

# Non-organ-specific autoantibodies in renal transplant recipients: relation to BK virus infection

Cristina Costa<sup>1</sup>, Giovanni Antonio Touscoz<sup>2</sup>, Massimiliano Bergallo<sup>1</sup>, Francesca Sidoti<sup>1</sup>, Maria Elena Terlizzi<sup>1</sup>, Sara Astegiano<sup>1</sup>, Chiara Merlino<sup>1</sup>, Giuseppe P. Segoloni<sup>3</sup>, Rossana Cavallo<sup>1</sup>

<sup>1</sup>Department of Public Health and Microbiology, Virology Unit, University of Turin, Italy;

<sup>2</sup>Gastro-Hepatology Unity, Laboratory of Digestive and Hepatic Physiopathology;

<sup>3</sup>Department of Internal Medicine, Renal Transplant Unit, Molinette Hospital, Turin, Italy

## SUMMARY

Polyomavirus BK reactivation is common in renal transplant recipients and may cause nephropathy with significant graft dysfunction. The induction of anti-double stranded DNA (anti-dsDNA) antibodies by BKV has been described in experimental animals and during primary infection, and has been implicated in the pathogenesis of systemic lupus erythematosus. This study evaluated the occurrence of anti-dsDNA antibodies and non-organ-specific autoantibodies (NOSA) by indirect immunofluorescence before transplantation and at 3 and 6 months post-transplantation in 90 renal transplant recipients and the association with BKV reactivation, demographic and clinical features. Moreover, the relation to HCMV infection, as detected by pp65-antigenemia, was also evaluated. Post-transplantation NOSAs were present in 23/90 (25.6%) and anti-dsDNA antibodies in 17/90 (18.9%). BK viremia was detected in at least one serum sample in 22 patients: 9 anti-dsDNA antibody-positive vs 13 negative ( $p < 0.01$ ). No significant correlation between the occurrence of NOSAs and anti-dsDNA antibodies and demographic and clinical features was found. No significant association with pp65-antigenemia-positivity was found, although antigenemia was positive in 6/23 NOSA-positive patients (26.1%). Although a relation seems to exist between BKV and the occurrence of anti-dsDNA antibodies in renal transplant patients, the lack of correlation with other epidemiological and clinical features does not allow any conclusion. The role of autoimmune response in this context and the relation with other patient-related factors and infectious agents should be further investigated.

**KEY WORDS:** Anti-dsDNA antibodies, BK virus, Non-organ-specific antibodies, Renal transplantation, Systemic lupus erythematosus

Received November 13, 2007

Accepted February 19, 2008

## INTRODUCTION

Human polyomavirus BK (BKV) belongs to the *Polyomaviridae* family and was first isolated in 1971 (Gardner *et al.*, 1971) from the urine of a renal transplant recipient. Approximately 80% of

the adult population worldwide are seropositive for BKV. Following primary infection that is characterized by low or no morbidity, BKV establishes latency prevalently within the renourinary tract. Reactivation may occur in both healthy subjects and immunocompromised patients, such as renal transplant recipients (10-68%) (Hirsch and Steiger, 2003). Reactivation may determine asymptomatic viremia, hemorrhagic cystitis (particularly in bone marrow transplant recipients), and BKV-associated nephropathy (BKVAN) in kidney graft recipients (Hirsch *et al.*, 2005, Nickeleit *et al.*, 2003).

