Epidemiology and Morbidity of Epstein-Barr Virus Infection in Pediatric Renal Transplant Recipients: A Multicenter, Prospective Study

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Background. The epidemiology and morbidity of Epstein-Barr virus (EBV) infection in pediatric renal transplant recipients have been characterized insufﬁciently.

Methods. In a prospective, multicenter study among 106 pediatric kidney allograft recipients aged 11.4 ± 5.9 years, we investigated the epidemiology of EBV infection and the relationship between EBV load, EBV serology, and EBV-related morbidity (posttransplant lymphoproliferative disease [PTLD] or symptomatic EBV infection, defined as flu-like symptoms or infectious mononucleosis).

Results. EBV primary infection occurred in 27 of 43 (63%) seronegative patients and reactivation/reinfection in 28 of 63 (44%) seropositive patients. There was no association between the degree or duration of EBV load and EBV-related morbidity: The vast majority (17 of 18 [94%]) of patients with a high, persistent EBV load remained PTLD-free throughout a follow-up of 5.0 ± 1.3 years, while 2 of 3 (66%) patients with EBV-related PTLD exhibited only a low EBV load beforehand. Eight of 18 (44%) patients with a high, persistent EBV load remained asymptomatic during a follow-up of 5.3 ± 2.9 years. Multivariate analysis identified the EBV high-risk (D+/R–) serostatus (odds ratio [OR], 7.07; P < .05), the presence of human leukocyte antigen (HLA)–DR7 (OR, 5.65; P < .05), and the intensity of the immunosuppressive therapy (OR, 1.53; P < .01) as independent risk factors for the development of a symptomatic EBV infection.

Conclusions. Presence of EBV high-risk seroconstellation, HLA-DR7, and intensity of immunosuppressive therapy are signiﬁcant risk factors for a symptomatic EBV infection, whereas there is no close association between the degree or duration of EBV load and EBV-related morbidity.

Clinical Trials Registration. NCT00963248.

Keywords. Epstein-Barr virus; post-transplant lymphoproliferative disease; pediatric renal transplantation; human leukocyte antigen; immunosuppression.
based solely on the presence of a high, persistent EBV load, may put patients at risk of transplant rejection and subsequent graft failure. Previous retrospective analyses [6–8] and 2 small prospective studies [9,10] among pediatric renal transplant recipients revealed controversial findings. In particular, previous studies focused on selected PTLD patients in which EBV load was evaluated retrospectively. However, data on prospectively monitored EBV load and its relationship with EBV-related morbidity in an unselected cohort of pediatric renal transplant recipients are lacking. We therefore performed the present large-scale, prospective, multicenter study on the epidemiology and morbidity of EBV infection in pediatric kidney allograft recipients and focused on the relationship between EBV load, EBV-specific antibody response, and EBV-related morbidity. Additionally, we analyzed potential determinants of a symptomatic EBV infection such as the impact of immunosuppressive treatment and human leukocyte antigen (HLA) allele typing as a possible genetic predisposition.

**MATERIALS AND METHODS**

**Study Design and Patient Population**

This prospective, multicenter trial was conducted in full accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines, and approved by the ethics committee of each contributing center. Written informed consent of patients’ parents or guardians was obtained prior to initiation of any study-related procedure.

Between July 2003 and June 2009, 106 patients were recruited for this study. Virological analyses were performed prospectively during the first posttransplant year. Additionally, patients’ clinical data were collected until database closure (July 2011), but at least for 2 years after engraftment. Patient characteristics are shown in Table 1. A subgroup analysis investigating the effect of an antiviral prophylaxis on EBV primary infection in high-risk (donor [D] EBV-seropositive [D⁺], recipient [R] EBV-seronegative [R − ] ) patients has been reported elsewhere [11]. Data of these 28 high-risk patients have also been included in the present analysis.

**Immunosuppressive Regimen**

Immunosuppressive medication was administered according to center practice as described previously [11]. A score modified according to Vasudev et al for assessment of the overall immunosuppressive load was calculated (Table 2) [12]. Immunosuppres- sive dosage, predose concentrations, and immunosuppressive score were documented at each sampling time point (see below).

