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ORIGINAL ARTICLE

Plasma cell infiltrates in polyomavirus nephropathy

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Summary

Polyomavirus (PV) associated nephropathy (PVAN) has become an important cause of allograft dysfunction. We studied plasma cells (PCs) – which have not yet been characterized – present in the cellular infiltrate of 20 PVAN cases using immunohistochemistry and morphometry. The results were correlated with morphological, clinical and anti-BK virus serological findings. PC-rich cellular infiltrates occurred in 50% of cases (>15% PCs in the cellular infiltrate) and in these IgM producing PCs were commonly seen (70%): IgM PC predominance in 50% of cases and a comparable number of IgM and IgG PCs in 20% of cases. We found a significant correlation not just between the absolute numbers ($P < 0.034$) and the percentage values of IgM PCs ($P < 0.004$ in relation to all cells) and the serum IgM-Ab anti-BKV activity, but also between the ratio of IgG/IgM PCs and the ratio of serum IgG/IgM-Ab activities ($P < 0.0001$). We showed that IgM PC counts in biopsies correlate with titers of circulating anti-BK virus IgM antibodies. Every case except one was C4d negative in peritubular capillaries (PTC). As IgG PCs characterize PC-rich rejection cases, we suggest that in the presence of IgM PCs in PC-rich infiltrate with PTC C4d negativity, a search for possible PVAN infection should be initiated.

Introduction

Polyomavirus (PV) associated nephropathy (PVAN) has become an important cause of allograft dysfunction and even graft loss in renal transplantation [1–12]. More and more information is available regarding its pathogenesis [1–18]. PVAN is also called BK virus nephropathy after the main causative agent, the polyoma-BK-virus strain [8,17]. PVAN is most likely caused by the reactivation of latent BK viruses [19–22], which under sustained and intensive immunosuppression, enter a replicative/productive cycle [3,6,9,14–17]. PVAN is typically found in kidney transplants and its development is associated with signs of viral activation i.e. decoy cells in the urine [3–6,8–10]. Prior to the mid 1990s, PVAN did not receive widespread clinical attention. Currently, PVAN is by far the most common viral disease affecting renal allografts [17] and has an estimated incidence approaching 10% [15].

The diagnosis of PVAN can be made by kidney biopsy, revealing intranuclear viral inclusion bodies in tubular epithelial cells associated with focal necrosis [4–7,14,16,17]. The necrosis of tubular cells is followed by the detachment from the tubular basement membranes (TBM) and their shedding into the urine. The inflammatory cellular infiltrate varies considerably; interstitial infiltrates consist of lymphocytes, histiocytes and occasionally abundant PCs and at some stage, polymorphonuclear leukocytes as well. A definite diagnosis relies on the immunohistochemical detection of virus-specific proteins, the hallmark of PVAN [4–7,14,16,17]. PVAN is frequently accompanied by tubulitis, hence making the differential diagnosis of concomitant, acute cellular rejection difficult by light microscopy alone [8,11]. The detection of Class II MHC antigens in the kidney biopsy may be of help [8]. In addition, plasma cell-rich (PR) rejection [23–29] is characterized by a predominance of IgG PCs [23,24].

The distinction between PVAN and a possible rejection process is important, as a decrease in immunosuppression is recommended in the treatment of PVAN [6,8,9,15,17], whereas in the case of a rejection, it demands an increase.

Whether the PR interstitial infiltrates seen in PVAN represent a specific inflammatory response or not is currently unclear, as is the types of PCs present in the cellular infiltrate. In the present work, we confirm that PR cellular infiltrates can be observed in a subset of PVAN and we will show that in such infiltrates, PCs predominantly produce IgM. This is in marked contrast to PR rejection processes, where IgG producing PCs predominate in the cellular infiltrate [23,24].

Materials and methods

Renal samples for our immunohistochemical analysis included 20 consecutive kidney allograft specimens with the PVAN group (PVAN). All the cases studied were transplants at the University Hospital in Basel, where both clinical and pathological management were performed between 1997 and 2005. Each patient was given immunosuppressive drugs, including tacrolimus and/or mycophenolate mofetil. The diagnosis of PVAN was established by histopathological evidence of PV-induced organ involvement, confirmed by the immunohistochemical detection of PV SV40 large T-antigen.

