Transplant Immunology 26 (2012) 123–127

Contents lists available at SciVerse ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Mannose-binding lectin deficiency is not associated with increased risk for polyomavirus nephropathy

Peter Liman [a,c], Nina Babel [a,c], Thomas Schachtner [a,c], Nadine Unterwalder [b], Julian König [a], Jörg Hofmann [e], Petra Reinke [a,c,d], Peter Nickel [a,c,*]

[a] Department of Nephrology and Intensive Care, Charité Campus Virchow, Augustenburger Platz 1, 13353 Berlin, Germany
[b] Institute of Medical Immunology, Charité Campus Virchow, Augustenburger Platz 1, D-13353 Berlin, Germany
[c] Interdisciplinary Transplant Research Group Nephrology/Medical Immunology, Charité Campus Virchow, Augustenburger Platz 1, D-13353 Berlin, Germany
[d] Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité Campus Virchow, Augustenburger Platz 1, 13353 Berlin, Germany
[e] Institute of Medical Virology, Charité Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany

ARTICLE INFO

Article history:
Received 12 September 2011
Received in revised form 6 December 2011
Accepted 7 December 2011

Keywords:
Mannose binding lectin
Polyomavirus nephropathy
Kidney transplantation

ABSTRACT

Background: Polyomavirus associated nephropathy (PVAN) affects up to 10% of kidney transplant recipients and is a major risk factor for graft loss. Mannose-binding lectin (MBL) is an important recognition molecule of the innate immune system, and its deficiency has been associated with susceptibility to various infections. In transplantation, on the other hand, high MBL levels have been associated with increased tissue damage in ischemia–reperfusion models and poorer graft and patient survival in solid organ transplant patients. To investigate the relation between MBL and BK virus infection, post-transplant (post-Tx) MBL levels were determined in a cohort of *de novo* kidney transplant patients with and without BK viremia.
Patients and methods: 41 *de novo* kidney transplant patients with high ($n=16$, group 1) or low level BK viremia ($n=25$, group 2) and 64 patients without BK viremia (group 3) were included. In every patient, functional MBL levels were determined at 1–3 time points (days 30, 90 or 180) post-Tx using an MBL oligomer ELISA.
Results: MBL levels remained unchanged between days 30 and 180 post-Tx independent of BKV viremia. Frequencies of MBL deficiency (<500 ng/mL) and MBL levels were not significantly different between the 3 groups. However, group 2 patients showed a trend towards lower MBL serum levels compared to group 1 patients, notably in patients without acute rejection ($p=0.076$).
Conclusion: MBL deficiency was not associated with higher risk for BK viremia. In contrast, we hypothesize that BK virus replication in patients with low MBL levels might imply lower risk for progression towards PVAN compared to patients with high MBL levels. This view is supported by recent data demonstrating local complement activation in BK nephropathy.

1. Introduction

With the advent of modern immunosuppressive therapy, polyomavirus-associated nephropathy (PVAN) has been recognized as a major complication after kidney transplantation with increased risk of graft loss [1,2]. In >95% of cases, PVAN is caused by the BK virus, which latently persists in renal tubular epithelial cells after primary infection in childhood and may become reactivated in states of immunodeficiency and tissue injury. Complete PVAN develops almost exclusively in transplanted kidneys, mostly during the early post-Tx period [3,4]. While 80% of adults worldwide are seropositive for BK virus [2], only one third of renal transplant patients show BK virus reactivation, and only 1 to 10% develop PVAN [1]. Early identification of patients at risk for PVAN is a highly desirable goal, as it would allow preemptive measures such as modification of immunosuppression with possible improvement of outcome. Established risk factors for PVAN include first of all high intensity of immunosuppression, but on the other hand also HLA-mismatch and alloimmune injury/rejection [1,5–7]. Recently, the importance of adaptive cellular and humoral immunity to BK virus has been demonstrated for viral replication and recovery from PVAN [6–11]. However, few is known about the contribution of innate immunity to defense against BK virus.