**Antiviral Chemoprophylaxis**

Antiviral prophylaxis was administered based on international guidelines for cytomegalovirus (CMV) prophylaxis in renal transplant recipients. Patients at high risk of CMV infection (D⁺/R−, D⁻/R⁺) received ganciclovir or, since the year 2006, valganciclovir. On the basis of the findings by Funch et al [13], some transplant centers have administered antiviral chemoprophylaxis since the year 2005 not only to patients at high risk of CMV infection, but also to those at high risk of EBV infection. Antiviral prophylaxis was administered based on international guidelines for CMV prophylaxis. Based on the findings by Funch et al [13], some transplant centers have administered antiviral chemoprophylaxis since the year 2005 not only to patients at high risk of CMV infection, but also to those at high risk of EBV infection.

Eighteen patients received CMV hyperimmunoglobulin in addition to valganciclovir or ganciclovir, but 3 patients were administered CMV hyperimmunoglobulin only. * Defined as eGFR at the time of discharge after initial transplant surgery.

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients (N = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at transplantation, y</td>
<td>11.4 ± 5.9</td>
</tr>
<tr>
<td>Male sex</td>
<td>68 (64%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>103 (97%)</td>
</tr>
<tr>
<td>Second RTx</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Living-related donation</td>
<td>41 (39%)</td>
</tr>
<tr>
<td>Cold ischemia time, h</td>
<td>10.1 ± 7.1</td>
</tr>
<tr>
<td>HLA mismatch, No.</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>EBV-naive at RTx</td>
<td>43 (41%)</td>
</tr>
<tr>
<td>EBV serostatus (n = 82) a</td>
<td></td>
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<tr>
<td>D⁺/R⁻</td>
<td>28 (34%)</td>
</tr>
<tr>
<td>D⁺/R⁺</td>
<td>39 (48%)</td>
</tr>
<tr>
<td>D⁻/R⁺</td>
<td>9 (11%)</td>
</tr>
<tr>
<td>D⁻/R⁻</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>CMV serostatus</td>
<td></td>
</tr>
<tr>
<td>D⁺/R⁻</td>
<td>23 (22%)</td>
</tr>
<tr>
<td>D⁺/R⁺</td>
<td>26 (25%)</td>
</tr>
<tr>
<td>D⁻/R⁺</td>
<td>11 (10%)</td>
</tr>
<tr>
<td>D⁻/R⁻</td>
<td>46 (43%)</td>
</tr>
<tr>
<td>EBV- and CMV-seropositive donor (n = 82) a</td>
<td>36 (44%)</td>
</tr>
<tr>
<td>Antiviral prophylaxis a</td>
<td>56 (53%)</td>
</tr>
<tr>
<td>Valganciclovir</td>
<td>29 (27%)</td>
</tr>
<tr>
<td>Ganciclovir</td>
<td>24 (23%)</td>
</tr>
<tr>
<td>CMV hyperimmunoglobulin a</td>
<td>21 (20%)</td>
</tr>
<tr>
<td>Initial immunosuppressive regimen</td>
<td></td>
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<tr>
<td>IL-2 receptor antagonist</td>
<td>24 (23%)</td>
</tr>
<tr>
<td>TAC</td>
<td>56 (53%)</td>
</tr>
<tr>
<td>CSA</td>
<td>50 (47%)</td>
</tr>
<tr>
<td>MMF</td>
<td>105 (99%)</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>106 (100%)</td>
</tr>
<tr>
<td>Baseline eGFR a, mL/min/1.73 m²</td>
<td>68.5 ± 19.8</td>
</tr>
</tbody>
</table>

Data are given as mean ± a standard deviation, if not indicated otherwise.