In general, two needle biopsy cores were obtained for morphologic study: one was used for light microscopy and immunohistochemical analysis after formalin fixation, and the other for immunofluorescence after quick-freezing in an optimal cutting temperature embedding medium (Miles Laboratories, Elkhart, IN, USA). All biopsies met the Banff criteria for adequacy [30] with a mean of 16 glomeruli for light microscopy and 10 for immunofluorescence. The procedure for light microscopy immunofluorescence and immunohistochemistry was performed in the usual, standard way. Fresh, frozen tissue was analyzed by immunofluorescence microscopy (IF) using a conventional panel of antibodies directed against IgG, IgM, IgA, C3c, C4c, C1q, fibrinogen (rabbit anti-human FITC-conjugated polyclonal antibodies; all from DAKO A/S, Glostrup, Denmark, except fibrinogen, from Quartett GmbH, Berlin, Germany).The MHC class II expression of tubular epithelial cells was evaluated by direct IF using a FITC-conjugated mouse anti-human monoclonal antibody (mAb); DAKO A/S; dilution, 1:3 in PBS; 30-min incubation at room temperature (RT). The deposition of C4d was detected by employing the indirect IF technique with a primary affinity-purified mAb (mouse anti-human; dilution, 1:50; 30-min incubation at RT, from Quidel San Diego, CA) and a FITC-labeled affinity-purified secondary goat anti-mouse polyclonal IgG antibody (dilution, 1:200; 30-min incubation at RT, from Molecular Probes INC Eugene, OR, USA). Immunohistochemically, PV (SV40 large T antigen, which is common to JC virus, BK virus and the simian virus 40) was detected with the indirect peroxidase technique on formalin fixed, paraffin embedded samples. We used a mouse mAb (Calbiochem/Oncogene Research Products, Cambridge, MA, USA, overnight incubation at 4 °C, dilution, 1:2000) combined with the microwave technique for antigen retrieval (Tris–HCl buffer 0.5M, pH 10.5, 15 min at 95 °C) and the avidin–biotin technique as an indicator system. CD3 antigen was detected with an anti-human rabbit polyclonal antibody (dilution, 1:500), and CD20, CD68 antigens with mouse anti-human mAbs (L26, dilution, 1:800; PG-M1, dilution, 1:1600, overnight incubation at 4 °C, from DAKO A/S, Glostrup, Denmark) using the Ventana method on formalin fixed, paraffin embedded samples combined with the microwave technique for antigen retrieval (Citrat buffer pH 6.0, 30 min at 98 °C). For the morphometric study IgG, IgA and IgM were detected on formalin fixed, paraffin embedded biopsies using anti-human IgG, IgA and IgM rabbit polyclonal antibodies (overnight incubation at 4 °C, dilution, 1:80 000; DAKO A/S, Glostrup, Denmark) and the avidin-biotin technique as indicator system combined with 0.1% pronase digestion for antigen retrieval (IgG and IgM for 45 min, IgA for 45 and 90 min).

The detection of BKV-specific IgG and IgM was performed by enzyme immuno assay (EIA) using 1:400 diluted sera on BK virus-like particle-coated microtiter plates [31]. In brief, BKVLP were purified by density gradient centrifugation from Sf9 insect cells transfected with the pFast-Bac VP1expression vector bearing the VP1 coding sequence (Dunlop strain). Standard 96-well EIA plates with high coating properties were used for coating overnight at 4 °C. The wells were subsequently washed five times with 0.1% Tween-20, treated with a blocking buffer (PBS pH7.4, 4.0% bovine serum albumin, 0.1% Tween-20) at RT for 2 h, and washed three times. The protein preparations were incubated for 1 h at RT with 100 µl of 400-fold diluted patient sera, washed five times, incubated with 1:10,000 diluted secondary antibodies at RT for 1 h, and once again washed five times. The secondary anti-human IgG and anti-human IgM antibodies were purchased from Sigma-Aldrich. The detection reaction using o-phenylenediamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was stopped after 30 min at RT by adding 1 N sulphuric acid. Optical densities were measured at 492 nm using an automated plate reader (Tecan Group Ltd., Männedorf, Switzerland). Equivalents of 1.0 pmol of purified antigen or Sf9 extract from pFast Bac lacking VP1 were run in parallel with each serum and OD values were taken as background.

The BK virus load was measured 6 months before and after biopsy, as described in a previous paper [32].

Morphometric analysis

The numbers of CD3 positive T lymphocytes, CD20 positive B lymphocytes, CD68 positive macrophages, IgG, IgA, and IgM positive PCs present in the cortical and/or medullary interstitial area were counted under high magnification (400×) in 10 randomly selected non-overlapping areas of cellular infiltrates using a 10-squared eyepiece micrometer (area: 0.1 mm^2). Among the 20 polyoma virus infected cases, 14 biopsy specimens contained both cortex and medulla; in five cases, only cortex was available, and in one case, only medulla was available for immunohistochemical study. In those specimens where cortex and the medulla could be studied, five of the randomly selected fields with interstitial inflammation were defined in the cortex and five in the medulla. Afterwards, the number of counted cells was expressed as cells per unit area (1 mm^2). To define PR and plasma cell poor (PP) groups of PVAN, we combined the IgG, IgA and IgM positive PC numbers counted in 1 mm^2 and calculated the percentage of the total PC number. The median number of PCs was used to define a subgroup of PR cases (>15% PCs of all infiltrating cells). The criterion for predominance of a particular PC type was defined as a difference of least 20% in the cell number per 1 mm^2.

Statistical analysis

The results were expressed as mean, median and SD values. Differences between groups were examined for statistical significance using the Mann–Whitney or Chi-square test, where appropriate. Nonparametric Spearman's rank correlation coefficient (rho) was calculated for each quantitative variable. *P*-values <0.05 were considered significant.

Results

Selected clinical, laboratory, and histological data of our PVAN cases are presented in Table 1. In our study, there were more male patients than female patients. The median age of the patients was 48.5 years (range: 12–73 years), the median time interval between transplantation and index biopsy was 233 days (range: 40–980 days), and median serum creatinine levels measured 284 μmol/l (range: 250–842 μmol/l). In all 20 cases, SV40 large T antigen was detected both in the cortex and in

Table 1. Clinical and descriptive morphological characteristics of polyomavirus (PV) associated nephropathy (PVAN) cases.

