Chapter 5

EBV reactivations in recipients of allogeneic stem cell transplants are frequently caused by re-infections with exogenous EBV strains

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Abstract

Recipients of allogeneic stem cell transplants often have Epstein-Barr virus (EBV) reactivations which may progress to EBV-associated lymphoproliferative disorders. It is not known whether these EBV reactivations are true reactivations of the endogenous EBV strain or re-infections with an exogenous EBV strain. Fifty-three recipients of matched related or matched unrelated donor grafts were studied. EBV monitoring was based on a realtime TaqMan™ EBV DNA PCR assay in plasma. In 17 patients EBV DNA PCR monitoring was performed in peripheral blood mononuclear cells (PBMCs) as well. From all patients and family donors mouth washes (MWs) were collected pre-transplant. Both pre-transplant EBV DNA from MWs and post-transplant EBV DNA from plasma or PBMCs was successfully obtained in 6 patients. A nested PCR targeting the EBV LMP-1 C-terminus gene was used to determine sequence variations enabling EBV strain typing. In 3 of 6 patients the post-transplant EBV sequence pattern differed from the pre-transplant pattern, indicating a re-infection post-transplant with an exogenous strain instead of a reactivation of the original endogenous EBV strain. In the other 3 patients the endogenous strain was identified. EBV reactivations frequently result from a re-infection with an exogenous EBV strain instead of a true reactivation of the endogenous strain.

Introduction

Recipients of allogeneic stem cell transplants (SCT) often have Epstein-Barr virus (EBV) reactivations[1]. Patients with EBV reactivations are at risk for developing EBV-associated lymphoproliferative disorders (EBV-LPD)[2], especially when other risk factors are present like: 1) T cell depletion (TCD) of stem cells using monoclonal antibodies (Moabs) directed at T cells or T and NK cells or using E-rosetting, 2) use of unrelated or ≥ 2 HLA antigen mismatched related donors, 3) use of Antithymocyteglobulin for prophylaxis or treatment of acute graft-versus-host disease (aGVHD) and 4) treatment of aGVHD with anti CD3 Moabs[1,3].
Currently it is not known whether these EBV reactivations are true reactivations of the endogenous EBV strain or re-infections with an exogenous EBV strain. EBV strains can be identified by examining size variation of the EBV nuclear antigen (EBNA) proteins (ebnotyping) or genes[4,5]. Ebnotyping requires the production of EBV transformed lymphoblastoid cell lines (LCLs). This technique is laborious and not all EBV strains are capable of efficient transformation of B cells[4]. Others have used DNA restriction fragment length polymorphisms (RFLP) of EBV DNA[6,7] or sequence analysis of the latent membrane protein (LMP)-1 gene[8-11] to characterise EBV strains. Here we report results of the LMP-1 sequencing approach for EBV strain identification in recipients of partially T cell depleted allogeneic SCT, using a nested PCR and direct sequencing targeting the EBV LMP-1 C-terminus gene to determine sequence variations.

Materials and Methods

Patients For this study data of 53 consecutively treated patients receiving either stem cells from matched related donors (MRD, n=26) or from matched unrelated donors (MUD, n=27) were analysed. EBV monitoring was performed weekly by quantitative EBV DNA detection in plasma samples until day 180 post-transplant. In 17 patients EBV DNA detection was performed in PBMCs as well. From all patients and family donors mouth washes (MWs) were collected pre-transplant to acquire endogenous EBV DNA. In 6 patients pre- and post-transplant EBV DNA could be obtained and analysed.

Patients with acute leukaemia in first complete remission, chronic myeloid leukaemia (CML) in first chronic phase and untreated severe aplastic anaemia (SAA) were considered low-risk regarding transplant related mortality (TRM) and relapse. All patients with other diseases were considered high-risk. Transplantations were performed between April 2001 and May 2002 at the Department of Haematology of the University Medical Centre Utrecht. Patients were treated according to clinical protocols approved by the local investigation review board after informed consent was obtained.