Abbreviations: CMV, cytomegalovirus; CSA, cyclosporine microemulsion; D, donor; EBV, Epstein-Barr virus; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; IL-2, interleukin 2; MMF, mycophenolate mofetil; R, recipient; RTx, renal transplantation; TAC, tacrolimus.

a Determination of EBV serology in donors has been mandatory only since 2006.

b A subgroup analysis on the effect of an antiviral prophylaxis on EBV primary infection in these 28 high-risk (EBV D⁺/R−) patients was published earlier [11].

c Antiviral prophylaxis was initially practiced in conformity with international guidelines for CMV prophylaxis. Based on the findings by Funch et al [13], some transplant centers have administered antiviral chemoprophylaxis since the year 2005 not only to patients at high risk of CMV infection, but also to those at high risk of EBV infection.

Data of these 28 high-risk patients have also been included in the present analysis.

Eighteen patients received CMV hyperimmunoglobulin in addition to valganciclovir or ganciclovir, but 3 patients were administered CMV hyperimmunoglobulin only.

* Defined as eGFR at the time of discharge after initial transplant surgery.
Table 2. Immunosuppressive Score

<table>
<thead>
<tr>
<th>Immunosuppressant</th>
<th>Vasudev Score a: Dose per Unit (mg/d)</th>
<th>Pediatric Score b: Dose per Unit (mg/m²/d)</th>
<th>Immunosuppressive Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC</td>
<td>2</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>CSA</td>
<td>100</td>
<td>58</td>
<td>1</td>
</tr>
<tr>
<td>SRL</td>
<td>2</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>MMF</td>
<td>500</td>
<td>290</td>
<td>1</td>
</tr>
<tr>
<td>AZA</td>
<td>100</td>
<td>58</td>
<td>1</td>
</tr>
<tr>
<td>Prednisone equivalent</td>
<td>5</td>
<td>2.9</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: AZA, azathioprine; CSA, cyclosporine microemulsion; MMF, mycophenolate mofetil; SRL, sirolimus; TAC, tacrolimus.

a See [12].

b The pediatric score, modified according to Vasudev et al for assessment of the overall immunosuppressive load, was calculated by adjustment of the adult score to a body surface area of 1.0 m², assuming an adult body surface area of 1.73 m². Induction therapy with an interleukin 2 receptor antagonist and anti-rejection treatment with steroid pulse therapy were included in this score and assigned to 2 immunosuppressive units each (see [11]).

If symptomatic EBV infection or PTLD was suspected, sampling was done at weekly intervals for at least 6 weeks and at monthly intervals thereafter.

**EBV Load**

EBV load was measured as described previously [11]. An EBV load of ≥10⁴ genomes/mL was defined as high viral load. A detectable EBV load in >50% of blood samples was considered to be persistent EBV load. The chronic high EBV load (CHL) carrier state was defined by the presence of a high (>10⁴ genomes/mL) and persistent (>50% of samples) EBV load.

**EBV Serology**

EBV-specific antibodies were analyzed as reported recently [11]. EBV seroconversion was defined as detection of at least 1 of the aforementioned EBV-specific antibodies in recipients EBV-seronegative at the time of transplantation. Patients receiving EBV antibody-containing CMV hyperimmunoglobulin or transfusions, who showed a single marginal or positive antibody result 6 weeks after administration but negative results thereafter, were regarded as seronegative.

**Additional Laboratory Assessments**

CMV pp65 antigen, CMV load, and human herpesvirus 6 (HHV-6) load as well as specific antibodies (anti-CMV immunoglobulin M [IgM]/immunoglobulin G [IgG]; anti–HHV-6 IgM/IgG) were measured in parallel to the EBV analyses. CMV infection was defined as positive pp65 antigenemia and/or positive quantitative polymerase chain reaction (PCR) and/or CMV seroconversion. HHV-6 infection was defined as positive quantitative PCR and/or HHV-6 seroconversion.

Transplant function was assessed as estimated glomerular filtration rate (eGFR) [15]. Pathological proteinuria was defined as urinary protein excretion >100 mg/m²/d in 24-hour collected urine. Histopathological changes of the graft were evaluated according to the current Banff classification of renal allograft pathology [16–19]. HLA allele typing was performed at the Department of Transplantation Immunology, University of Heidelberg. Recipients were typed by serological and molecular methods using Biostest Lymphotype HLA-AB 120 special (Dreieich, Germany) and CTS-PCR-SSP HLA kits (Heidelberg, Germany), respectively [20].

