Transpl Int (1995) 8: 169–173

ORIGINAL ARTICLE

Kenji Hoshino
Takayuki Nishi
Hironobu Adachi
Hisao Ito
Yasuhiko Fukuda
Kiyohiko Dohi
Takeshi Kurata

Human herpesvirus-6 infection in renal allografts: retrospective immunohistochemical study in Japanese recipients

Received: 25 May 1994
Received after revision: 5 October 1994
Accepted: 13 October 1994

K. Hoshino · T. Nishi ·
H. Adachi · H. Ito (✉)
First Department of Pathology,
Tottori University,
Faculty of Medicine,
86 Nishi-machi, Yonago/Tottori 683,
Japan

Y. Fukuda · K. Dohi
Second Department of Surgery,
Hiroshima University,
School of Medicine,
1-2-3 Kasumi, Minami-ku, Hiroshima 734,
Japan

T. Kurata
Department of Pathology,
National Institute of Health,
1-23-1 Koyama, Shinjuku, Tokyo 162,
Japan

Abstract This study was conducted to determine the incidence and clinical significance of human herpesvirus-6 (HHV-6) infection in renal allografts. A total of 105 biopsy specimens from 72 recipients were immunohistochemically examined for the presence of HHV-6 antigen, which localized in the distal tubular epithelial cells and in a few lymphocytes infiltrating into the interstitium. HHV-6 antigen in the tubular epithelia was detected in 63 (61.2 %) specimens. Categorically, a higher incidence of the antigen was noted in specimens of accelerated rejection (3/4, 75.0 %), acute rejection (28/3, 73.7 %), and cyclosporin nephropathy (8/11, 72.7 %). The antigen was present and absent an almost equal number of times in the categories of chronic rejection, intraoperative and routine protocol biopsies. Repeated biopsies were performed in six cases showing HHV-6 antigen, only one of which underwent transplant nephrectomy due to severe chronic rejection. Single or multinucleated giant cells in distal tubuli occurred in 10 (9.5 %) specimens in a scattered manner. All of them were diagnosed as acute or chronic rejection. The giant cells showed no immunoreactivity for HHV-6, cytomegalovirus, or herpes simplex virus. These results indicate overall that HHV-6 infection is common in renal allografts and might be reactivated in acute rejection or cyclosporin nephropathy. The presence of HHV-6 antigen, however, does not necessarily correlate with a poor prognosis for the renal graft nor with the occurrence of giant cells in distal tubuli.

Key words HHV-6 infection, renal allografts, immunohistochemistry

Introduction

Human herpesvirus-6 (HHV-6), initially designated as "human B-lymphotropic virus", was isolated from peripheral blood mononuclear cells of patients with acquired immunodeficiency syndrome (AIDS) and lymphoproliferative disorders [9, 21]. HHV-6 belongs to the human herpesvirus family and is approximately 160–200 nm in diameter. It has an enveloped virion containing an icosahedral nucleocapsid with 162 capsomeres and a large, central, double-stranded DNA genome [4, 9]. Tropism of HHV-6 has been shown not only for T lymphocytes, but also for B lymphocytes, monocytes/macrophages, megakaryocytes, glioblastoma cells, and fibroblasts [1, 11, 13, 14, 23]. Seroconversion occurs in almost all infected infants aged 5 months to 2 years, and the virus remains a latent source of infection in a substantial proportion of adults [12, 26]. HHV-6 has been found to be a causal agent of exanthem subitum [28] as well as of subacute necrotizing lymphadenitis [7, 12]. Reactivation of, or reinfection by, HHV-6 may occur in an immunosuppressive state as other herpesvirus do [12] and it may also accelerate the progression of AIDS [15].