BKV genome, a double-stranded circular DNA,

### Corresponding author

Dr. Cristina Costa

Dept. of Public Health and Microbiology

Virology Unit

University of Turin,

Via Santena, 9 - 10126 Turin (Italy)

E-mail: cristina.costa@unito.it

can be divided into three functional regions (early, late and non-coding control region). Large T-antigen, a regulatory protein encoded by the early region, binds both viral and host cell dsDNA. Capsid proteins VP1, VP2, and VP3, whose genetic information is encompassed by the late region, also have the potential to bind DNA. As the ability of DNA alone to induce antibody production is poor, the interaction of DNA with a non-self protein seems necessary to immunogenicity. Based on these observations a "hapten-carrier" mechanism for virus-induced autoimmunity has been proposed by which the self (chromatin)-non-self (viral protein) complex evokes a humoral T cell-dependent immune response to chromatin with production of anti-dsDNA and anti-histone antibodies (Van Ghelue *et al.*, 2003). This has been described in experimental animals (Flaegstad *et al.*, 1988, Rekvig *et al.*, 1997) and during primary infection in children (Fredriksen *et al.*, 1993). Moreover, a role for BKV in the pathogenesis of systemic lupus erythematosus (SLE) has been suggested. SLE is a systemic autoimmune disease of unknown aetiology that is serologically characterized by the presence of a wide array of autoantibodies, particularly directed against nuclear constituents, including antibodies against the nucleosome. Currently, the disease activity of SLE is associated with the presence of anti-dsDNA antibodies, which therefore are used as a diagnostic marker of SLE (Tan *et al.*, 1982b; Carson, 1991; Reeves *et al.*, 1994).

Aim of this study was to investigate the occurrence of anti-dsDNA antibodies and non-organ-specific autoantibodies (NOSAs) in relation to BKV reactivation and to demographic characteristics, therapeutic protocols and human cytomegalovirus (HCMV) infection.

## MATERIALS AND METHODS

### *Study population*

All 90 patients (62 M/ 28 F; mean age  $\pm$  SD, 54.72  $\pm$  13.51 years; range, 21-79) who underwent renal transplantation at the Renal Transplant Unit, Molinette Hospital of Turin, over a one year period were retrospectively studied. To evaluate a homogeneous group, patients with histologically confirmed BKVAN were excluded. The underlying diseases that led to transplantation were as

follows: chronic renal failure due to glomerulonephritis in 19 patients, polycystic nephropathy in 7, vesicoureteral reflux or excretory tract congenital malformations in 6, interstitial nephropathy in 4, vascular nephropathy in 4, haemolytic uremic syndrome in 3, nephroangiosclerosis in 3, chronic pyelonephritis in 3, Alport's syndrome in 2, nephrolithiasis in 2, toxic nephropathy in 1, and unknown in the remaining patients. Immunosuppressive therapy was as follows: tacrolimus (FK506), steroid (S), and mycophenolate mofetil (MMF) in 23 patients; FK506 and S in 22 patients; cyclosporine A (CyA) and MMF in two patients; CyA, S, and MMF in 11 patients; CyA and S in nine patients; S and MMF 12 patients; S in three patient; sirolimus and S in three patients; sirolimus, S, and MMF in five patients. A renal biopsy was undertaken in all 23 patients with a clinical or laboratory suspicion of acute or chronic rejection: an episode of acute rejection (interstitial or vascular) was found in 3 patients, while no episode of chronic rejection was found. If acute kidney rejection occurred, they were given methylprednisolone. In all patients, medical history was negative for systemic autoimmune disorders. Serum sample collection was as follows: one serum specimen before transplantation (in all the patients, but two) and two samples post-transplantation (at the third and the sixth month). Overall, 268 serum samples were tested. Clinical charts of all the patients and the Renal Transplant database of the Piedmont Region were reviewed. The presence of HCMV infection was evaluated by pp65-antigenemia test, as previously described (Bergallo *et al.*, 2006a).

### *BKV DNA quantification in serum samples*

Extraction procedure was performed as previously described (Bergallo *et al.*, 2006b). BKV DNA quantification was performed by PCR Real Time TaqMan using a commercial kit (BKV Q-PCR Alert Kit; Nanogen Advanced Diagnostic, Milano, Italy) for the detection of the target viral gene encoding for the large T-antigen, with the 7300 Real Time PCR System (Nanogen).