PVAN cases case numbers	Sex	Age (years)	Serum Creat μmol/l	Time of biopsy post-Tx (days)	HLA-DR	C4d	Tubulointerst. inflamm./acute rejection^{AR} Banff 97 [30]	Chronic allograft nephropathy Banff 97 [30]	PVAN in medulla (M+)/cortex (C+)	Stage of PVAN [15]	Decoy cells first detected in urine post-Tx (days)
P 1. 14 447	M	52	309	335	Focal	Neg	t3, i1, c0^{AR}	ct3, ci3, cv0	M + C+	C	68
P 2. 14 541	M	52	331	377	Focal	Neg	t2, i2, c0^{AR}	ct3, ci3, cv1	M + C+	C	67
P 3. 15 124	M	59	406	653	Neg	Neg	t1, i2, c0	ct3, ci3, cv1	M + C+	C	304
P 4. 15 653	M	53	514	170	Neg	Neg	t2, i1, c0	ct0, ci0, cv1	M + C+	B	81
P 5. 15 935	M	47	842	932	Neg	Neg	t0, i1, c0	ct3, ci3, cv0	M + C+	C	249
P 6. 17 236	F	52	284	40	Focal	Diffuse	t1, i1, c0^{AR}	ct1, ci1, cv0	M + C+	B	17
P 7. 17 746	M	46	120	295	Focal	Neg	t1, i0, c0^{AR}	ct0, ci0, cv0	C+	A	25
P 8. 17 806	F	40	150	386	Neg	Neg	t0, i0, c0	ct2, ci2, cv0	M + C+	B	134
P 9. 19 564	M	48	391	88	Neg	Neg	t0, i0, c0	ct2, ci2, cv3	M + C+	A	6
P 10 19 601	M	73	190	175	Neg	Neg	t0, i0, c0	ct0, ci0, cv0	M+	A	**
P 11. 20 200	M	62	192	178	Neg	Neg	t3, i2, c0	ct0, ci0, cv0	M + C+	B	65
P 12. 20 210	F	39	285	980	Neg	Neg	t3, i2, c0	ct2, ci2, cv0	M + C+	B	621
P 13. 20 244	F	40	160	372	Neg	Neg	t2, i1, c0	ct3, ci3, cv0	M + C+	C	189
P 14. 20 245	M	46	192	114	Focal	Neg	t2, i1, c0^{AR}	ct1, ci0, cv0	C+*	B	**
P 15. 20 363	F	59	174	196	Neg	Neg	t0, i0, c0	ct1, ci0, cv0	C+*	A	48
P 16. 20 592	M	49	525	226	Neg	Neg	t3, i3, c0	ct0, ci0, cv0	M + C+	B	114
P 17. 20 596	F	12	63	221	Neg	Neg	t2, i1, c0	ct1, ci0, cv0	M + C+	B	**
P 18. 21 288	M	30	349	182	Focal	Neg	t1, i1, c0^{AR}	ct2, ci2, cv0	M + C+	C	31
P 19. 21 554	M	20	215	188	Focal	Neg	t2, i1, c0^{AR}	ct2, ci2, cv0	M + C+	C	18
P 20. 21 705	M	58	180	726	Neg	Neg	t1, i2, c0	ct1, ci2, cv0	M + C+	B	43

AR concomitant acute T cell mediated rejection: tubulointerstitial inflammation with focal HLA-DR positivity, *only cortex in the biopsy, **not tested before diagnosis.

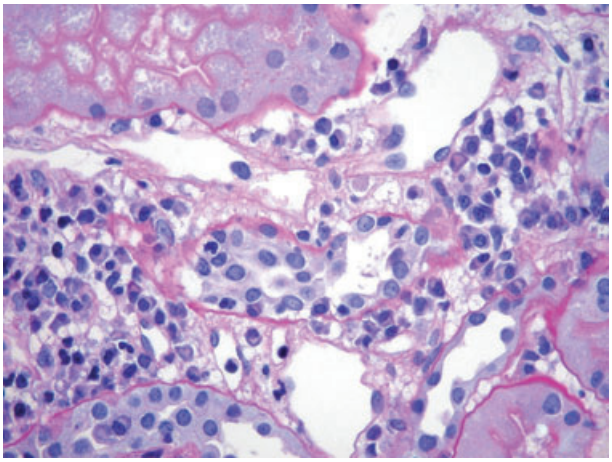

Figure 1 Plasma cell tubulitis. There are numerous plasma cells within the tubular lumen. Massive interstitial plasma cell infiltration. PAS, ×320.

the medulla. Tubulitis of varying degree was observed in 15/20 cases. In seven out of 20 cases, focal HLA-DR was present. The HLA-DR positivity of the tubular epithelium was independent of the degree of tubulitis. In our study, tubulitis associated with focal HLA-DR positivity was regarded as concomitant T cell mediated acute rejection, and it was found in six cases. Every PVAN case except one (Table 1) was negative for C4d in peritubular capillaries (PTC). In case 6, diffuse PTC C4d positivity was found together with mild tubulitis and focal HLA-DR positivity. In general, various degrees of tubular atrophy (15 cases) and interstitial fibrosis (12 cases) were seen. Proliferative and sclerosing transplant vasculopathy was present in four cases, mild transplant glomerulitis in two cases, glomerulonephritis in one case, and Calcineurin inhibitor associated toxicity in two cases. The inflammatory cell infiltrate varied considerably in extent and cellular composition. A common feature was PCs within the tubules (plasma cell tubulitis), observed in those cases that were rich in PCs (Fig. 1). The tubulointerstitial inflammation was not limited to the virus-infected tubules, but spread more extensively around the infected tubules (Fig. 2), and always involved both the medulla and the cortex (Table 1). The stage of PVAN was determined in each case and the results are shown in Table 1.