Transplantation procedures, pre-emptive treatment of CMV reactivations and treatment of CMV disease were performed as described[12].

Conditioning regimen The conditioning regimen consisted of cyclophosphamide (60 mg/kg/day) on each of two successive days, followed by total body irradiation (TBI) (600 cGy/day) on each of 2 successive days, with partial shielding of the lungs (total lung dose 850 cGy). The graft was infused after the second TBI fraction (day 0). Antithymocyte-globulin (ATG) (Thymoglobulin™, Sangstat, Amstelveen, the Netherlands) was given to MUD patients before cyclophosphamide was infused, in a dose of 2 mg/kg/day for 4 days.

TCD In vitro TCD of bone marrow (BM) from unrelated donors was performed using the immunorosette depletion technique as described[12]. MRD patients received a peripheral blood stem cell (PBSC) graft. TCD of these G-CSF stimulated PBSC grafts was performed by positive selection of CD34+ cells (CliniMacs™, Miltenyi Biotec, Bergisch Gladbach, Germany). After these maximal TCD procedures the residual number of T cells was counted and nonmanipulated T cells (from a small BM/PBSC fraction that was set apart) were added to obtain the desired fixed low number of T cells (1-2 x 10^5 T cells/kg recipient weight).

HLA-matching In MRD recipient/donor pairs HLA-A, B and Cw matching was based on serological typing and HLA-DRB1 and DQB1 matching on low resolution molecular typing with sequence specific primers (SSP). In MUD recipient/donor pairs HLA-A, B and DRB1 matching was based on high resolution sequence based typing and HLA-Cw and DQB1 matching on low resolution molecular typing with SSP.

CMV monitoring CMV monitoring was based on a realtime TaqMan™ CMV DNA PCR assay in EDTA-plasma[13] and was performed weekly until day 180 post-transplant in CMV-seropositive recipient/donor pairs. In patients with active GVHD or with CMV reactivation, defined as a viral load (VL) > 400 copies (c) /ml, monitoring was performed twice a week. Pre-emptive antiviral treatment of CMV reactivations was instituted when VL was > 1000 c/ml.

EBV serostatus Serum samples were tested for IgG antibodies to Epstein-Barr viral capsid antigen (VCA) by an indirect immunofluorescence assay using slides coated with EBV-infected P3HR-1 cells. Individuals were considered positive when reactivity against EBV VCA could be detected at a serum dilution of 1:10 or higher.

EBV monitoring EBV monitoring was based on a realtime TaqMan™ EBV DNA PCR assay in plasma and was performed weekly until day 180 post-transplant in all patients. In 17 patients EBV DNA PCR monitoring was performed in PBMCs as well. In patients with active GVHD or with EBV reactivation, defined as a VL > 50 c/ml plasma, monitoring was performed twice a week. Pre-emptive treatment of EBV reactivations was instituted when the EBV VL was > 1000 c/ml and consisted of rituximab (Mabthera™, Roche, Basel, Switzerland), an anti CD20 monoclonal antibody, given as a single infusion (375 mg/m^2) as described[1,14]. Immunosuppressive medication was decreased or stopped if possible. Furthermore, valacyclovir, given to all patients prophylactically in a dose of 500 mg twice a day, was increased to 1 gram 3 times a day.

Mouth washes From all patients and family donors MWs were collected pre-transplant by gargling the mouth with 15 ml phosphate buffered salt (PBS) during 1 minute. After centrifugation of the MWs for 10 minutes at 900g, the cell pellet (resuspended in 400 μL PBS) and the supernatant were stored separately at -70° C until use. The cell pellet was used to detect EBV by realtime Taqman™ EBV DNA PCR assay. When EBV was detected in these MWs, also post-transplant MWs were collected.