### *Detection of antibodies to dsDNA by the Crithidia Test*

This was done by an indirect immunofluorescence test (*Crithidia luciliae* immunofluorescence test, CLIFT) using the hemoflagellate *Crithidia lu-*

*ciliae* that presents a giant mitochondrion (the kinetoplast) which is composed of pure dsDNA. A commercial test system was used (Anti-nDNA antibody test system, Scimedx, Denville, N.J.) according to the manufacturer's instructions. For screening, serum samples were diluted 1:10 in PBS. In case of positivity, titration was made setting up doubling dilutions of serum. The immunofluorescence was evaluated at 400x magnification using a Diaplan microscope (Leitz, Germany). Two investigators independently and blindly read all the specimens.

#### Detection of NOSAs by indirect immunofluorescence

The following NOSAs were evaluated: anti-nuclear antibodies (ANA), smooth-muscle antibodies (SMA), anti-mitochondrial antibodies (AMA), and liver-kidney microsome antibodies type 1 (LKM-1), as previously described (Roitt and Doniach, 1972). Briefly, sera diluted 1:40 in PBS were tested on cryostat sections of snap-frozen rat liver, kidney, and stomach. Fluorescein isoth-

iocyanate-conjugated rabbit anti-human immunoglobulin (Polyclonal Rabbit anti-human IgA, IgG, IgM, Kappa, lambda/FITC, Dako-Cytomation, Glostrup, Denmark), diluted 1:100 in PBS, was used as secondary antibody. In order to better define the ANA pattern, all serum samples were also evaluated on commercially available HEp-2 cell lines (Incstar; Diasorin, Saluggia, Italy) at a 1:40 dilution.

#### Statistical analysis

Statistical analysis was performed using the chi-square test and the unpaired *t* test of Student, as appropriate. Results were considered significant when the P value was below 0.05. The MedCalc statistical software (version 9.2.1.0) was used.

## RESULTS

Results are summarized in Table 1. During the follow-up post-transplantation, BK viremia was detected in at least one serum specimen of 22/90

TABLE 1 - Demographic, clinical and virological parameters in relation to autoantibody positivity.

	Anti-dsDNA pos. (N = 17)	Anti-dsDNA neg. (N = 73)	p	NOSA pos. (N = 23)	NOSA neg. (N = 67)	p
Sex			n.s.			n.s.
M (N= 62)	12 (19.4%)	50 (80.6%)		15 (24.2%)	47 (75.8%)	
F (N = 28)	5 (17.9%)	23 (82.1%)		8 (28.6%)	20 (71.4%)	
Age years (mean±SD)	53.47±9.45	55.11±14.58	n.s.	53.26±13.32	56.05±13.13	n.s.
BK viremia (N=22)	9 (40.9%)	13 (59.1%)	<0.01	5 (22.7%)	17 (25.4%)	n.s.
Viral load (mean±SD)*	93943.2±174362.2	37494.4±114734.1	n.s.	189.2±107.5	86287.6±162923.5	n.s.
HCMV pp65-antigenemia (N=19)	2 (10.5%)	17 (89.5%)	n.s.	6 (31.6%)	13 (68.4%)	n.s.
Therapeutic protocol**			n.s.			n.s.
FK506+S+MMF (N=23)	3 (13.0%)	20 (87.0%)		9 (39.1%)	14 (60.9%)	
FK506+S (N=22)	7 (31.8%)	15 (68.2%)		8 (36.4%)	14 (63.6%)	
CyA+S+MMF (N=11)	5 (45.4%)	6 (54.6%)		3 (27.3%)	8 (72.7%)	
CyA+S (N=9)	2 (22.2%)	7 (77.8%)		0 (0%)	9 (100%)	
MMF+S (N=12)	0 (0%)	12 (100%)		2 (16.7%)	10 (83.3%)	
Other protocols (N=13)	0 (0%)	13 (100%)		0 (0%)	13 (100%)	
sCreatinine (mg/dL) mean±SD***	2.42±0.92	2.27±0.83	n.s.	2.4±1.24	2.4±0.86	n.s.