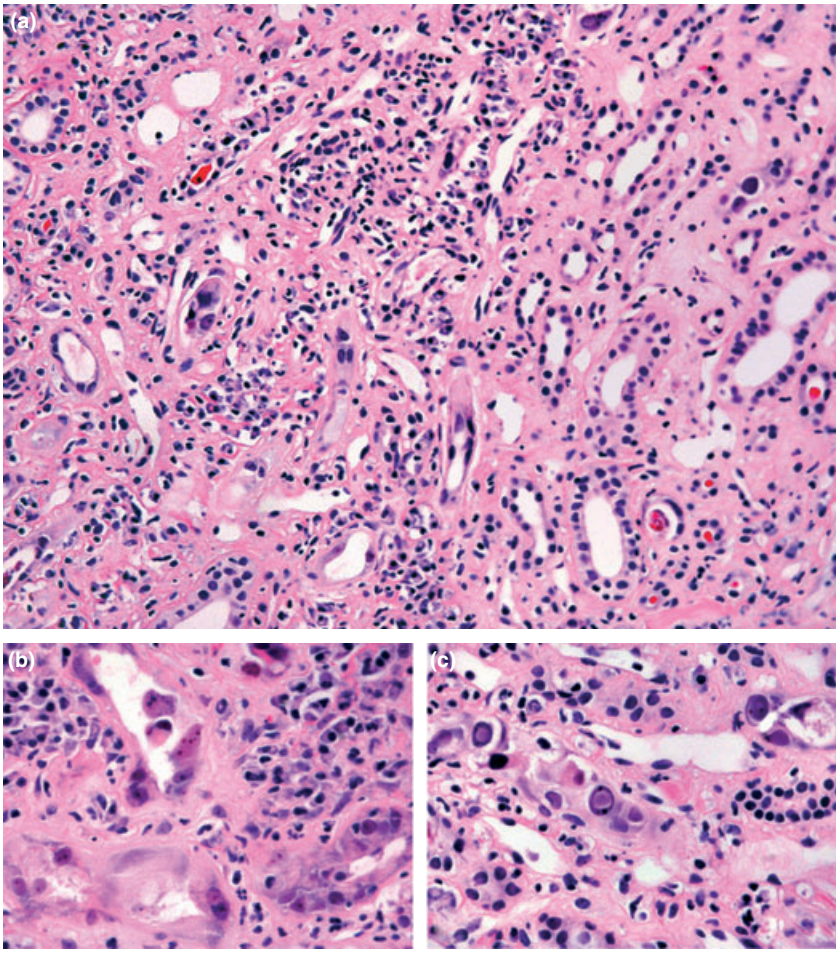

Figure 2 Plasma cell-rich polyomavirus nephropathy. (a) Note the abundant plasma cells present around the virus infected tubules in the medulla. H–E, ×130. (b, c) Typical viral inclusions in the tubular epithelial cells. H–E, ×200.

Based on the immunohistochemical analysis of the cell types, a heterogeneous cell infiltrate (Figs 3 and 4, Table 2) was found: CD3 positive T cells (median: 666/mm^2) exceeded the number of CD20 positive B cells (median: 261/mm^2), and CD68 positive macrophages (median: 83/mm^2). The absolute number of PCs varied from case to case, and the median number of PCs was 177/mm^2 (range: 0–907/mm^2), which represented 15% (range: 0–39%) of all infiltrating cells. Among the PCs, the number of IgM PCs 116/mm^2 (range: 0–530/mm^2) exceeded the number of IgG PCs [median: 50/mm^2 (range: 0–495/mm^2)], but the difference did not reach a significant level ($P = 0.33$). The number of IgA PCs [median 13/mm^2 (range: 0–95/mm^2)] was significantly less compared with the number of IgM PCs ($P = 0.001$) and IgG PCs ($P = 0.001$). Quite similar results were obtained when we examined the distribution of PC types in percentage terms (Table 2).

According to our definition, 10 of 20 cases of PVAN were rich in PCs (>15% of inflammatory cells). The results of the cell distribution are shown in Fig. 5 and Table 2. In the PR group, the number of IgM PCs [median: 232/mm^2 (range: 0–530/mm^2)] again exceeded the number of IgG PCs [median: 85/mm^2 (range: 0–495/mm^2)], but the difference between the number of IgM PCs and IgG PCs was not significant ($P = 0.57$). The number of IgA PCs [median: 16/mm^2 (range: 7–43/mm^2)] was significantly smaller compared with the number of IgM PCs ($P = 0.007$) and IgG PCs ($P = 0.009$). When we examined the individual cases, we found that five of the PR cases displayed a predominance of IgM (5/10, 50%), 3 IgG PCs (3/10, 30%) and in two cases, the same proportion of IgG and IgM positive PCs (2/10, 20%) was present. Other than what we expected, a statistical analysis revealed no significant differences (Table 2).

Interestingly, in two cases with IgG predominance, IgG was also found in the TBM of a few tubules with SV40 positive cells (Fig. 6). Case 3 was in the PP group (7.1% of PCs of all cells) and case 16 in the PR group (38.27% PCs of all cells), yet none of these cases showed signs of acute rejection, and were HLA-DR and PTC C4d nega-