Peripheral blood mononuclear cells In 17 patients EBV DNA PCR monitoring post-transplant was performed in PBMCs as well. EBV reactivation in PBMCs was defined as a VL > 50 c/10^6 cells. PBMCs were isolated from 9 ml of EDTA-anticoagulated peripheral blood by Ficoll-Paque density centrifugation (Amersham Pharmacia Biotech AB, Uppsala, Sweden) at 1000g for 10 minutes, without brake. PBMCs were washed twice with PBS and stored at -70° C.

DNA isolation DNA from plasma was isolated with the MagNA Pure LC™ total nucleic acid isolation kit - large volume (Roche Diagnostics, Almere, the Netherlands). DNA from MWs-cell pellets or PBMCs was isolated with the MagNA Pure LC™ DNA isolation kit I by an automated nucleic acid extractor (MagNA Pure extractor, Roche Diagnostics, Almere, the Netherlands).

Realtime TaqMan™ EBV DNA PCR A quantitative realtime EBV DNA PCR assay was performed as described by Niesters et al[15]. Test results were quantified by comparison with a standardised control (EBV B95-8, Advanced Biotechnologies Incorporated, Columbia, Md.). As an internal control a standardised quantity of phocine herpes virus was added to each sample. EBV test results < 50 c/ml were considered negative. In order to quantify EBV DNA in MWs-cell pellets and PBMCs an albumin realtime TaqMan™ PCR was performed in these samples as well. The primers used for the albumin PCR were 5'-TGA.AAC.ATA.CGT.TCC.CAA.AGA.GTT.T-3' (forward primer) and 5'-TCT.CTC.CTT.CTC.AGA.AAG.TGT.GCA.TA-3' (reverse primer). A fluorogenic probe (5'-TGC.TGA.AAC.ATT.CAC.CTT.CCA.TGC.AGA-3') was synthesised by PE Biosystems (Nieuwerkerk ad IJssel, the Netherlands) with a FAM reporter molecule attached to the 5' end and a TAMRA quencher linked at the 3' end. VL in PBMCs is depicted as $c/10^6$ cells.

EBV strain typing Because EBV DNA encoding the EBV LMP-1 gene is most variable, a nested PCR targeting the EBV LMP-1 C-terminus gene was used to determine sequence variations. The PCR was performed using a modified primer scheme according to Knecht et al[8]. Sequences of the outer primers 5 and 12 (primer 12 was modified) were: 5'-CTA.CAA.CAA.AAC.TGG.TGG.ACT-3', position (168.843-168.823) and 5'-AGA.CAG.TGT.GGC.TAA.GGG.AGT-3', position (168.059-168.039), respectively. Sequences of the inner primers 8' and 11 were: 5'-TGC.TCT.CAA.AAC.CTA.GGC.GCA-3', position (168.609-168.589) and 5'-TGA.TTA.GCT.AAG.GCA.TTC.CCA-3', position (168.075-168.095), respectively. The nested PCR product of the LMP-1 C-terminus PCR was used for sequencing as described[11]. Positions of the nucleotides were compared to the reference strain B95-8[16].

Statistical analysis Differences between groups were compared using Fisher's exact test or Pearson chi-square analyses, whichever was appropriate, in case of discrete variables. In case of continuous variables a Students t-test or Mann Whitney U test, whichever was appropriate, was used. Probability of EBV reactivation was calculated by the cumulative incidence procedure, death without EBV reactivation being the competing risk. The following variables were included in the analysis of prognostic factors: age, risk status (low vs high), recipient CMV serostatus (negative vs positive), donor type (related vs unrelated), graft characteristics (CD34+ cell count, T/B cell ratio), CMV reactivation (no vs yes), aGVHD (No-I vs II-IV). Univariate analyses were performed using the log rank test. Variables which showed to influence EBV reactivation at a level of $p<0.1$ were used in a multivariate Cox regression analysis. P values from regression models were calculated with the Wald test. The

post-transplant variables 'CMV reactivation' and 'aGVHD' were as well analysed as time-dependent covariates. Calculations were performed using SPSS/PC+ 10.0 (SPSS Inc, Chicago Il, USA).