\*In at least one concomitant serum sample (mean viral load was calculated considering the highest value in each patient concomitant to the detection of autoantibodies). \*\*Statistical significance was analysed considering only the protocols associated with the occurrence of anti-dsDNA or NOSAs. \*\*\*Mean serum creatinine levels were calculated considering the highest value in each patient concomitant to the detection of autoantibodies. FK506, tacrolimus; S, steroid; MMF, mycophenolate mofetil; CyA, cyclosporine A; sCreatinine, serum creatinine.

(24.4%) patients (median viral load, 387 genome copies/ml; range 125-560604).

None of the patients were positive to anti-dsDNA before transplantation. Post-transplantation, anti-dsDNA antibodies developed in 17/90 (18.9%) patients (titer: 1:10 in 13; 1:20 in two; 1:40 in two); in three of them anti-dsDNA were detected at 3 and 6 months. No significant difference in terms of demographic (age, gender) or clinical (renal function as evaluated by serum creatinine level, occurrence of acute rejection) parameters between anti-dsDNA-positive and -negative patients was found. Considering the 22 patients with BK viremia, anti-dsDNA antibodies were present in 9 vs 13 who did not develop anti-dsDNA antibodies, while in BK viremia-negative patients 8 were anti-dsDNA positive vs 60 negative ( $p < 0.01$ ). No significant correlation between the occurrence of anti-dsDNA antibodies and HCMV infection was found. No immunosuppressive protocol was significantly associated with the occurrence of anti-dsDNA antibodies.

Overall NOSAs were present in 23/90 patients (25.6%) post-transplantation. ANAs were present in 16 patients (homogeneous pattern in 11; speckled in 5; one patient positive to both ANA and SMA), two already positive before transplantation. SMA were present in seven patients (including the one positive to both ANA and SMA), three already positive before transplantation. AMAs were negative in all the patients before and after transplantation. LKM-1 were present in one patient negative to both anti-dsDNA antibodies and BKV-DNA (this patient was anti-HCV-positive). No significant association was found between NOSAs and demographic, clinical, and virological parameters. Similarly, no significant correlation was found with the occurrence of acute rejection and the presence of anti-dsDNA and NOSAs (data not shown).

## DISCUSSION

In this study, anti-dsDNA antibodies and NOSAs occurred in 18.9% and 25.6%, respectively, of a population of renal transplant recipients. The occurrence of NOSAs and the relation to viral infections have been studied in both bone marrow and solid organ transplant recipients (Hebart *et al.*, 1996, Toyoda *et al.*, 1997, Rider *et al.*, 1997).

In a study by Varani *et al.* on 40 liver transplant recipients, five patients developed NOSAs (4 SMA, 1 ANA), all with high pp65-antigenaemia levels (Varani *et al.*, 2002). Regarding allogeneic bone marrow transplantation, Hebart *et al.* noted a high prevalence of natural (including 12 SMA-positive patients/95) and disease-relevant (including 11 ANA/95) autoantibodies in the first 100 days post-transplant and that these were significantly correlated with the status of HCMV infection.

In our study, NOSA appearance were not significantly associated with BKV and HCMV infection. However, considering the occurrence of NOSAs after transplantation, 6/23 (26.1%) patients with NOSAs were pp65-positive and 5/23 (21.7%) BK viremia-positive.

The occurrence of anti-dsDNA antibodies was significantly correlated with BK viremia (9/17 anti-dsDNA-positive patients were BKV-positive), while only 2/17 were pp65-positive. In all the cases, anti-dsDNA were present at low titer. Anti-dsDNA, usually at high titer ( $> 1:160$ ), is a highly specific marker for SLE, which correlates with clinical severity and nephritis. Besides their diagnostic relevance, anti-dsDNA could function as initiators of glomerulonephritis typical for SLE. However, not all of the autoantibodies produced during the course of autoimmune diseases are pathogenic. In fact, it has been suggested that the qualitative aspects of autoantibodies may be important in their ability to induce cellular and tissue injuries (Ramos *et al.*, 2003).