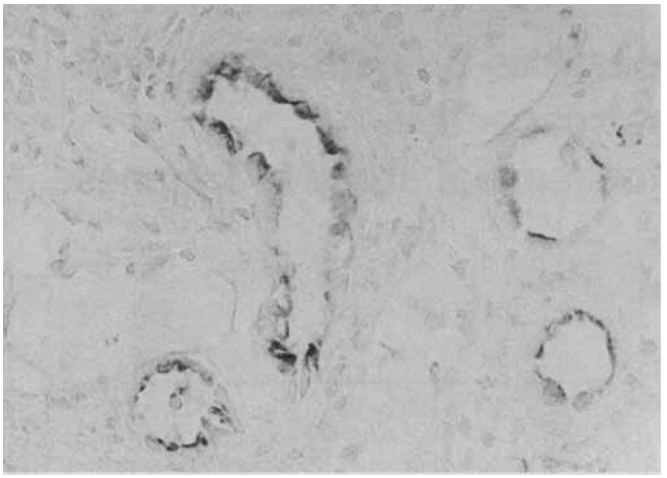

Fig. 1 Immunoreactivity for human herpesvirus-6 in distal tubular epithelia of transplanted kidney (× 270)

Recently, HHV-6 infection has been detected in kidney [2, 10, 12, 16–18, 27, 29], liver [25], and bone marrow [3, 5, 6] transplant recipients. It is well known that herpesvirus infections such as human cytomegalovirus (CMV), Epstein-Barr virus (EBV), and herpes simplex virus (HSV) cause morbidity and mortality in transplant patients. Yet, little information is available on HHV-6 infection, which has been diagnosed exclusively by serological tests. Only two reports have described immunohistochemical findings in a small number of cases [12, 18]. In addition, we have noticed the presence of single or multinucleated giant cells in distal tubuli in biopsy specimens of renal allografts. This is unique, having never been previously described, and its pathological significance remains unexplained.

Given this background, we immunohistochemically examined HHV-6 infection in human allotransplanted kidneys. The results obtained were compared with histopathological findings and the clinical course of the patients to clarify the significance of HHV-6 infection in renal transplantation.

Materials and methods

A total of 105 needle biopsy specimens from 72 renal transplant recipients (53 males and 19 females) were selected from the files of our department. All of the recipients underwent renal transplantation in the Second Department of Surgery, Hiroshima University School of Medicine, and in its related hospitals until April 1993. The biopsies were taken between September 1992 and August 1993. Antibodies for HHV-6 were not tested serologically. All of the patients were treated with basic immunosuppressive therapy (cyclosporin and prednisone), and when acute rejection occurred, they were given methyprednisolone as ordinary rescue therapy.

Needle biopsies were taken at any time from 1 h to 10 years post-transplantation when renal function deteriorated or as routine protocol. In order to clarify the relationship between HHV-6 infection and histological findings, we excluded specimens diagnosed with acute or chronic rejection and with combined cyclosporin toxicity and acute or chronic rejection. The specimens were all fixed in 10 % formalin and embedded in paraffin wax. The histological diagnosis was made by one of the authors (H. I.).

For the detection of HHV-6 protein, the avidin-biotin-peroxidase complex (ABC) method of Hsu et al. [8] was used. Briefly, the sections were first deparaffinized and incubated with 0.025 % trypsin for 120 min at 37 °C. Incubation with anti-HHV-6 antibody or rinsing in PBS at each step was performed for at least 30 min at room temperature. Endogenous peroxidase activity was inactivated by immersing the specimens in 0.03 % hydrogen peroxide in absolute methanol for 20 min. The sections were counterstained with 3 % methyl green. Biotinylated anti-mouse IgG and avidin-biotinylated horse radish peroxidase complex (ABC) were purchased from Vector Laboratories (Calif., USA).

Anti-HHV-6 mouse monoclonal antibodies (OHV1 and OHV3) were kindly supplied by Dr. Koichi Yamanishi (Department of Virology, Institute of Microbial Diseases, Osaka University, Osaka), and lyophilized preparation was diluted 1 : 100. The preparation and characterization of the antibodies have been described previously [19, 20]. Briefly, OHV1 recognizes four glycosylated proteins with 106, 102, 65 and 63 kDa and OHV3 two glycosylated proteins with 98 and 92 kDa. The antibody has been shown not to crossreact with HSV, CMV, or other types of viruses. In cases of giant cells in tubuli, the serial sections were also immunostained with the antibody for CMV (monoclonal; Chemical International, USA) and HSV (polyclonal; DAKO Immunoglobulins, Copenhagen, Denmark).

