Immune response to herpesvirus infections in immunocomprised children
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Chapter 3

Spontaneous outgrowth of EBV-transformed B cells reflects EBV-specific immunity in vivo; a useful tool in the follow-up of EBV-driven immunoproliferative disorders in allograft recipients

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Abstract
During immunosuppressive medication, Epstein-Barr virus (EBV) infection is associated with a risk of developing posttransplant lymphoproliferative disease (PTLD). The appropriateness of a spontaneous EBV B cell transformation (SET) assay as a monitor of EBV-specific immunity was evaluated to investigate if it safely allows reducing immunosuppressive medication, thereby decreasing the risk of developing PTLD. PBMCs were isolated longitudinally from 20 pediatric renal allograft recipients treated with prednisone and cyclosporine combined with either azathioprine or mycophenolate mofetil. Most significantly, EBV-peptide-specific CD8$^+$ T cells were detectable in blood from patients with negative SET assays, coinciding with significantly lower EBV loads, whereas these cells were less frequent in the blood from patients with positive SET assays. Reducing the levels of immunosuppression resulted in normalization of the SET assays. Therefore, the SET assay is a reflection of the interaction between viral replication, transformation of B cells, and EBV-specific immunity in vivo and hence a valuable screening test for EBV-driven lymphoproliferative phenomena in allograft recipients.

Introduction
The remarkable improvement in the survival rates of solid organ transplant patients is to a large extent due to the increased use of immunosuppressive medication. Most significantly, the introduction of the immunosuppressive agent cyclosporine has led to the increased success of allograft function over longer time ranges. Intensified use of immunosuppression has however concomitantly resulted in an increase of the complications from infectious disease in allograft recipients during primary infections and in reactivation of latent viruses of the Herpesviridae.

In the setting of immunosuppressive medication, both primary Epstein-Barr virus (EBV) infection and, to a lesser extent, EBV reactivation, are associated with the risk of developing post-transplant lymphoproliferative disease (PTLD) [1]. PTLD is a constellation of various potentially fatal diseases, ranging from polyclonal B-cell proliferation, which regresses when immunosuppression is stopped, to the aggressive monoclonal B-cell lymphomas that usually appear to be resistant to therapy [2,3].

Insight into the mechanism of viral reactivation is rudimentary. While reactivation of herpes simplex virus (HSV)-type 1 and varicella-zoster virus (VZV) may occur as oro-labial disease or shingles during an acute bacterial infection or moments of severe physical or emotional stress, reactivation of EBV and cytomegalovirus (CMV) occur only rarely, if ever, in healthy individuals [4]. Cytotoxic CD8$^+$ T lymphocytes (CTLs) are thought to be essential for suppression of the viral replication and dissemination of viral agents [5]. Without these CTLs, EBV-infected B cells would proliferate in an unlimited fashion, inducing the B cells to produce their own growth factors among which are IL-6 or (viral) IL-10 [6].

The interaction between EBV and host immune factors can be evaluated by assessing spontaneous EBV transformation of PBMCs [7]. We designed a prospective study in a cohort of renal transplant patients on different regimens of immunosuppressive drugs to evaluate the value of the spontaneous EBV B-cell transformation (SET) assay as a monitor of EBV-specific immunity in pediatric recipients of kidney allografts.
Materials and Methods

Study design
From January 1998 onward, we enrolled 20 patients with a renal allograft. Informed consent for a 2-monthly blood check for immune parameters was obtained from the parents of the children enrolled in the studies, as is required by the institutional medical ethical committee, in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

The patients were all under immunosuppressive regimens of prednisone (7.5 mg/m²) and cyclosporine (plasma trough levels range: 100-150 ng/ml); or tacrolimus, when cyclosporin-related side-effects were found), and either azathioprine (AZA: 1-2 mg/kg), or mycophenolate mofetil (MMF: 1.2 g/m²/day in two doses). The choice among the regimes was arbitrary and non-randomized. EBV-reactivation was defined by serology: i.e. at least a fourfold increase in titer of anti-early antigen (EA) in the absence or disappearance of anti-Epstein-Barr virus-encoded nuclear antigen (EBNA)-1 antibodies in immune (anti-viral capsid antigen (VCA) IgM negative, anti-EA antibody negative, and anti-EBNA1 antibody positive) individuals [8].