**Statistical Analysis**

Data were analyzed using PASW (SPSS) Statistics 18.0. Results for continuous variables are given as mean ± standard deviation, while categorical parameters are expressed as number and percentage of patients. Normal distribution of the data was evaluated by the Shapiro-Wilk test. Differences between 2 groups were determined by the Student t test or, if normality failed, by the Mann-Whitney rank-sum test. Rates in groups were compared with Fisher exact test. Univariate analysis...
(logistic regression) was performed to examine the association of immunosuppressive treatment with the development of EBV infection and the association of HLA alleles or antiviral prophylaxis with the development of a symptomatic EBV infection. Statistically significant factors were then included in a multivariate analysis. Differences in means or proportions with a 2-tailed $P < .05$ were considered statistically significant.

**RESULTS**

**EBV-Related Clinical Symptoms**

Over the 1-year observation period, EBV primary infection occurred in 27 of 43 (63%) initially seronegative patients, and EBV reactivation/reinfection in 28 of 63 (44%) initially seropositive recipients. Patients with primary EBV infection were more often symptomatic (14/27 [52%]) than those undergoing EBV reactivation/reinfection (3/28 [11%]; $P < .01$). Three patients (2.8%) developed an EBV-related B-cell PTLD after asymptomatic (n = 2) or symptomatic (n = 1) EBV primary infection. Histological examination revealed a monomorphic, monoclonal diffuse large B-cell lymphoma.

The overall frequency of a CMV infection in the first post-transplant year was 19 of 106 (18%). The frequency of a CMV coinfection in patients with a symptomatic (0/17 [0%]) and an asymptomatic EBV infection (5/38 [13%]) was not significantly different ($P = .31$). The overall frequency of an HHV-6 infection in the first year after transplant was 27 of 106 (26%). Similarly, the rate of an HHV-6 coinfection was comparable ($P = 1.00$) between patients with a symptomatic (2/17 [12%]) and an asymptomatic (6/38 [16%]) EBV infection.

**EBV Load**

There was no significant association between the extent of EBV load and EBV-related morbidity (Figure 1). Two of three patients (66%) with PTLD exhibited a low EBV load beforehand, while 8 of 18 (44%) patients with a CHL carrier state remained asymptomatic during a mean follow-up period of 5.3 ± 2.9 years (range, 2.0–8.6 years). Ten of 18 (56%) patients with a CHL had a symptomatic EBV infection; only 1 of these 18 patients (6%) developed a PTLD at 6 months after transplant, 4.5 months after the symptomatic EBV infection. Hence, the vast majority of patients (17 of 18 [94%]) with a CHL status in the first year after transplant remained PTLD-free throughout a mean observation period of 5.0 ± 1.3 years (range, 3.0–6.7 years). The rate, type, and dosage of antiviral prophylaxis between patients with and without CHL status were comparable (see Supplementary Table 1).

Patients with symptomatic EBV infection tended to have ($P = .07$) a persistent EBV load more often than asymptomatic recipients (Figure 1A), which resulted in a numerically higher ($P = .06$) median total EBV exposure, expressed as EBV load.