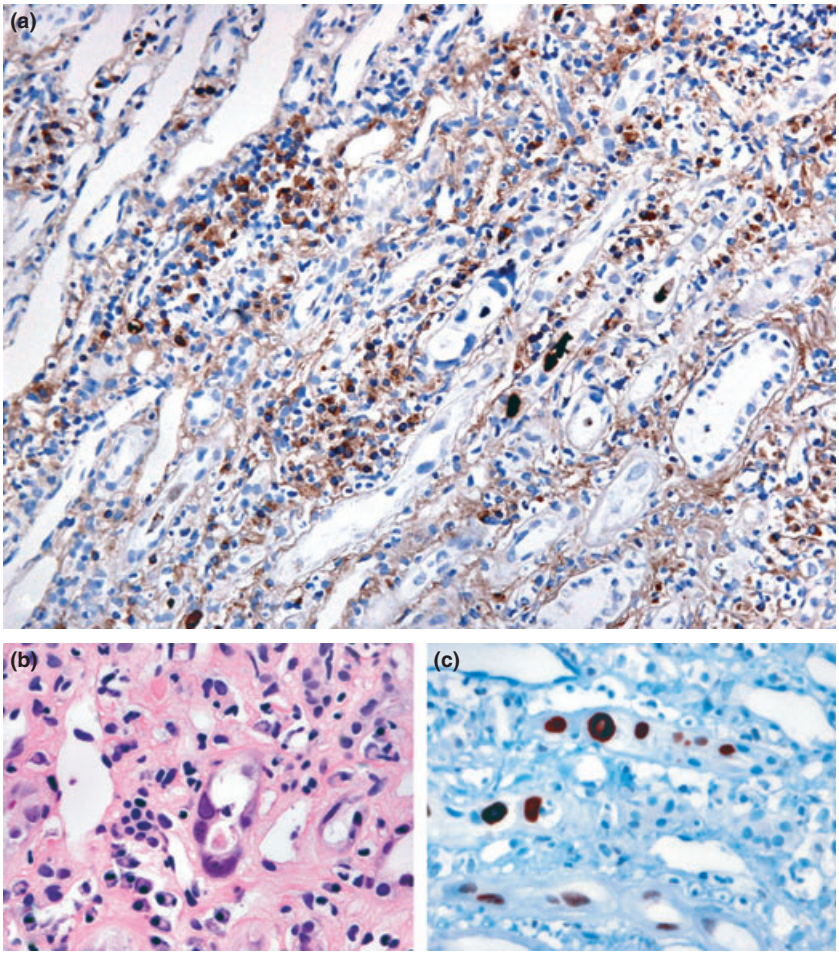

Figure 3 Plasma cell-rich polyomavirus nephropathy with the same field as that shown in Fig. 1(a) Most of the plasma cells produce IgM (anti-IgM) ×130. (b) Nuclear inclusion bodies, the hallmark of polyomavirus (PV) associated nephropathy (PVAN). H–E, ×250. (c) Immunohistochemical staining for SV 40 large T cell antigen confirms the diagnosis of PVAN. Immunohistochemistry, ×200.

tive. In every other case, no IgG deposits were seen in the tubular basement membranes.

Here, no correlation was found between PC predominance or PC type and C4d or HLA-DR positivity, or with age, gender, serum creatinine of the patients, or any other morphological finding including the PVAN stage. The time-interval correlation between transplantation and biopsy on the one hand, and the total number of cells and the total number of PCs on the other hand was statistically significant ($P < 0.05$), but no correlation with the duration of decoy cell shedding in the urine and other morphological parameters was found. The absolute numbers as well as the percentage values of PCs (in relation to all cells) correlated with the serum IgM-Ab activity (absolute number: $P < 0.034$, percentage: $P < 0.004$). The correlation between the absolute number or the percentage value of IgM positive PCs and the serum IgM-Ab activity lay in a similar range (absolute number: $P < 0.002$, percentage: $P < 0.01$). The percentage value of IgM positive PCs, however, correlated inversely with the serum IgG-Ab activity ($P < 0.007$). No correlation between the absolute number or the percentage of IgG positive PCs and the serum IgG-Ab activity was observed. Notably, there was a close correlation between the ratio of IgG/IgM positive PCs and the ratio of serum IgG/IgM-Ab activities ($P < 0.0001$) (Fig. 7). The BK virus load in plasma only displayed a correlation tendency with the total number of IgM PCs ($P < 0.08$). We found no correlation with the other variables used in our study.

Discussion

Abundant PCs have been reported in PVAN by ourselves and others [2,5,6,8,12,13,17]. The present study shows that PR cellular infiltrates are quite common in PVAN. Half of our cases of PVAN had more than 15% PCs (of all cells in the cellular infiltrate). We found that PC tubulitis is commonly observed in PVAN, which accords with our longstanding experience [6], but to date, it has not been properly highlighted in the literature. Meehan *et al.* [13] found abundant interstitial PCs in 75% of untreated PVAN cases compared to 21% in acute rejection cases. The infiltrating PCs were polyclonal [13], as in our study (data not shown). Tubular inflammation involved