Laboratory tests for EBV infection
Specific IgM and IgG antibodies against EBV-VCA and EBV-EA were determined by indirect immunofluorescence. Antibodies to EBNA1 were determined by anticomplement immunofluorescence (Gull Laboratories, Salt Lake City, USA) ELISA.

Quantification of EBV load
In a limited number of samples, their was sufficient material to retrospectively quantify the virus concentration of EBV in blood, using an adaptation of the previously reported quantitative PCR for EBV-DNA [9], combined with the electrochemiluminescence assay as described before by R. Boom and coworkers [10]. Viral loads were determined as number of copies per ml blood.

Lymphocyte subsets
Absolute numbers of B cells (CD19+) and T cells (CD3+, CD4+, CD8+) were determined longitudinally by standard FACScan procedures on a FACScan (BD Biosciences, San Jose, CA); the MoAbs were produced by Sanquin Research (Amsterdam, The Netherlands).

Spontaneous EBV B cell transformation (SET) assay
After standard purification steps and several washes, PBMCs were cultured in two 96-well plates at 1x10⁵ PBMCs per well for 4 to 6 weeks. At least 60 wells were plated per patient (range 60-248, median 114). By the use of this low cell number per well, spontaneous transformation of PBMCs in healthy seropositive controls cannot be observed, as described before [7].

Proliferation of B cells was in no cases observed in PBMCs from individuals with acute EBV (IgM+, EBV-DNA load >10,000 copies/ml blood; n=5), healthy EBV-seropositive (VCA IgG/EBNA1 IgG+; n=20), and seronegative donors (VCA IgG+/EBNA1 IgG-; n=20). The outgrowth of B cell blasts of these healthy individuals was only observed when infected in vitro by exogenous EBV, prior to the addition of PHA. In the standard SET assay, positive controls (exogenous EBV added) were included in each experiment. Culture medium containing PHA (1 μg/ml) was refreshed weekly. PHA was added to the cultures to exhaust T cells in these long-term cultures and thereby exclude the influence of cytotoxic T cells on the lymphoproliferation in vitro to ensure that the outcome of the SET assay is purely a reflection of the capacity of EBV to transform B cells in vivo [11]. As shown in Fig. 1, B-cell numbers remained constant over time, with no difference between the MMF- or AZA-treated patients. The number of positive wells were scored by light microscopy after 4-8 weeks of culture and expressed as a percentage of total wells. Resulting B cell lines were tested for clonality using IgH DNA rearrangement [12]. Toxic effects of AZA or MMF per se on B-cell transformation and outgrowth capacity was excluded in this assay by the positive EBV transformation result upon addition of exogenous infectious virus.
Figure 1 The absolute number of total lymphocytes and CD19+ B cells in pediatric renal transplant patients. There was no significant difference, neither in absolute CD4+ nor in CD8+ T-cell sub-populations between the AZA- and MMF-subgroups. Furthermore, B-cell numbers remained constant over time. Numbers are expressed as mean \pm SD (10^6 cells/ml) over time.

Enumeration of cytotoxic effector cells

HLA-typing information of the allograft recipients as performed prior to transplantation was used to define the HLA-A*0201-positive (n=8) and HLA-B8 positive (n=1) patients in our cohort. None of the HLA-A*0201 individuals were HLA-B8 positive and vice-versa. In HLA-A*0201 positive individuals, EBV-specific CD8+ T cells were quantified by use of an APC-labeled tetramer (kind gift from Dr. D. van Baarle) containing HLA-A*0201, β2-microglobulin and the peptide GLCTLVAML, which is derived from the lytic protein BMLF-1. BMLF-1-specific CD8+ T cells can be detected with this tetramer during acute infection as well as during latency [13]. In the same manner, the EBV-specific CD8+ T cells of the HLA-B8-positive individual were quantified by use of a tetramer containing the peptide RAKFKQKL, derived from the lytic protein BZLF1. PBMCs from an HLA-A*0201/HLA-B8 seropositive healthy donor were included in each experiment to standardize the assay. To identify CD8+ T cell subsets, cells were subtyped by use of a combination of CD45RA and CCR7 MoAbs. For staining with the mouse anti human CCR7 mAb, a three step staining protocol was performed consisting of incubation with the CCR7 antibody (Pharmingen, San Diego, CA) for 30 min, washing, incubation with biotinylated goat anti mouse IgM (Pharmingen) for 30 min, followed by incubation with streptavidin-PE (BD Biosciences). Next, PBMCs were incubated with 10% (v/v) normal mouse serum (Sanquin), directly conjugated mAbs and tetrameric complexes for 30 min.