The specificity of immunostaining was examined as follows; normal mouse IgG was used as the first layer and 3,3′ diaminobenzidine-tetrahydrochlorides or H_2O_2 was omitted from the incubation medium. The control slides were invariably negative for immunostaining. As positive controls, we simultaneously immunostained specimens of previously confirmed subacute necrotizing lymphadenitis caused by HHV-6 infection.

The data obtained were subjected to statistical analysis using the chi-square test with 2 × 2 contingency tables.

Results

HHV-6 antigen was found in variable numbers in the cytoplasm and cell membranes of the distal tubular epithelial cells (Fig. 1) and in a few lymphocytes infiltrating into the interstitium of transplanted kidneys. No immunoreactivity was detected in the glomeruli or vascular endothelia.

Table 1 shows the pathological diagnoses and the incidence of HHV-6 antigen in the 105 biopsy specimens examined. These include accelerated rejection ($n = 4$), acute rejection ($n = 38$), chronic rejection ($n = 24$), cyclosporin nephropathy ($n = 11$), and tubular degeneration of intraoperative 1-h biopsy ($n = 6$). A routine protocol biopsy consisting of 22 specimens showed near-normal findings or mild tubular degeneration. HHV-6 antigen was detected in the distal tubular epithelia in 64 (61.0 %) out of 105 specimens. A higher incidence of the antigen – over 70 % – was noted in the categories of accelerated rejection (3/4, 75.0 %), acute rejection (28/38, 73.7 %), and cyclosporin nephropathy (8/11,

Table 1 Correlation between pathological findings and the presence of HHV-6 antigen and giant cells in tubuli of renal allografts

Pathological findings	Number of specimens	HHV-6-antigen [Number of specimens with giant cells]	
		Positive	Negative
Accelerated rejection	4	3 (75.0 %)	1 (25.0 %)
Acute rejection	38	28 (73.7 %) [6]	10 (26.3 %)
Chronic rejection	24	11 (45.8 %) [3]	13 (54.2 %) [1]
Cyclosporin nephropathy	11	8 (72.7 %)	3 (27.3 %)
Intraoperative biopsy	6	3 (50.0 %)	3 (50.0 %)
Protocol biopsy	22	11 (50.0 %)	11 (50.0 %)
Total	105	64 (61.0 %) [9]	41 (39.0 %) [1]

Table 2 Clinical courses of six cases showing HHV-6 antigen (*AR* acute rejection, *CR* chronic rejection, *CyA* cyclosporin nephropathy, *IH* intraoperative one-hour biopsy, *IW* one week, □ HHV-6-positive, ● HHV-6-negative)

Case	Age	Sex	Clinical course
1	38	F	5W: ● AR, ● AR, □ AR
2	35	M	1H: □ AR, 7W: □ CR, 15W: ● CR
3	47	F	4W: □ AR, 7W: ● AR, 13W: ●
4	46	M	3W: □ CyA, 7W: ● AR, 13W: □ AR
5	27	M	242W: □ AR or CR, 260W: □ CR, 273W: □ CR
6	47	F	1W: □ AR, 2W: □ AR, 3W: □ AR

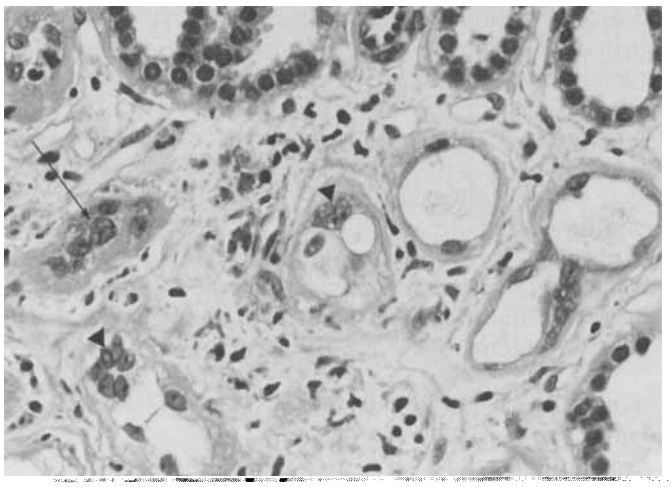

Fig. 2 Single *(arrow)* and multinucleated *(arrow heads)* giant cells occurring in distal tubuli of transplanted kidney (H & E, × 270)

72.7 %). The presence or absence of the antigen was almost equal in the categories of chronic rejection, 1-h biopsy, and routine protocol biopsy. The incidence of HHV-6 antigen was significantly higher ($P < 0.05$) in acute rejection than in chronic rejection.