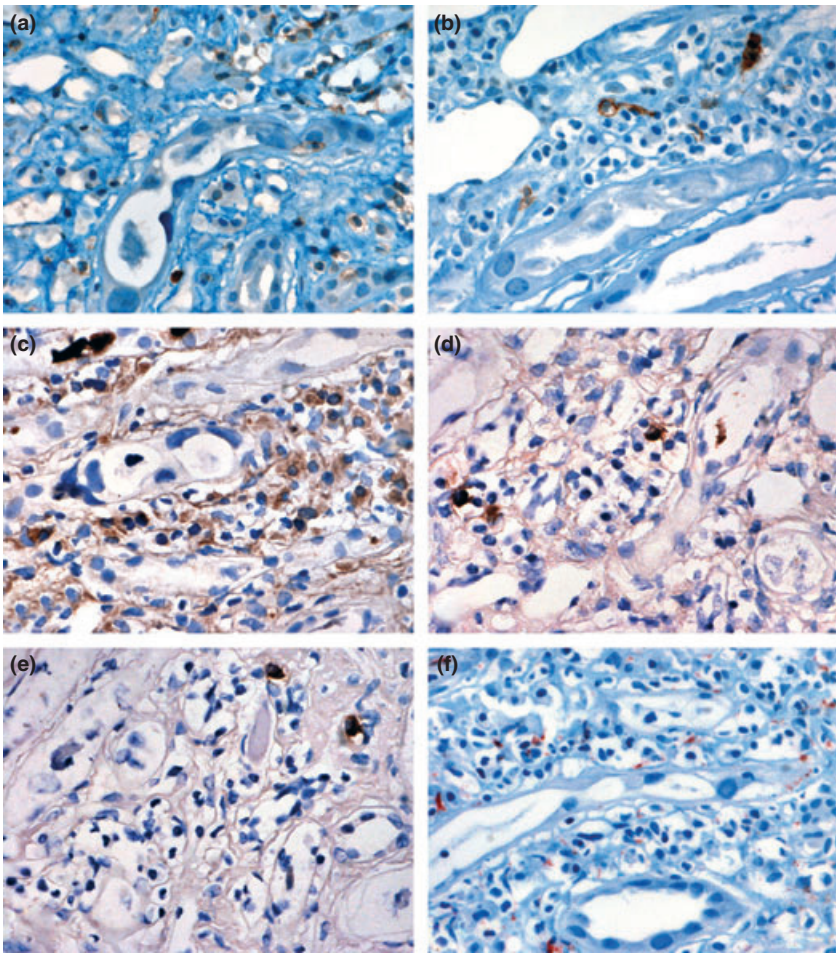

Figure 4 Plasma cell-rich polyomavirus nephropathy with the same field as that shown in Fig. 1 at high magnification. (a) CD3 positive T cells, (b) CD 20 positive B cells, (c) IgM positive plasma cells, (d) IgA positive plasma cells, (e) IgG positive cylinder, (f) CD 68 positive macrophages. Note that IgM positive plasma cells are predominant in the cellular infiltrate. Immunohistochemistry, ×200.

Table 2. Quantitative results of the cellular infiltrates and serum antibody activity.

Groups	PVAN All cases, $n = 20$	PVAN All cases (%)	Plasma cell rich cases (PR), $n = 10$	Plasma cell poor (PP) cases, $n = 10$	Mann–Whitney test
	P	P%	PR	PP	
Total number of cells*	1549 ± 873 (340–3665) 1362		1646 (340–3665) 1506	1452 (366–2928) 1314	ns
CD3 pc	822 ± 422 (165–1657) 666	56 (35–92) 55	738 (165–1538) 648	907 (336–1657) 768	PR% vs. PP% $P = 0.005$
CD 20 pc	331 ± 300 (26–1285) 261	19 (4–35) 20	316 (31–1285) 173	346 (26–820) 316	ns
CD 68 pc	116 ± 120 (2–534) 83	8 (0.5–24) 6	137 (12–534) 84	96 (2–211) 81	ns
Total number of plasma cells*	279 ± 266 (0–907) 177	17 (0–39) 15	455 (61–907) 502	103 (0–388) 63	PR vs. PP $P = 0.0025$
IgG pc	111 ± 154 (0–495) 50	37 (0–88) 33	188 (14–495) 85	34 (0–119) 18	PR vs. PP $P = 0.0126$
IgM pc	147 ± 158 (0–530) 116	54 (0–100) 54	246 (0–530) 232	48 (0–174) 13	PR vs. PP $P = 0.0091$
IgA pc	20 ± 23 (0–95) 13	9 (0–30) 7	20 (7–43) 16	21 (0–95) 12	ns
IgG/IgM pc	1.3 (0–9.8) 0.5	n.a.	0.9 (0.03–3.1) 0.6	1.7 (0–9.8) 0.5	ns
S-IgM (in units)	1.5 (0.04–2.8) 1.4	n.a.	2.1 (0.4–2.8) 2.2	0.8 (0.04–2.8) 0.7	PR vs. PP $P = 0.013$
S-IgG (in units)	2.1 (0.1–3.3) 2.3	n.a.	2.3 (0.3–3.2) 2.4	1.8 (0.1–3.3) 1.8	ns
S-IgM/IgG	4.2 (0.1–40.6) 1.5	n.a.	1.9 (0.1–8.6) 1.2	7.0 (0.3–40.6) 2.3	ns
Virus load BKVL/ml	130 968 (3156–13 116 320) 48 357	n.a.	951 148 (11 500–3 760 000) 93 030	1 783 244 (3156–13 116 320) 18 558	ns

*Number of cells (n/mm^2) mean ± SD (range), median/pc = number of positive cells (n/mm^2).

Figure 5 Graph showing the occurrence of plasma cells with mean number/mm^2 ± SD in polyomavirus (PV) associated nephropathy (PVAN).

Figure 6 IgG deposits in tubular basement membranes of tubules that have SV40 positive cells. Double immunofluorescent staining for SV40 and IgG, ×320.

infected tubular profiles with greater severity than those without any signs of infection [13], which we could confirm. A detailed study of the PC types present in the cellular infiltrate has not yet been performed.

Here, we showed that in the PR biopsies there were significantly more PCs and, among them, IgM producing PCs were commonly seen (70% of PR cases). In half of these PR cases (50%), IgM PCs predominated the cellular infiltrate and in 20%, a comparable number of IgM and IgG PCs were present. This intragraft finding was consistent with those obtained in the sera of the patients as we found a significant correlation between the absolute

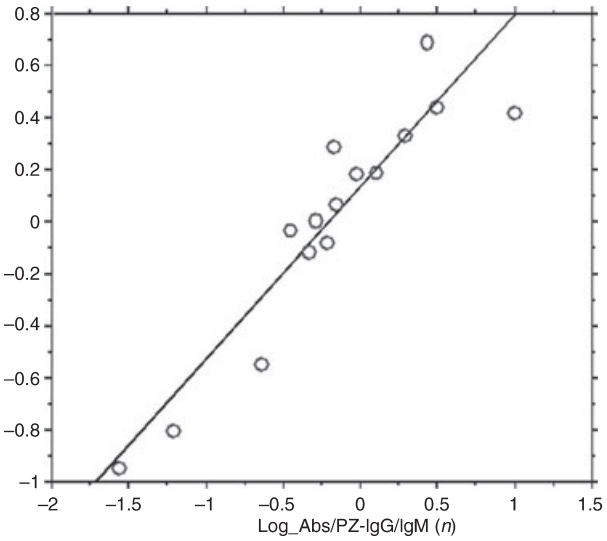

Figure 7 Correlation between the ratio of plasma cells (log absolute number of plasma cells IgG/IgM) and the serum antibody activities (log serum activity IgG/IgM).