Determination of functionality of the EBV-specific CD8+ T cells

Functionality of EBV-specific CD8+ T cells as defined by production of IFN-γ was determined by enzyme-linked immunospot assay (ELIspot) (Mabtech, Nacka, Sweden). Thawed PBMCs of HLA-A*0201 positive donors were resuspended in RPMI, containing 10% FCS and antibiotics. For the EBV-specific ELIspot, resuspended PBMCs isolated from HLA-A*0201 positive donors were stimulated with the HLA-A*0201 restricted peptide GLCTLVAML (10 μg/ml), as well as with the HLA-B8 restricted peptide RAKFKQLL (negative control) (10 μg/ml) at 37°C and 5% CO2 in α-IFN-γ precoated silent screen 96-wells plates (Nalge Nunc, Rochester, NY). In the same manner, resuspended PBMCs isolated from the HLA-B8 positive donor were stimulated with the HLA-B8 restricted peptide RAKFKQLL (10 μg/ml), as well as with the HLA-A*0201 restricted peptide GLCTLVAML (negative control) (10 μg/ml). PBMCs were cultured in final amounts of 250000, 125000 and 62500 PBMC/well. After 18 h, cells were removed by washing with PBS and wells were incubated with biotin-labeled α-IFN-γ for 3 h at RT. Thereafter, the wells were incubated with streptavidin conjugated alkaline phosphatase (ALP) for 2 h at RT. Finally, spots were visualized by incubation with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT; Sigma Chemical, St. Louis, MO). Spots were quantified by the AELVIS ELIspot reader and software (AELVIS, Hannover, Germany). Results are expressed as spot forming units (SFU) per 10^6 CD8+ T cells (mean of duplicate cultures) after correction for background staining. All peptides used in this study were purchased from the IHB-LUMC peptide synthesis library facility (Leiden, The Netherlands). The peptides were generated by standard Fmoc techniques and purified by ether precipitation and HPLC techniques. The peptides were
dissolved in dimethylsulfoxide (DMSO; Merck, Darmstadt, Germany) in a concentration of 5 mg/mL. PBMCs from an HLA-A*0201/HLA-B8 seropositive healthy donor were included in each experiment to standardize the assay.

**Statistics**
Student’s t test and ANOVA were applied for normally distributed data, and the Wilcoxon two-sample test and Kruskal-Wallis test for non-normally distributed data. Fisher’s exact test was used for statistical analysis of symptoms in the patient cohorts.

**Results**

**Clinical description of the study groups**
The study groups were formed according to the immunosuppressive regimen of the participants. In addition to the formerly standard of corticosteroids with cyclosporine (or tacrolimus (FK506) in case of cyclosporine toxicity), patients received either (1) azathioprine (AZA), or (2) mycophenolate mofetil (MMF). Both groups’ members were of similar age and received the same duration of follow-up (Table 1). Although five patients had a primary EBV infection after undergoing transplantation, episodes of symptomatic EBV infection during the period of sequential monitoring occurred only once. Symptoms were acute fever, hepatitis, and EBV-related biopsy-proven acute rejection of the allograft (AZA#2). The number of patients suspected of EBV-reactivation in our cohort by serological criteria was seven in total (35%), of whom five were in the AZA-group and two in the MMF-group (Table 1).

<table>
<thead>
<tr>
<th>Table 1 Study population</th>
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<tbody>
<tr>
<td>n</td>
</tr>
<tr>
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</tr>
<tr>
<td>AZA 10</td>
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<td>MMF 10</td>
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</table>

1 EBV reactivation is defined as at least a fourfold increase in titer of anti-EA in the absence or disappearance of anti-EBNA-1 antibodies in immune individuals