Mannose-binding lectin (MBL) is a serum protein and important recognition molecule of the innate immune response. Oligomeric serum MBL multivalently binds to a wide range of microbial components such as sugar structures, phospholipids, nucleic acids and non-glycosytated proteins, enabling phagocytosis or cell lysis by activation of MBL-associated serine proteases, C4 and the classical

* Corresponding author at: Department of Nephrology and, Intensive Care, Charité Campus Virchow, Augustenburger Platz 1, D-13353 Berlin, Germany. Tel./fax: +49 30 450 553 370/904.
E-mail address: peter.nickel@charite.de (P. Nickel).

0966-3274/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.trim.2011.12.004

complement pathway [12]. Moreover, MBL binds to immunoglobulins and may therefore enhance adaptive immunity [13].

MBL levels in the peripheral blood are determined largely genetically by polymorphisms of its encoding *MBL2* gene. These polymorphisms cause defects in MBL polymerization or protein expression resulting in MBL deficiency with low serum levels and impaired MBL activity [12]. MBL deficiency is a risk factor for infections by enveloped viruses such as herpes simplex, HIV and hepatitis B virus [14]. In kidney transplant patients with high-risk CMV serostatus, MBL deficiency has been associated with CMV infection/disease [15]. Recently, MBL deficiency has been linked to infections by non-enveloped viruses as well, such as HPV and astrovirus [16–18]. Regarding the BK virus, one study associated MBL with protection from BK virus infection in high risk HPV positive women [19]. In transplantation, however, MBL seems to play an important role far beyond its antiinfectious properties, as animal models and studies in organ transplant patients have associated high MBL levels with increased ischemia–reperfusion injury and graft rejection, which was impressively illustrated by studies showing poorer graft survival in kidney, and poorer patient survival in kidney–pancreas transplant patients with high MBL levels [13].

2. Objective

The aim of the present study was to exclude an association of BK virus infection with underlying MBL deficiency in kidney transplant patients. To this end, we determined MBL levels in *de novo* kidney transplant patients with and without BK virus infection during the early post-Tx period. As BKV-DNA load in plasma/serum of more than 10^4 copies/ml has been shown to have a high predictive value for PVAN, we compared MBL levels in patients with undetectable and low-level BK viral load with patients that developed high-level BK viremia with viral loads $>10^4$ copies/mL for more than 3 consecutive weeks [20].

3. Materials and methods

3.1. Patients and screening for BKV replication

Patients receiving *de novo* kidney transplants between 2005 and 2010 at our center were included if at least 2 BKV PCR determinations during the first 6 months after transplantation had been performed and frozen sera of post-Tx days 30, 90 and/or 180 were available for MBL analysis. Patients with pneumocystis jirovecii pneumonia were excluded from the study. In every patient, quantitative BK viral load was determined at least 2 times during the first 6 months after transplantation (median 4, maximum 12 times). Screening algorithm for BKV viral load was monthly for the first 3–6 months, then once every 3 months until month 12 post-Tx and additionally if clinically indicated, i.e. in cases with transplant deterioration. High level BK viremia was defined as BK viral load $>10^4$ copies/mL that was detectable for at least 3 weeks, and low BK viral load $<10^4$ copies/mL or $>10^4$ copies/mL for less than 3 weeks [20].

3.2. Methods

For MBL determination, at least 1 serum sample was collected from each patient either at post-Tx days 30, 90 or 180. To investigate stability of MBL values during the time of observation, serum samples were collected in 39/105 patients at all 3 time points days 30, 90 and 180 post-Tx. All samples were stored at −80 °C until the time of analysis. In all serum samples, functional MBL was measured using an MBL Oligomer enzyme-linked immunosorbent assay kit (Antibody Shop, Gentofte, Denmark) as described elsewhere [21]. In line with former studies, median post-Tx MBL concentrations lower than 500 ng/mL were classified as MBL deficiency and values below 1000 ng/mL as partial MBL deficiency [15,21,22].