number or the percentage value of IgM positive PCs and the serum IgM-Ab activity against PV. Furthermore, a strong correlation was found between the ratio of IgG/IgM PCs and serum-IgG/serum-IgM activities against PV. The correlation between the IgM positive PCs and the serum IgM-Ab activity supports the notion that the PC infiltrate in the kidney induced by the PV infection may be part of an antiviral host humoral immune response and may be the source of intragraft antibodies. It supports our hypothesis that serum antibodies may partly derive from local intragraft production. In addition, serum antibody levels also reflect the induced immunological response in lymphoid organs, taking into account the high systemic exposure to plasma BK virus loads.

Rhandawa *et al.* studied the immunoglobulin G, A and M responses to BK virus in renal transplants [33]. Among patients with paired serum samples, most of those with BK viruria or viremia displayed a substantial increase in IgG, IgA and IgM antibody levels, indicating that transplant recipients are capable of mounting anamnestic antibody responses to BK. The detection of IgA and IgM class antibodies or an increase in IgG antibody levels was strongly associated with active viral replication. We also found that patients with PVAN have significantly higher serum-IgG and serum-IgM activities than patients without PVAN (data not shown). Although the humoral immune response to PV infection is not well characterized, the evidence we have of specific antibody production in the sera of patients with PV infection suggests that humoral immunitity plays a role in the pathogenesis of PVAN.

Bracamonte *et al.* [18] showed that a significant proportion (16/30 cases) of patients with PVAN have tubular basement membrane immunodeposits (TBMID) on kidney biopsy. They described the presence of greater numbers of PCs in biopsies with TBMID compared with the other cases. These authors propose that the antibody response may target viral antigens shed from infected tubular epithelial cells and deposited along the TBM; alternatively, the deposits may be composed of antigen-antibody complexes. In our study, TBMID was rarely seen; just two of the 20 biopsies with PVAN displayed tubular IgG deposition. We should mention here that these two were cases with IgG predominance (one in PP and one in PR group), suggesting that the IgG PCs in these cases may also be associated with PVAN infection. It is also supported by the observation that none of these cases showed signs of acute rejection, and were PTC C4d negative. The finding that some of our PVAN cases had comparable numbers of IgM and IgG positive PCs, while some have an IgG predominance, may be attributable to the fact that the level of IgM is high in the primary immune response, but later, during the secondary immune response, IgG secretion greatly exceeds IgM production. Hence, cases with predominant IgM positive PCs may represent an earlier stage of the disease, which is supported by our serological anti-PV data and IgG predominance at a later stage.

Plasma cell-rich cellular infiltrates in the renal allograft may also occur in transplant rejection [23,24,27] with and without C4d in peritubulular capillaries [27]. In sharp contrast to PVAN, in rejection cases PCs are predominantly IgG and smaller IgA producers [23,24]. On the basis of these findings, we suggest that the presence of IgM PCs seen in PVAN biopsies may also be helpful in the differential diagnosis of PR rejection [23–29] and PVAN. The differentiation between rejection and PVAN is crucial. In the former, immunosuppression should be increased, but in the latter, the opposite strategy is recommended [6,8,9,15,17]. Taking these observations and our results on the PC types into account, we strongly believe that the presence of IgM PCs, especially the predominance of IgM producing PCs in a PR cellular infiltrate could be an indicator of PV infection. This is particularly relevant in cases with negative PTC C4d and a strong clinical suspicion of PVAN, but negative immunohistochemistry for SV40 large T antigen may possibly be attributable to sampling errors.

Authorship

ÉK did the morphometry, and wrote the paper, HHH did the antibody measurements, JE did the statistical analyses, UD did the immunohistochemistry, HH did the

immunohistology for tubular BM, and MJM designed the research plan.