**Detection of EBV-specific immunity by the SET assay**
The use of the SET assay was evaluated as a measurement of the potential infectious activity of EBV in direct relation to the cytotoxic potential of PBMCs to suppress EBV-induced B cell infection and transformation in vivo. The SET assay is both sensitive and specific. The SET assays remained negative in healthy seropositive controls and recently infected individuals ([14], P.A. Baars, unpublished). All individuals turned positive after in vitro infection by exogenous EBV, prior to the addition of PHA. Furthermore, reproducibility of the SET assay is high. For example, PBMCs from three donors, each obtained 2 days apart, resulted in SET positivities of 6.9 and 7.6%, 1.2 and 1.4% and twice 0%, respectively. An illustration of the value of this assay in the follow-up of immunosuppressive medication is shown in Fig. 2. In this patient (AZA#1) with the highest positive SET assay results measured over 6 consecutive months (outgrowth of PBMCs was detected in up to 74% of the wells), a decrease in positive wells only occurred after reduction of the immunosuppressive medication (Fig. 2).

In the AZA group, five patients were found with outgrowth of B cell lymphoblasts (B-LCL), versus two in the MMF group (Table 2). Some remained positive over several months, while others were positive during short periods of 2-4 months. The B-LCL of these six patients showed an oligoclonal pattern that changed over time, thus making early development and
detection of persistent PTLD less likely (not shown). A weak correlation between positive SET assay and negative EBNA-serology (and vice-versa) in EBV-positive patients was observed in our cohort (r=-0.40) (Table 2).

![Figure 2 Spontaneous outgrowth of endogenously infected EBV-transformed B cells (SET assay) in relation to immunosuppression.](image)

Also, when anti-EBNA1 and anti-EA serology versus outcome of the SET assays were analyzed longitudinally after EBV infection, no definite correlation was observed (Fig. 3). This is best illustrated in the same patient with the strongly positive SET assays (AZA#1, see also Fig. 2); anti-EBNA1 remained negative, anti-EA became positive at the moment the SET assays normalized (Fig. 3A). Another patient with negative anti-EBNA1 serology and rising titers of anti-EA (AZA#9) who could be serologically expected to have incomplete EBV clearance and potential reactivation ([8]; see Materials & Methods) remained negative in the SET assays over time (Fig. 3B). The results of the SET assay seemed to correspond to the plasma levels of cyclosporine. In four patients, the spontaneous outgrowth disappeared when lower plasma levels of cyclosporine were accepted (data not shown). However, no correlation could be observed between the use of ATG as induction therapy and the positivity of SET assays (data not shown).

**Table 2 Correlation of EBV reactivation defined by serology and defined by spontaneous EBV-B cell transformation**

<table>
<thead>
<tr>
<th>AZA #</th>
<th>SET&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Anti-EA</th>
<th>Anti-EBNA</th>
<th>MMF #</th>
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<th>Anti-EBNA</th>
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</tr>
</tbody>
</table>

<sup>a</sup>—no positive wells detected in SET assay; + = positive wells detected in SET assay
SET assay as a measurement of EBV-specific immunity

Figure 3 Anti-EBNA1 and anti-EA serology versus outcome of the SET assays were measured longitudinally after EBV infection, to study a possible correlation between the outcome of the SET assays and EBV reactivation as defined by classical serology. (A) In a patient with strongly positive SET assays (AZA#1), anti-EBNA1 remained negative and anti-EA became positive at the moment the SET assays normalized. (B) Another patient with negative anti-EBNA1 serology and rising titers of anti-EA (AZA#9), who could be serologically expected to have incomplete EBV clearance and potential EBV reactivation, remained negative in the SET assays over time.

EBV-specific T-cell immunity

Since reactivation defined by serology correlated only weakly to the outcome of the SET assay, EBV-specific CD8+ T cell immunity was determined by both tetramer staining and by production of IFN-γ after stimulation with virus-specific peptides. An example of the enumeration of EBV-specific T cells by tetramer staining is shown in Fig. 4. EBV-specific CD8+ T cells could be detected in only one of three HLA-A*0201 positive patients with positive SET assays, whereas these EBV-specific CD8+ T cells could be detected in all of the HLA-A*0201 or HLA-B8 patients with negative SET assays (Table 3). The frequencies of EBV-specific CD8+ T cells were significantly different between the two groups (0.12 ± 0.21 for SET+ vs. 0.42 ± 0.13 for SET−; P=0.04). The detection of EBV-specific CD8+ T cells in the patient with a positive SET assay appeared to be of minor significance in terms of immunity, since EBV-specific CD8+ T cells were detected only at one single moment in a series of 7 time points studied (data not shown). Representative time curves of patients with negative and positive SET assays are shown in Fig. 5. Data of all patients analyzed are summarized in Table 3. After EBV infection, EBV-peptide-specific CD8+ T cells were found in the blood from the patient with negative SET assays up to a frequency of 0.6% of total CD8+ T cells. These EBV-specific T cells were mainly CCR7+. Furthermore, IFN-γ producing EBV-specific CD8+ T cells could be detected in all patients with tetramer-positive cells. The numbers of IFN-γ producing EBV-specific CD8+ T cells as measured by ELispot analysis were significantly different between the two groups (92 ± 19 for SET+ vs. 1416 ± 1704 for SET−; P=0.02).