A needle biopsy was taken three times or more in six cases, the clinical course and histological findings of which are summarized in Table 2. The appearance of HHV-6 antigen was variable; in case no. 1 it was first negative and then positive, in cases 2 and 3 it was first positive and then negative, and in cases 5 and 6 it remained unchanged. By April 1993, renal function was sufficient in all cases except for one, case no. 5, who underwent transplant nephrectomy due to severe chronic rejection 7 years after transplantation.

Next, we examined the correlation between single or multinucleated giant cells in distal tubuli (Fig. 2) and the HHV-6 antigen. A few giant cells were scarcely distributed; they were found in only 10 (9.5 %) out of 105 specimens. These ten cases were diagnosed as either acute ($n = 6$) or chronic ($n = 4$) rejection (Table 1). No giant cells were detected in the category of cyclosporin nephropathy, 1-hour biopsy, or protocol biopsy. The giant cells showed no intranuclear (owl's eye) or cytoplasmic inclusion bodies with chromatin homogeneity or "ground glass" appearance, which microscopically indicate CMV or HSV infection. Serial sections revealed no immunoreactivity for HHV-6, CMV, or HSV antigen in the giant cells.

Discussion

Although serological studies are of limited diagnostic value in differentiating between latent and active viral infection, several groups investigating HHV-6 infection in renal allograft recipients have examined serum antibody titer. Morris et al. [17] followed HHV-6 antibody in 17 patients and classified them into three categories as follows; (1) primary HHV-6 infection after transplantation in four (23.5 %) patients, (2) reactivation with increased antibody titer in ten (58.8 %) patients, and (3) no serological evidence of infection in three patients.

Merlino et al. [16] found seroconversion corresponding to category 1 of Morris et al. in 10 (18.9 %) of 53 recipients after renal transplantation. Out of 40 seropositive patients before transplantation, 25 (47.2 %) had a significant increase in antibody titer. Okuno et al. [18] examined 21 patients, all of whom had detectable antibody to HHV-6 before transplantation. An increased serum titer was found in 8 (38.1 %) recipients suffering a severe rejection reaction. Yoshikawa et al. [29] found HHV-6 viremia occurring in 9 (14 %) of 65 recipients around 2–4 weeks post-transplantation. An additional 27 recipients showed a significant rise in antibody titer. These results indicate that HHV-6 infection is closely related to the renal transplant, the virus being reactivated in many cases in the early post-transplant period. However, the origin of the virus – whether transmitted by the donor itself, the recipient, or another person – could not be determined.

Novel immunological and molecular techniques allow direct detection of the virus in vivo. Kikuta et al. [10] demonstrated HHV-6 DNA using a PCR technique in peripheral blood mononuclear cells from eight of nine kidney transplant patients in contrast to none of five healthy individuals. Our immunohistochemical study revealed a relatively high incidence of the HHV-6 antigen, even in intraoperative biopsies, in which reactivation of the virus may be unlikely. Okuna et al. [18] also isolated HHV-6 from three renal tissue specimens obtained during transplant surgery, but not from their blood at that time. Considering these findings together, one may conclude that HHV-6 probably remains latent in the kidney after primary infection and may be reactivated by immunosuppression after renal transplantation.

Immunohistochemical staining with a specific antibody provides useful and reliable information about the virus expression in vivo. Yet, surprisingly, only two reports have briefly reported on immunohistochemical findings of HHV-6 antigen in renal allografts. Okuno et al. [18] detected HHV-6 antigen in the epithelial cells of the medullary tubuli and in lymphocytes infiltrating into renal interstitium in five (55.6 %) of nine transplanted kidneys, removed due to severe rejection. Kurata et al. [12] examined 19 biopsy specimens showing acute and/or chronic rejection in which HHV-6 antigen was detected in 11 (57.9 %) cases; both the incidence and localization were similar to our findings. Moreover, the immunohistochemical incidence of the HHV-6 antigen coincided relatively well with that of increased serum antibody titer, as described above.