![Figure 1.](https://academic.oup.com/cid/article-abstract/56/1/84/417171) Epstein-Barr virus (EBV) load in patients with EBV-related post-transplant lymphoproliferative disease (PTLD), or symptomatic or asymptomatic EBV infection. A, Percentage of patients with EBV-related PTLD (black bars; n = 3; a), symptomatic (gray bars; n = 17; b), or asymptomatic (white bars; n = 35; c) EBV infection, who developed a high (>10⁴ genomes/mL) and/or persistent (detectable viral load in >50% of samples) EBV load. B, Peak EBV load and EBV load exposure (area under the concentration-time curve [AUC]) observed over the 1-year study period in patients with PTLD (black squares; n = 3; a), symptomatic (gray triangles; n = 7; b), or asymptomatic (white circles; n = 35; c) EBV infection. The horizontal line indicates the median value. EBV load peak: (a) vs (b), $P = .54$; (a) vs (c), $P = .10$; (b) vs (c), $P = .25$. Persistent EBV load: (a) vs (b), $P = .57$; (a) vs (c), $P = 1.00$; (b) vs (c), $P = .07$. B, Peak EBV load and EBV load exposure (area under the concentration-time curve [AUC]) observed over the 1-year study period in patients with PTLD (black squares; n = 3; a), symptomatic (gray triangles; n = 7; b), or asymptomatic (white circles; n = 35; c) EBV infection. The horizontal line indicates the median value. EBV load AUC: (a) vs (b), $P = .75$; (a) vs (c), $P = 1.00$; (b) vs (c), $P = .13$. This figure contains data of 28 high-risk (EBV D/R²) patients in whom a subgroup analysis on the effect of an antiviral prophylaxis on EBV primary infection was performed previously [11]. Abbreviations: AUC, area under curve; EBV, Epstein-Barr virus; PTLD, posttransplant lymphoproliferative disease.
area under the concentration-time curve (AUC; Figure 1B). The rate, type, and dosage of antiviral prophylaxis were similar \((P = 1.00)\) among PTLD patients \((2/3 [67%])\), patients with symptomatic EBV infection \((8/17 [47%])\), or those with asymptomatic EBV infection \((18/35 [51%]; \text{see also Supplementary Tables 2 and 3})\).

Significantly more patients with EBV primary infection showed a high and/or persistent EBV load than patients with EBV reactivation/reinfection (Figure 2A). Thus, median EBV load peak and median EBV AUC were 7 and 14 times higher, respectively, in patients with EBV primary infection than in those with EBV reactivation/reinfection (Figure 2B). The rate of antiviral prophylaxis did not differ significantly \((P = .79)\) between patients with EBV primary infection \((13/27 [48%])\) and those with EBV reactivation or reinfection \((15/28 [54%])\).  

**EBV Serology**

EBV seroconversion in EBV-naive patients occurred at 7.5 ± 2.3 months after transplant and at 4.9 ± 3.3 months after initial EBV viremia. The mean peak anti-EBV IgG titer was numerically \((P = .10)\) higher in symptomatic \((322 ± 355 \text{ U/mL})\) than in asymptomatic \((184 ± 252 \text{ U/mL})\) patients with EBV infection. Interestingly, 5 of 27 (19%) patients contracting EBV primary infection did not undergo EBV seroconversion (IgG, IgM, early antigen, viral capsid antigen, Epstein-Barr virus nuclear antigen [EBNA]) during the first posttransplant year. Five of 63 (8%) patients experienced an at least transient loss of their EBV-specific antibodies; in 2 of them, EBV reactivation/reinfection was diagnosed shortly after EBV-specific antibody loss. Overall, the rate of EBV reactivation/reinfection did not differ between patients with \((2/5 [40%])\) and without \((26/58 [45%])\) EBV-specific antibody loss \((P = 1.00)\).

**Impact of Immunosuppressive Therapy**

The type of calcineurin inhibitor (CNI) did not influence the incidence of EBV infection: 33% tacrolimus (TAC) – vs 39% cyclosporine microemulsion (CSA)–treated patients contracted an EBV primary infection \((P = .86)\), and 29% TAC–vs 32% CSA–treated patients experienced an EBV reactivation/reinfection \((P = .76)\). Additionally, the rate of EBV infection did not differ between patients with \((15/24 [63%])\) and without \((40/82 [49%]; P = .34)\) interleukin 2 receptor antibody induction. CsA or equivalent prednisone dosage and TAC or CSA predose levels had no relevant impact on the development of EBV infection (data not shown). However, univariate analysis revealed that a higher TAC dosage (odds ratio [OR], 1.34; 95% confidence interval [CI], 1.05–1.71; \(P = .02\)) and overall immunosuppressive load (OR, 1.23; 95% CI, 1.05–1.46; \(P = .01\)) estimated by means of the modified Vasudev score, constituted significant risk factors of EBV infection, whereas a higher MPA predose concentration (OR, 0.59; 95% CI, .41–.84;
EBV Infection in Pediatric Renal Transplantation