Finally, significantly higher EBV viral loads (up to 92,580 copies/ml blood) were detected in patients with positive outcomes of the SET assays than in patients with negative outcomes of the SET assays (25,327 ± 34,859 vs. 3,387 ± 4,820 resp.; P=0.03; calculated as mean of highest EBV viral load detected in patients during follow-up) (Table 3).
Table 3 Parameters of EBV immunity in SET− vs. SET+ patients

<table>
<thead>
<tr>
<th>patient</th>
<th>peak EBV load (copies/ml blood)</th>
<th>peak % tetramer pos. CD8+ T cells</th>
<th>peak ELISpot (SFU/10⁶ CD8+ T cells)</th>
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<td>SET− (mean ± SD)</td>
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<td>0.42 ± 0.13</td>
<td>1416 ± 1704</td>
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<tr>
<td>SET+ (mean ± SD)</td>
<td>25620 ± 34859</td>
<td>0.12 ± 0.21</td>
<td>92 ± 19</td>
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*after seroconversion
n.a. = not applicable
n.d. = not done; patient was HLA-A*0201/B8*

Discussion

The development and evaluation of techniques for early detection and prevention of life-threatening lymphoproliferative diseases has become indispensable with the increased risk of PTLD associated with EBV infection due to the use of more potent immunosuppression. The effect of immunosuppressive medication on immune parameters relevant for EBV reactivation was prospectively studied in a cohort of pediatric renal allograft recipients. Serologic definition of EBV-reactivation was compared with an assay system in which spontaneous proliferation and outgrowth of EBV-transformed B cell lines were determined. We presume that in this SET assay, the test result is a reflection of the in vivo balance between infectious EBV, B cells, and cytotoxic lymphocytes (consisting of natural killer cells and CTL function) in the blood. The relevance of the SET assay was demonstrated by the fact that immunosuppression seemed to be directly linked to B-LCL generation in vitro, as illustrated by a patient in whom reduction of medication resulted in a complete normalization of the subsequent SET assays (Fig. 2).

As shown in this study, EBV-specific CD8+ T cell immunity could be detected more frequently in the patients who did not show spontaneous outgrowth of B cells in the SET assays in comparison to the patients with positive SET assays (Table 3), which correlated to IFN-γ production upon virus-specific stimulation and significantly lower EBV loads in the blood, reflecting the ability of these CD8+ T cells to control the virus. This correlation of the outcome of the SET assay with EBV-specific T cell immunity (Fig. 5) is stronger than the correlation —if
any- with EBV reactivation as defined by classical serology (Fig. 3). In the latent phase of viral infection, at least three different virus-specific memory populations can be discriminated: 1) CD45RO+ CD27+ CCR7+; 2) CD45RO+ CD27+ CCR7-; and 3) CD45RA+ CD27- CCR7- [15,16]. These subsets have important characteristics. Cytotoxic potential increases with loss of CD27 and the expression of CD27 appears to differ between CD8+ T cells directed to different viruses [17]. The EBV-specific CD8+ T cells detected in the patients were mainly CCR7-, which typifies them as effector-memory and effector-like CD8+ T cells [16]. EBV is a lymphocytotropic virus that resides largely in the lymph nodes. Although the loss of CCR7 correlates to high cytotoxic potential and increased IFN-γ production [16,18] these CCR7- cells have lost the capacity to re-enter secondary lymphoid organs by ligation to the ligands MIP3β (CCL19) and SLC (CCL21). This loss would impede their ability to cope with EBV-infected cells. However, it cannot be excluded that the virus-specific lymphocytes, e.g. EBV-specific T cells, detected in blood are part of different subsets than the virus-specific cells within the secondary lymphoid organs. Furthermore, during EBV reactivation, effector-type T cells regain expression of CCR7 [19] and are thereby facilitated to re-enter secondary lymphoid organs such as lymph nodes and tonsillar tissue, which are the target sites of these EBV-specific cells [20].