In a study on 20 patients with SLE, one or several episodes of BKV reactivation were found in 80% of patients versus 0% of 32 healthy subjects (Van Ghelue *et al.*, 2003). On the other hand, in a study on BKV reactivation in SLE nephritis, we found no significant difference in terms of clinical, histopathological and prognostic features in patients with or without BKV reactivation and the prevalence of BKV reactivation was similar in SLE nephritis patients and controls (Colla *et al.*, 2007). Anti-dsDNA antibodies can occur in normal subjects ( $< 1%$  [Koffier *et al.*, 1969]) and in other inflammatory diseases, such as scleroderma and rheumatoid arthritis, but usually at very low titre (Tan, 1982a). Based on the clinical setting and the reciprocal interaction between virus replication and immune response, Hirsch considered the pathogenic role of BKV infection as divided

into four patterns: cytopathic, cytopathic inflammatory, immune reconstitution, autoimmune (Hirsch, 2005). The induction of anti-dsDNA antibodies by BKV in the clinical context of renal transplantation could be attributable to an aberrant immune response triggered by the hapten-carrier complex T large antigen-host cell DNA, although no direct pathogenic role of this finding was demonstrable in our group of patients, as no significant correlation with demographic and clinical, including immunosuppressive protocol was found.

Different methodologies are available for the detection of anti-dsDNA antibodies. In contrast to the ELISA method, CLIFT has a relatively low sensitivity and high specificity; while in contrast to a parametric test, such as radioimmune assay, it should be considered a semi-quantitative test. Although there have been occasional reports on the putative presence of histones in the kinetoplast, and the presence of lipoprotein IgG complexes in the sample leading to false positive results, the CLIFT remains one of the preferred methods if good practices in indirect immunofluorescence are respected (Rouquette and Desgruelles, 2006).

In conclusion, although we have found an association between BKV reactivation and the occurrence of anti-dsDNA antibodies in renal transplant patients, evidence that these autoreactive responses are themselves pathogenic is lacking and should be specifically addressed in other studies, implying a biological and clinical insight on the significance of this finding.