Determinants of Symptomatic EBV Infection

In the univariate analysis, the presence of HLA-A19 (OR, 3.19; 95% CI, 1.00–10.1; \( P = .04 \)) or HLA-DR7 (OR, 4.77; 95% CI, 1.60–14.2; \( P = .01 \)) alleles in the recipient was a significant risk factor for a symptomatic EBV infection. In the multivariate analysis, in which HLA-A19, HLA-DR7, the modified Vasudev score, antiviral prophylaxis, and donor and recipient EBV serostatus were considered as covariates, only HLA-DR7, the Vasudev score, and an EBV high-risk constellation (EBV D+/R−) were identified as independent significant factors (Figure 4). Carriers of HLA-DR7 developed a symptomatic EBV infection significantly more often (9/14 [64%]) than HLA-DR7–negative patients (8/41 [20%]; \( P = .01 \)).

Transplant Function and Acute Rejection

No significant difference was found in the rate of treated or biopsy-proven acute rejections (BPARs) in the first year after transplant than EBV infection–free recipients. The overall immunosuppressive load (modified Vasudev score) was comparable between patients with and without CHL both at the time of discharge after engraftment (12.0 ± 2.9 vs 11.0 ± 3.0; \( P = .21 \)) and at 12 months after transplant (7.5 ± 1.7 vs 6.8 ± 2.1; \( P = .15 \)).
transplant between patients contracting an EBV infection (primary infection or reactivation/reinfection) and those remaining EBV infection-free. The incidence of BPARs including borderline changes in the first year after transplant was comparable in patients with EBV infection 19/58 (33%) and those without (15/48 [31%]; \(P = .91\)). The mean eGFR loss during the first and second posttransplant year did not differ significantly between patients with EBV infection and those without (first year, \(-3.7 \pm 11.6\) vs \(-0.8 \pm 8.6\) mL/min/1.73 m\(^{2}\), \(P = .14\); second year, \(-8.2 \pm 11.5\) vs \(-4.7 \pm 10.2\) mL/min/1.73 m\(^{2}\), \(P = .11\)). Additionally, mean urinary protein excretion was comparable between these 2 groups during the first 2 years after transplant (first year, 157 \pm 149 vs 122 \pm 177 mg/m\(^{2}\)/d, \(P = .27\); second year, 116 \pm 109 vs 157 \pm 169 mg/m\(^{2}\)/d, \(P = .71\)).

**DISCUSSION**

We report here the largest prospective, multicenter study investigating the epidemiology and morbidity of EBV infection in pediatric renal allograft recipients. A subgroup analysis investigating the influence of an antiviral prophylaxis on EBV primary infection in high-risk patients was reported recently [11]. Data of these high-risk patients have also been included in the present analysis. The main finding of our study is that presence of high-risk serostatus for EBV infection, HLA-DR7, and intensity of immunosuppressive therapy were significant risk factors for EBV-related morbidity, whereas degree or duration of EBV load was not. Fifty-six percent of CHL carriers exhibited EBV-associated flu-like symptoms or infectious mononucleosis, while 44% remained asymptomatic. Only 1 of 18 (6%) CHL carriers developed a PTLD, while the vast majority (94%) of CHL carriers remained PTLD-free throughout a mean observation period of 5 years. A previous retrospective study in 36 pediatric liver transplant recipients with CHL observed that 39% had EBV disease (17%) or PTLD (22%) prior to development of the CHL state [21]. In 69% of patients, the CHL carrier status resolved without progression to PTLD, and 28% retained their CHL status without evidence of EBV-related clinical symptoms. Only 1 patient (1/10 [10%]) contracted PTLD while being a CHL carrier [21]. However, another retrospective analysis, conducted by the same investigators, among 20 pediatric heart transplant recipients showed progression to PTLD in 45% of CHL carriers [22]. Hence, the significance of the CHL carrier status as a risk factor for the later development of PTLD appears to depend on the patient population and the intensity of immunosuppressive therapy. Taken together, the presence of a high or persistent EBV load alone in pediatric renal transplant recipients appears not to be predictive for the later development of a PTLD. Other factors may determine this risk, such as the intensity of immunosuppressive therapy [23], the EBV virulence [24], the nature of EBV-infected B cells [25, 26], the EBV-specific T-lymphocyte response [27], and a genetic predisposition [28].