The clinical significance of HHV-6 infection has not, however, been sufficiently elucidated. The unique biological properties of HHV-6 – in particular, its immunotropic nature and its positive interaction with human immunodeficiency virus (HIV) [15] – suggest that it may have detrimental effects on the immune system and negatively influence the clinical course of renal grafts. Okuno et al. [18] reported a close association between HHV-6 activation and acute rejection. In contrast, despite the higher frequency of rejection in patients with an active HHV-6 infection, no significant correlation could be demonstrated by either Merlino et al. [16] or Yoshikawa et al. [29]. In the present study, the incidence of HHV-6 antigen was significantly higher in acute rejection than in chronic rejection, suggesting a close association with the former. We failed to demonstrate any apparent correlation between HHV-6 infection and graft survival because of the small number of cases resected. HHV-6 antigen was also detected at a high incidence in the intraoperative and protocol biopsies, but the graft survival rate has been excellent in our cases: over 90 % in the last 3 years (data not presented). Thus, HHV-6 antigens might not necessarily be detrimental to the graft.

Of particular interest is the higher incidence of the HHV-6 antigen in cyclosporin nephropathy, suggesting that excessive immunosuppresion might reactivate the virus. However, the relationship between cyclosporin nephropathy and HHV-6 infections remains to be elucidated.

We found single or multinucleated giant cells in distal tubuli that showed no immunoreactivity for HHV-6, CMV, or HSV. The presence of giant cells is a unique finding that has, as far as our hunt of the literature showed [22, 24, 30], heretofore not been described. Their histogenesis and clinical significance could not be clarified in this study. These cells were, however, exclusively detected in the specimens showing acute or chronic rejection and not in those with cyclosporin nephropathy or intraoperative or protocol biopsies. These results point to a rejection-related nature of the giant cells.

In summary, we have immunohistochemically demonstrated the relatively high incidence of the HHV-6 antigen in renal allografts. HHV-6 may be reactivated in conditions of acute rejection and cyclosporin nephropathy. Further prospective studies should be conducted employing molecular and immunological techniques to clarify the immunopathogenic role of HHV-6 in renal transplant recipients.

Acknowledgement This work was support in part by the Ministry of Health and Welfare, Japan.

