory cells showed a T-cell dominance of 1.8:1 to 4.8:1 in four patients (two with AR and two without) and a B-cell dominance of 1.1:1 to 2.4:1 in three patients with AR. In patient 5, the T-cell:B-cell ratio was reversed in a repeated biopsy following steroid pulse treatment. The ratio of CD45RO-positive cells to CD8-positive cells was 2.1:1 to 3.8:1. Granzyme B-positive cells were rare, even in samples with AR. Tubular HLA-DR expression was negative in three patients and positive in only a few tubules of one patient (patient 5) (Table 2).

Clinical course and its relationship with renal histology

Immunosuppression was decreased following diagnosis in all but one patient. In patient 2, tacrolimus was switched to cyclosporine. Immunosuppression in patient 5 was decreased following methylprednisolone pulse therapy. In three patients, concurrent or subsequent occurrence of other viral infections was present following the diagnosis of PV nephropathy (cytomegalovirus in patients 1 and 7, Epstein–Barr virus in patients 7 and 8, and Varicella-zoster virus in patient 7). During the mean follow-up of 17.8 months (range: 1–37 months), four patients finished with graft failure, with one patient dead. The graft failed in three of six patients with concurrent AR and in two of three patients with previous episodes of

Table 2 Immuno-profile of interstitial inflammatory cells. Values are numbers of cells per mm^2. *ND* not done, or unsatisfactory

Patient no.	2	3	4	5-1	5-2	6	7	9
CD68	211	203	ND	96	467	343	517	255
CD20	159	727	148	771	205	210	73	597
CD45RO	638	657	505	614	429	385	351	245
CD8	310	259	181	163	ND	ND	ND	ND

AR. Grafts were lost in three of five patients that were showing moderate-to-severe inflammatory infiltration and in all patients with moderate-to-severe interstitial fibrosis, but loss of graft did not correlate with the number of virally infected cells per tubule.

Discussion

With the recent increase of PV nephropathy in renal allografts, much information has been obtained relating to risk factors and histopathology [12, 18]. One of the risk factors is heavy immunosuppression. In this study, seven of nine patients received cyclosporine-based immunosuppression, with MMF in the case of four patients, unlike in other reports, where the use of tacrolimus or MMF, or their combination, was popular [3, 8, 14, 16]. Of these seven patients, all received the recently introduced micro-emulsion form of cyclosporine, which resulted in more effective absorption and maintenance of higher cyclosporine blood levels than with the use of the conventional form [15]. Concurrent or subsequent activation of other viruses in three patients can also be explained by the fact that these patients were under severe immunosuppression. Another risk factor is the number of AR episodes. Three patients had at least one rejection episode (33.3%), which is a lower percentage than that in other reports [2, 3].

In this study, we examined the validity of tubulitis and inflammatory phenotype in the diagnosis of concurrent AR. Tubulitis was present in seven of nine patients and was associated with AR in six. The degree of tubulitis was higher in the biopsy samples with AR than in those without ("t" scores: 2.9 vs 1), which was also true for interstitial inflammation ("i" scores: 2.3 vs 1.7). However, tubulitis was severe in the samples with many virus-infected cells, and the degree of tubulitis was increased, but not concordant with the degree of interstitial inflammation. Furthermore, there was no correlation between the sites of viral inclusions and those of tubulitis or interstitial inflammation. These results may suggest that tubulitis is not a marker for AR, but may be related to active viral replication or may reflect interstitial inflammatory activity [6, 18]. Interstitial nephritis, disproportionate to tubular changes, may be a feature that favors AR. The inflammatory phenotype also did not seem to be an adjunct to concurrent AR. T-cell dominance was present in two of five patients that were showing histological features of AR, whereas all patients without AR also showed T-cell dominance. Furthermore, in one patient with AR, the T-cell:B-cell ratio was reversed after steroid pulse treatment. Tubular HLA-DR expression was positive in only one of two samples with AR and was limited to a few tubules. The higher and consistent HLA-DR-positive rate in patients with concomitant AR, as reported by Nickeleit et al. [12], may be attributed to the sensitivity of staining, since immunofluorescence was employed on frozen sections instead of immunohistochemistry on paraffin sections, as in our study. It seems that vascular rejection, if present, is the most reliable histological marker for AR in the context of PV nephropathy. In that regard, C4d staining may be helpful in demonstrating humoral rejection [5].

The effects of anti-rejection treatment on concurrent AR are controversial. In a study by Randhawa et al. [17], grafts failed in 67% of the12 patients treated, whereas eight patients that were treated by reduction of immunosuppression from the outset were free of graft loss for 0.2–10 months. However, Nickeleit et al. [11] proposed that graft function might be restored if the concurrent AR was treated properly. The discrepancy in treatment results may be attributed to the diagnostic criteria used for AR and the different treatment policy. Tubulitis was presumed to be a marker for rejection in Randhawa's series [17], and immunosuppression was decreased in only a fraction of patients following treatment failure, whereas tubular HLA-DR expression was an adjunctive marker in addition to tubulointerstitial nephritis, and immunosuppression was decreased soon after the steroid pulse in Nickeleit's study [11]. Successful anti-rejection treatment results, with subsequent decreased immunosuppression in one patient who fulfilled the biopsy criteria of AR and HLA-DR-positivity, support the notion that some patients may have therapeutic benefits.

It is not clear what mediates the progression of renal fibrosis and leads to renal failure in PV nephropathy. Barri et al. [2] reported that the severity of interstitial inflammation, interstitial fibrosis, and the presence of crescents, could be responsible for graft loss or chronic graft dysfunction. In agreement with that report, grafts failed more frequently in patients with severe interstitial inflammation than in those with mild inflammation, but were lost in all three patients that were showing moderate-to-severe interstitial fibrosis. Quantification of interstitial inflammation and fibrosis may be necessary in biopsy samples for prediction of the progression of PV nephropathy. A major pitfall in the prediction of prognosis in our study lies in the clearance of virally infected cells, or subclinical rejection, which is not checked systematically during follow-up [13]. Although the degrees of tubulitis and interstitial inflammation were higher in AR, the presence of tubulitis or inflammatory phenotype is not helpful in the diagnosis of concurrent AR. Further studies will be required to find better markers for coexisting AR in patients with PV nephropathy and to establish strategies for treatment.

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