## REFERENCES

- BERGALLO M., COSTA C., GRIBAUDO G., TARALLO S., BARO S., NEGRO PONZI A., CAVALLO R. (2006a). Evaluation of six methods for extraction and purification of viral DNA from urine and serum samples. *New Microbiol.* **29**, 111-119.
- BERGALLO M., COSTA C., TARALLO S., DANIELE R., MERLINO C., SEGOLONI G.P., NEGRO PONZI A., CAVALLO R. (2006b). Development of a quantitative-competitive PCR for quantification of human cytomegalovirus load and comparison with antigenaemia, viraemia and pp67 RNA detection by nucleic acid sequence-based amplification. *Panminerva Med.* **48**, 119-127.
- CARSON A.D. (1991). The specificity of anti-DNA antibodies in SLE. *J. Immunol.* **146**, 1-2.
- COLLA L., MESIANO P., MORELLINI V., BESSO L., CAVALLO R., BERGALLO M., COSTA C., MERLINO C., MARCUCCIO C., FOP F., LANFRANCO G., SEGOLONI G.P., CANAVESE C., STRAITTA P. (2007). Human polyomavirus BK in patients with lupus nephritis: clinical and histological correlations. *Lupus* **16**, 881-886.
- FLAEGSTAD T., FREDRIKSEN K., DAHL B., TRAAVIK T., REKVIK O.P. (1988). Inoculation with BK virus may break immunological tolerance to histone and antigens. *Proc. Natl. Acad. Sci. USA* **85**, 8171-8175.
- FREDRIKSEN K., SKOGSHOLM A., FLAEGSTAD T., TRAAVIK T., REKVIK O.P. (1993). Antibodies to dsDNA are produced during primary BK virus infection in man, indicating that anti-dsDNA antibodies may be related to virus replication in vivo. *Scand. J. Immunol.* **38**, 401-406.
- GARDNER S.D., FIELD A.M., COLEMAN D.V., HULME B. (1971). New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet.* **1**, 1253-1257.
- HEBART H., EINSELE H., KLEIN R., FISCHER I., BUHLER S., DIETZ K., JAHN G., BERG P.A., KANZ L., MULLER C.A. (1996). CMV infection after allogeneic bone marrow transplantation is associated with the occurrence of various autoantibodies and monoclonal gammopathies. *Br. J. Haematol.* **95**, 138-144.
- HIRSCH H.H. (2005). BK virus: opportunity makes a pathogen. *Clin. Infect. Dis.* **41**, 354-360.
- HIRSCH H.H., STEIGER J. (2003). Polyomavirus BK. *Lancet Infect. Dis.* **3**, 611-623.
- KOFFIER D., CARR I.R., ANGELO V., FIEZI T., KUNDEL H.G. (1969). Antibodies to polynucleotides: Distribution in human serums. *Science.* **166**, 1648-1649.
- NICKELEIT V., SINGH H.K., MIHATSCH M.J. (2003). Polyomavirus nephropathy: morphology, pathophysiology, and clinical management. *Curr. Opin. Nephrol. Hypertens.* **12**, 599-605.
- RAMOS M.A., PIÑERA C., SETIÉN M.A., BUELTA L., DE COS M.A., DE FRANCISCO A.L., MERINO R., ARIAS M. (2003). Modulation of autoantibody production by mycophenolate mofetil: effects on the development of SLE in (NZB x NZW)<sub>F1</sub> mice. *Nephrol. Dial. Transplant.* **18**, 878-883.
- REEVES W.H., SATOH M., JINSONG W., CHOU C., AJMANIK A. (1994). Antibodies to DNA, DNA binding proteins and histones. *Rheum. Dis. Clin. North Am.* **20**, 1-28.
- REKVIK O.P., MOENS U., SUNDSFJORD A., BREDHOLT G., OSEI A., TRAAVIK T. (1997). Experimental expression in mice and spontaneous expression in human SLE of polyomavirus T antigen. *J. Clin. Invest.* **99**, 2045-2054.
- RIDER J.R., OLLIER W.E., LOCK R.J., BROOKES S.T. (1997). Human cytomegalovirus infection and systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **15**, 405-409.
- ROITT I.M., DONIACH D. (1972). Immunofluorescent test for the detection of antibodies. In: *WHO booklet of*

- immunological studies*. Geneva: World Health Organization.
- ROUQUETTE A.M., DESGRUELLES C. (2006). Detection of antibodies to dsDNA: an overview of laboratory assays. *Lupus* **15**, 403-407.
- TAN E.A. (1982a). Autoantibodies to nuclear antigens: Their biology and medicine. *Adv. Immunol.* **33**, 167-240.
- TAN E.M., COHEN A.S., FRIES J.F., MASI A.T., MCSHANE D.J., ROTHFIELD N.F., SCHALLER J.G., TALAL N., WINCHESTER R.J. (1982b). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1271-1277.
- TOYODA M., GALFAYAN K., GALERA O.A., PETROSIAN A., CZER L.S., JORDAN S.C. (1997). Cytomegalovirus infection induces anti-endothelial cell antibodies in cardiac and renal allograft recipients. *Transplant. Immunol.* **5**, 104-111.
- VAN GHELUE M., MOENS U., BENDIKSEN S., REKVIK O.P. (2003). Autoimmunity to nucleosomes related to viral infection: a focus on hapten-carrier complex formation. *J. Autoimmun.* **20**, 171-182.
- VARANI S., MURATORI L., DE RUVO N., VIVARELLI M., LAZZARETTO T., GABRIELLI L., BIANCHI F.B., BELLUSCI R., LANDINI M.P. (2002). Autoantibody appearance in cytomegalovirus-infected liver transplant recipients: correlation with antigenemia. *J. Med. Virol.* **66**, 56-62.