**Figure 4 Characterization of EBV-specific CD8+ T cells.** (A) Approximately 0.45% of the CD8+ T cells were specific for the EBV GLC-epitope derived from BMLF-1, as shown by tetramer staining gated on lymphocytes. (B) The EBV-specific CD8+ T cells of this patient were CCR7-, and mainly of the effector memory phenotype (i.e. CCR7+/CD45RA+). The summarized data of the characterization of the EBV-specific CD8+ T cells of the HLA-A*0201 positive patients is shown in Table 3. (C) Both CCR7+ and CCR7- populations were detected in total CD8+ T cells from the patient.

MMF has been reported to be very potent in its immunomodulating effects to prevent acute renal, lung and heart allograft rejection without many side-effects [21-23]. Although its use may imply stronger immunosuppression, its in-vivo effects can be positively judged, as is also reflected by our findings related to EBV. A difference in the incidence of reactivation of EBV, either defined by serology or SET assay, was established between the two subgroups in our cohort, which differed only in the immunosuppressive medication that was prescribed (AZA versus MMF) (Table 2). The SET assays remained largely negative in the MMF group. Moreover, the effect of MMF on EBV reactivation does not seem to be specific for EBV, since the number of CMV reactivations was also reduced in the MMF-group (data not shown). It has been presumed that MMF inhibits antibody production [24] and this could influence the determination of EBV reactivation by classical serology. However, anti-EBNA antibodies as well as anti-VCA antibodies could be detected in the patients treated with MMF without significant differences in titers (Table 2). Some reports even suggest potentiating anti-viral
effects of MMF [25,26]. Yet, since dissemination of reactivated VZV and CMV have been reported in patients treated with MMF [27,28], we do not think that MMF has a general antiviral effect. In our cohort an almost fatal primary VZV infection occurred in an MMF-treated patient resulting in multi-organ failure and acute allograft rejection (MMF#6). Thus, a profound explanation for this phenomenon is still lacking. Larger study cohorts are needed to determine the significance of the differences in lymphoproliferative disorders between patients treated with MMF or AZA.

Figure 5 EBV-specific CD8\(^+\) T cell immunity in relation to spontaneous EBV-transformed B cell outgrowth (SET). (A) Significantly higher EBV viral loads were present in the blood from patients with positive SET assays (SET +), compared with EBV viral loads in the blood from patients with negative SET assays (SET –) (25,328 ± 34,859 vs. 3,387 ± 4,820 resp., P<0.05). (B) Accordingly, higher amounts of EBV-specific CD8\(^+\) T-cells (up to 0.6% of CD8\(^+\) T cells) (circles) could be detected in the patients with negative SET assays, reflecting the capability of these cells to control viral replication. These cells exhibited effector functions, demonstrated by IFN-\(\gamma\) release in the EBV-peptide specific ELIspot assay (open squares). Data on time curves are derived from two representative patients. \(t=0\) represents time of EBV infection.

In conclusion, the SET assay is a valuable overall screening test for lymphoproliferative phenomena driven by EBV in allograft recipients. The absence of EBV-specific CD8\(^+\) T cells strongly correlated to spontaneous outgrowth of EBV-transformed B cells and high EBV loads, and vice versa. Furthermore, reducing the levels of immunosuppression resulted in normalization of the SET assays. Therefore, the SET assay indeed appears to reflect the interplay between viral replication and transformation of B cells, and EBV-specific immunity of the host in vivo. PCR-based quantification of EBV concentrations in blood must be evaluated in larger studies to fully understand its prognostic potential [29-31]. Determination of EBV viral load to predict the risk of development of PTLD is probably only indicative in combination with data on EBV-specific T-cell immunity [31]. After all, the final goal is to find applicable parameters to reduce immunosuppressive medication in a patient-tailored approach. The present study is a step toward such an approach.

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SET assay as a measurement of EBV-specific immunity

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