We observed that EBV-specific serological analyses are diagnostically not conclusive in immunosuppressed patients, which is consistent with previous observations [29, 30]. Our observation that the mean anti-EBV IgG titer was numerically higher in symptomatic than in asymptomatic patients is in line with the assumption that EBV-related symptoms are mainly caused by the host’s anti-EBV immune response rather than the cytopathic effect of the virus itself [31, 32].

We sought to determine the impact of immunosuppressive therapy on the development of an EBV infection and observed that a higher TAC dosage and the overall immunosuppressive load, estimated by the modified Vasudev score, constituted significant risk factors of EBV infection, whereas a higher MPA predose concentration was associated with a lower risk. The latter finding is interesting, because MPA has been shown to inhibit proliferation of EBV-transformed B-cell lines in vitro [33] and to strongly potentiate the anti-herpesvirus activities of antiviral drugs [34]. A prospective clinical study in 115 adult kidney transplant recipients investigated the relationship between clinical parameters and EBV load [35]; patients receiving mycophenolate mofetil (MMF) carried a significantly lower risk of EBV viremia compared to those without MMF (hazard ratio, 0.52; 95% CI, .31–.88; \(P = .014\)). The potential protective effect of MMF is backed by another trial examining EBV load in 172 adult heart transplant recipients. The overall incidence of EBV viremia was significantly \((P < .01)\) associated with the use of CNI and azathioprine, and with the absence of MMF treatment [35].

We also analyzed potential determinants of a symptomatic EBV infection in this patient population and identified an EBV high-risk constellation (EBV D’/R’), HLA-DR7, and the Vasudev score as independent significant risk factors. Hence, carriers of the HLA-DR7 allele appear to have a genetic predisposition of developing a symptomatic EBV infection. Previous in vitro studies have shown that HLA-DR7 induces an efficient T-helper cell reaction through presentation of EBNA1 epitopes on antigen-presenting cells [36, 37]. Also, a multicenter case-control study comparing a group of 155 adult PTLD patients with 1996 controls after solid-organ transplantation revealed a significant negative association between the expression of HLA-DR7 (OR, 0.46; 95% CI, .28–.78; \(P < .004\)) and PTLD [28]. Therefore, it is tempting to speculate that pediatric renal transplant recipients expressing HLA-DR7 develop a symptomatic EBV infection more often due to a more pronounced anti-EBV immune response, which causes the EBV-related symptoms rather than the cytopathic effect of the virus itself [31, 32]. In the long term, this stronger host’s anti-EBV immune response may afford protection against PTLD.
A previous study had observed an association between subclinical EBV infection and impaired graft function in pediatric renal transplant patients [38]. In our study, no significant difference of eGFR loss was found between patients with and without EBV infection. However, our study was not designed to detect such a relationship, and we also did not perform protocol biopsies of the renal allograft.

In conclusion, our data in pediatric renal transplant recipients indicate that presence of high-risk serostatus for EBV infection, HLA-DR7, and intensity of immunosuppressive therapy are significant risk factors for EBV-related morbidity, whereas degree or duration of EBV load is not. The vast majority of patients with a high, persistent EBV load (ie, CHL carrier), remain PTLD-free throughout a 5-year follow-up. The finding that HLA-DR7–positive patients develop a symptomatic EBV infection more frequently than HLA-DR7–negative recipients is possibly due to a more pronounced anti-EBV immune response, which, in the long run, may protect against PTLD.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary materials are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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References