References

1. Ablashi DV, Salahuddin SZ, Josephs SF, Imam F, Lusso P, Gallo RC (1987) HBLV (or HHV-6) in human cell lines. Nature 329: 207
2. Asano Y, Yoshikawa T, Suga S, Yazaki T, Hirabayashi S, Ono Y, Tsuzuki K, Oshima S (1989) Human herpesvirus 6 harbouring in kidney (letter). Lancet II: 1391
3. Asano Y, Yoshikawa T, Suga S, Nakashima T, Yazaki T, Fukuda M, Kojima S, Matsuyama T (1991) Reactivation of herpesvirus type 6 in children receiving bone marrow transplantation for leukemia. N Engl J Med 324: 634–635
4. Biberfeld P, Kramarsky B, Salahuddin SZ, Gallo RC (1987) Ultrastructural characterization of a new human B lymphotropic DNA virus (human herpesvirus 6) isolated from patients with lymphoproliferative disease. J Natl Cancer Inst 79: 933–941
5. Carrigan DR, Drobyski WR, Russler SK, Tapper MA, Knox KK, Ash RC (1991) Interstitial pneumonitis associated with human herpesvirus-6 infection after marrow transplantation. Lancet 338: 147–149
6. Cone RW, Hackman RC, Huang MW, Bowden RA, Meyers JD, Metcalf M, Zeh J, Ashley R, Corey L (1993) Human herpesvirus 6 in lung tissue from patients with pneumonitis after bone marrow transplantation. N Engl J Med 329: 156–161
7. Eizuru Y, Minematsu T, Minamishima Y, Kikuchi M, Yamanishi K, Takahashi M, Kurata T (1989) Human herpesvirus 6 in lymph nodes. Lancet I: 40–41
8. Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29: 577–580
9. Josephs SF, Salahuddin SZ, Ablashi DV, Schachter F, Wong-Staal F, Gallo RC (1986) Genomic analysis of the human B-lymphotropic virus (HBLV). Science 234: 601–603
10. Kikuta H, Itami N, Matsumoto S, Chikaraishi T, Togashi M (1991) Frequent detection of human herpesvirus 6 DNA in peripheral blood mononuclear cells from kidney transplant patients. J Infect Dis 163: 925
11. Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K (1991) Latent human herpesvirus 6 infection of human monocytes/macrophages. J Gen Virol 72: 1401–1408
12. Kurata T, Iwasaki T, Sata T, Wakabayashi T, Yamaguchi K, Okuno T, Yamanishi K, Takei Y (1990) Viral pathology of human herpesvirus 6 infection. In: Lopez C, Mori R, Roizman B, Whitly RW (eds) Immunobiology and prophylaxis of human herpesvirus infections. Advances in experimental medicine and biology. Plenum Press, New York, pp 39–47
13. Luka J, Okano M, Thiele G (1990) Isolation of human herpesvirus-6 from clinical specimens using human fibroblast cultures. J Clin Lab Anal 4: 483–486
14. Lusso P, Markham PD, Tschachler E, di Marzo Veronese F, Salahuddin SZ, Ablashi DV, Pahwa S, Krohn K, Gallo RC (1988) In vitro cellular tropism of human B-lymphotropic virus (human herpesvirus-6). J Exp Med 167: 1659–1670
15. Lusso P, Gallo RC (1994) Human herpesvirus 6 in AIDS. Lancet 343: 555–556
16. Merlino C, Giacchino F, Segoloni GP, Ponzi AN (1992) Human herpesvirus-6 infection and renal transplantation. Transplantation 53: 1382–1383
17. Morris DJ, Littler E, Arrand JR, Jordan D (1989) Human herpesvirus 6 infection in renal transplant recipients. N Engl J Med 320: 1560–1561
18. Okuno T, Higashi K, Shiraki K, Yamanishi K, Takahashi M, Kokado Y, Ishibashi M, Takahara S, Sonoda T, Tanaka K, Baba K, Yabuuchi H, Kurata T (1990) Human herpesvirus 6 infection in renal transplantation. Transplantation 49: 519–522
19. Okuno T, Sao H, Asada H, Shiraki K, Takahashi M, Yamanishi K (1990) Analysis of a glycoprotein of human herpesvirus 6 (HHV-6) using monoclonal antibodies. Virology 176: 625–628
20. Okuno T, Shao H, Asada H, Shiraki K, Takahashi M, Yamanishi K (19929 Analysis of human herpesvirus 6 glycoproteins recognized by monoclonal antibody OHV1. J Gen Virol 73: 443–447
21. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B, Gallo RC (1986) Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science 234: 596–601
22. Sanfilippo F (1990) Renal transplantation. In: Sale GE (ed) The pathology of organ transplantation. Butterworths, Boston London Singapore, pp 51–101
23. Takahashi K, Sonoda S, Higashi K, Kondo T, Takahashi H, Takahashi M, Yamanishi K (1989) Predominant CD4 T-lymphocytes tropism of human herpesvirus 6-related virus. J Virol 63: 3161–3163
24. Wakabayashi T (1993) The pathology of the transplanted kidney (in Japanese). Pathology and Clinical Medicine 11: 428–433
25. Ward KN, Gray JJ, Efstathiou S (1989) Primary human herpesvirus 6 infection in a patient following liver transplantation from a seronegative donor. J Med Virol 28: 69–72
26. Ward KN, Gray JJ, Fotheringham MW, Sheldon MJ (1993) IgG antibodies to human herpesvirus-6 in young children: changes in avidity of antibody correlate with time after infection. J Med Virol 39: 131–138
27. Wrzos H, Gibbons J, Abt PL, Gifford RRM, Yang HC (1990) Human herpesvirus 6 in monocytes of transplant patients. Lancet 335: 486–487
28. Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, Kurata T (1988) Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet I: 1065–1067
29. Yoshikawa T, Suga S, Asano Y, Nakashima T, Yazaki T, Ono Y, Fujita T, Tsuzuki K, Sugiyama S, Oshima S (1992) A prospective study of human herpesvirus-6 infection in renal transplantation. Transplantation 54: 879–883
30. Zollinger HU, Mihatsch MJ (1978) Renal pathology in biopsy. Springer, Berlin Heidelberg New York