Serum BKV-loads were measured by TaqMan real-time polymerase chain reaction (PCR) targeting the large T antigen. Primers and probes were described elsewhere [23]. The nucleic acid was extracted from serum or urine samples using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. To assess an increased sensitivity (95% detection limit was 2000 copies/mL) all reactions were controlled for presence of inhibiting factors by the use of an internal co-amplified DNA. Sampling of patient material was approved by the Ethics Commission at the Charité University Hospital, Berlin. All patients gave informed consent.

3.3. Statistics

MBL values and frequency of MBL deficiency were compared between the groups using Kruskal Wallis test, Mann Whitney U (MWU) test and Chi square or Fisher's exact test. MBL levels at 30, 90 and 180 days post-Tx were compared using Friedman's test, respectively. Data were analyzed using PASW statistics 18.

4. Results

A total of 105 patients were included and categorized in 41 *de novo* kidney transplant patients with high level (group 1, $n=16$) or low level (group 2, $n=25$) BK viremia and 64 patients without BK virus replication during screening (group 3). 25/105 patients had living-related donors. Induction treatment consisted of basiliximab ($n=86$), thymoglobulin ($n=10$), alemtuzumab ($n=7$) or OKT3 ($n=2$), maintenance immunosuppression of calcineurin inhibitor (cyclosporine $n=51$, tacrolimus $n=54$), mycophenolic acid ($n=103$) and steroids ($n=104$).

Clinical characteristics of the patients are shown in Table 1. The average of peak BK viral loads in group 1 was 1691358 copies/mL versus 13013 copies/mL in group 2 ($p<0.001$). Time to BK virus replication was on average 158 days (group 1) versus 126 days (group 2) post-Tx ($p=0.285$). In 7/16 of group 1 patients intragraft BK virus infection was confirmed by biopsy. Treatment of group 1 patients with high level BK viremia consisted of reduction of immunosuppression in all patients, 6/16 patients were additionally treated by cidofovir. Patients of group 2 showed lower 6 (1.60 mg/dL, $p=0.375$) and 12 (1.66 mg/dL, $p=0.359$) months serum creatinine levels compared to patients of group 1 (1.82 mg/dL and 1.79 mg/dL, respectively). Patients of group 2 showed higher Cockroft Gault GFR at 6 (65.7 ml/min, $p=0.067$) and 12 months (66.8 ml/min, $p=0.120$) compared to group 1 patients (50.8 ml/min and 53 ml/min, respectively). 6 and 12 months proteinuria were not different between the groups (Table 1).The frequency of acute rejection was higher with 7/16 in group 1 versus 10/25 in group 2 and 14/ 64 in group 3 patients ($p=0.097$).

In the 38 patients with MBL determinations at all 3 time points day 30, 90 and 180 post-Tx stability of MBL levels during the early post-Tx phase was assessed. MBL levels

Table 1
Patient characteristics.

Patient characteristics	High level BK viremia	Low level BK viremia	Controls	*p*-value
N	16	25	64	–
Age (mean)	50.9 (±14.9)	46.5 (±9.5)	48.9 (±13.5)	0.622
Male (%)	12 (75%)	17 (68%)	40 (62.5%)	0.618
% MBL deficiency (<500 ng/mL)	25%	28%	22%	0.824
Maximum BKV viral load (mean, cop/mL)	1691358 ±4263759	13013 ±19498	–	<0.001
Time to BKV reactivation (mean, days post-Tx)	158 ± 161	126 ± 139	–	0.285
12 month serum creatinine (mean, mg/dL)	1.79 ± 0.62	1.66 ± 0.66	1.55 ± 0.44	0.360
12 months cockroft gault GFR (mean, ml/min)	53 ± 17.5	66.8 ± 28.0	60.1 ± 18.6	0.302
12 months proteinuria (mean, mg/24 h)	210 ± 256	312 ± 361	220 ± 166	0.334
HLA-mismatch (mean)	3.3 ± 1.8	3.0 ± 1.7	2.76 ± 1.8	0.502
Acute rejection (%)	7 (44%)	10 (40%)		0.097