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References

1. Purighalla R, Shapiro R, McCauley J, Randhawa P. BK virus infection in a kidney allograft diagnosed by needle biopsy. *Am J Kidney Dis* 1995; **4**: 671.
2. Mathur VS, Olson JL, Darragh TM, Yen TS. Polyomavirus-induced interstitial nephritis in two renal transplant recipients: case reports and review of the literature. *Am J Kidney Dis* 1997; **29**: 754.
3. Binet I, Nickeleit V, Hirsch HH, *et al.* Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. *Transplantation* 1999; **67**: 918.
4. Drachenberg CB, Beskow CO, Cangro CB, *et al.* Human polyoma virus in renal allograft biopsies: morphological findings and correlation with urine cytology. *Hum Pathol* 1999; **30**: 970.
5. Howell DN, Smith SR, Butterly DW, *et al.* Diagnosis and management of BK polyomavirus interstitial nephritis in renal transplant recipients. *Transplantation* 1999; **68**: 1279.
6. Nickeleit V, Hirsch HH, Binet IF, *et al.* Polyomavirus infection of renal allograft recipients: from latent infection to manifest disease. *J Am Soc Nephrol* 1999; **10**: 1080.
7. Randhawa PS, Finkelstein S, Scantlebury V, *et al.* Human polyoma virus-associated interstitial nephritis in the allograft kidney. *Transplantation* 1999; **67**: 103.
8. Nickeleit V, Hirsch HH, Zeiler M, *et al.* BK-virus nephropathy in renal transplants-tubular necrosis, MHC-class II expression and rejection in a puzzling game. *Nephrol Dial Transplant* 2000; **15**: 324.
9. Nickeleit V, Klimkait T, Binet IF, *et al.* Testing for polyomavirus type BK DNA in plasma to identify renal allograft recipients with viral nephropathy. *New Engl J Med* 2000; **342**: 1309.
10. Drachenberg RC, Drachenberg CB, Papadimitriou JC, *et al.* Morphological spectrum of polyoma virus disease in renal allografts: diagnostic accuracy of urine cytology. *Am J Transplant* 2001; **1**: 373.
11. Celik B, Shapiro R, Vats A, Randhawa PS. Polyomavirus allograft nephropathy: sequential assessment of histologic viral load, tubulitis, and graft function following changes in immunosuppression. *Am J Transplant* 2003; **3**: 1378.
12. Drachenberg CB, Papadimitriou JC, Hirsch HH, *et al.* Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load. *Am J Transplant* 2004; **4**:2082.
13. Meehan SM, Kadambi PV, Manaligod JR, Williams JW, Josephson MA, Javaid B. Polyoma virus infection of renal allografts: relationships of the distribution of viral infection, tubulointerstitial inflammation, and fibrosis suggesting viral interstitial nephritis in untreated disease. *Hum Pathol* 2005; **36**: 1256.
14. Drachenberg CB, Hirsch HH, Ramos E, Papadimitriou JC. Polyomavirus disease in renal transplantation. Review of pathological findings and diagnostic methods. *Hum Pathol* 2005; **36**: 1245.
15. Hirsch HH, Brennan DC, Drachenberg CB, *et al.* Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation* 2005; **79**: 1277.
16. Liptak P, Kemeny E, Ivanyi B. Histopathology of polyomavirus-associated nephropathy in renal allografts. *Nat Clin Pract Nephrol* 2006; **2**: 631.
17. Nickeleit V, Singh HK, Mihatsch MJ. Latent and productive polyomavirus infections of renal allografts: morphological, clinical, and pathophysiological aspects. *Adv Exp Med Biol* 2006; **577**: 190.
18. Bracamonte E, Leca N, Smith KD, *et al.* Tubular basement membrane immune deposits in association with BK polyomavirus nephropathy. *Amer J Transplant* 2007; **7**: 1552.
19. Heritage J, Chesters PM, McCane DJ. The persistence of papovavirus BK DNA sequences in normal human renal tissue. *J Med Virol* 1981; **8**: 143.
20. Chesters PM, Heritage J, McCane DJ. Persistence of DNA sequences of BK and JC virus in normal human tissues and in diseased tissues. *J Infect Dis* 1983; **147**: 676.
21. Kahan AV, Coleman DV, Koss LG. Activation of human polyomavirus infection-detection by cytologic technics. *Am J Clin Pathol* 1980; **74**: 326.
22. Markowitz RB, Thompson HC, Mueller JF, Cohen JA, Dynan WS. Incidence of BK virus and JC virus viruria in human immunodeficiency virus-infected and -uninfected subjects. *J Infect Dis* 1993; **167**: 13.
23. Nadasdy T, Krenacs T, Kalmar KN, Csajbok E, Boda K, Ormos J. Importance of plasma cells in the infiltrate of renal allografts. An immunohistochemical study. *Pathol Res Pract* 1991; **187**: 178.
24. Charney DA, Nadasdy T, Lo AW, Racusen LC. Plasma cell-rich acute renal allograft rejection. *Transplantation* 1999; **68**: 791.
25. Meehan SM, Domer P, Josephson M, *et al.* The clinical and pathologic implications of plasmacytic infiltrates in percutaneous renal allograft biopsies. *Hum Pathol* 2001; **32**: 205.
26. Desvaux D, Le Gouvello S, Pastural M, *et al.* Acute renal allograft rejections with major interstitial oedema and plasma cell-rich infiltrates: high γ-interferon expression and poor clinical outcome. *Nephrol Dial Transplant* 2004; **19**: 933.

27. Poduval RD, Kadambi PV, Josephson MA, *et al.* Implications of immunohistochemical detection of C4d along peritubular capillaries in late acute renal allograft rejection. *Transplantation* 2005; **79**: 228.
28. Adrogue HE, Soltero L, Land GA, Ramanathan V, Truong LD, Suki WN. Immunoglobulin therapy for plasma cell-rich rejection in the renal allograft. *Transplantation* 2006; **82**: 567.
29. Gartner V, Eigentler TK, Viebahn R. Plasma cell-rich rejection processes in renal transplantation: morphology and prognostic relevance. *Transplantation* 2006; **81**: 986.
30. Racusen LC, Solez K, Colvin RB, *et al.* The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; **55**: 713.
31. Ginevri F, Azzi A, Hirsch HH, *et al.* Prospective monitoring of polyomavirus BK replication and impact of pre-emptive intervention in pediatric kidney recipients. *Am J Transplant* 2007; **7**: 2727.
32. Hirsch HH, Mohaupt M, Klimkait T. Prospective monitoring of BK virus load after discontinuing sirolimus treatment in renal transplant patient with BK virus nephropathy. *J Infect Dis* 2001; **184**: 1494.
33. Randhawa PS, Gupta G, Vats A, Shapiro R, Viscidi RP. Immunoglobulin G, A, and M responses to BK virus in renal transplantation. *Clin Vaccine Immunol* 2006; **13**: 1057.