Results

Patients (Table 1). Fifty-three patients were monitored weekly for EBV reactivation by quantitative EBV DNA detection in plasma. Twenty-six patients received grafts from MRDs, 27 from MUDs. MRD recipients were significantly older and were more often diagnosed with aGVHD grade II-IV compared to MUD recipients (44 vs 35 year and 50% vs 22%, respectively). The incidence of CMV reactivations was 4% in the MRD group compared to 22% in the MUD group, however, this difference was not significant.

EBV reactivations in plasma samples (Table 2). Among MRD recipients 7 (27%) patients showed an EBV VL > 50 c/ml. All 7 patients were EBV-seropositive and had a seropositive donor. Two of these 7 had a VL > 1000 c/ml and received anti B cell therapy with rituximab which resulted in clearance of plasma EBV DNA in one patient. The second patient suffered from aGVHD grade III and was given rituximab 3 times with intervals of one week. EBV VL was not responding. She finally died from pulmonary bleeding during severe aGVHD, without symptoms of EBV-LPD.

Among MUD recipients 16 (59%) patients showed an EBV VL > 50 c/ml. Twelve patient/donor pairs were EBV-seropositive (R+/D+), 2 patients were seropositive while their donor serostatus was unknown, one patient was seropositive and had a negative donor, one patient/donor pair was seronegative (R-/D-). Nine of these 16 had a VL > 1000 c/ml and received anti B cell therapy with rituximab which resulted in clearance of plasma EBV DNA in 6 patients. One of these 6 patients had lymphadenopathy and an EBV VL > 1000 c/ml at the same day. Pathologic and immunohistochemical examination of a lymph node was consistent with EBV-LPD. The seventh patient most probable was suffering from EBV-LPD with central nervous system (CNS) involvement. This patient received intensive immunosuppression because of aGVHD grade III and was treated with rituximab once, thereafter further treatment was refused and death resulted from rapid neurological deterioration. The two other patients showed increasing VL after treatment with rituximab given

Table 1 Patient characteristics

	MRD (%)	MUD (%)	P-value
No. of patients	26	27	
Age (range)	44 (21-53)	35 (17-55)	0.002
Diagnosis			ns
AML	4 (15)	8 (30)	
ALL	4 (15)	6 (22)	
CML	3 (12)	6 (22)	
SAA	1 (4)	0	
Other	14 (54)	7 (26)	
Risk status			ns
Low	10 (39)	6 (22)	
High	16 (61)	21 (78)	
EBV serostatus			ns
R+/D+	23 (89)	19 (70)	
R+/D?	0	4 (15)	
R+/D-	1 (4)	3 (11)	
R-/D+	2 (8)	0	
R-/D-	0	1 (4)	
CMV serostatus			ns
R+/D+	10 (39)	8 (30)	
R+/D-	4 (15)	7 (26)	
R-/D+	1 (4)	4 (15)	
R-/D-	11 (42)	8 (30)	
aGVHD			0.035
No-I	13 (50)	21 (78)	
II-IV	13 (50)	6 (22)	
CMV VL>1000 c/ml			ns
Yes	1 (4)	6 (22)	
No	25 (96)	21 (78)	

R/D = recipient/donor.

Table 2 Characteristics of EBV reactivations in plasma samples of MRD and MUD recipients

	MRD (n=26)	MUD (n=27)	P-value
No. of patients with EBV VL>50 c/ml (%)	7 (27)	16 (59)	0.018
No. of patients with EBV VL>1000 c/ml (%)	2 (8)	9 (33)	0.021
Median time to EBV reactivation (d; range)	109 (24-172)	57 (17-180)	ns
Median maximum viral load (c/ml; range)	190 (135-9958)	1788 (85-139.495)	ns
Median time to EBV DNA clearance after rituximab in complete responders (d; range)	10 (7-34)		

d = days.

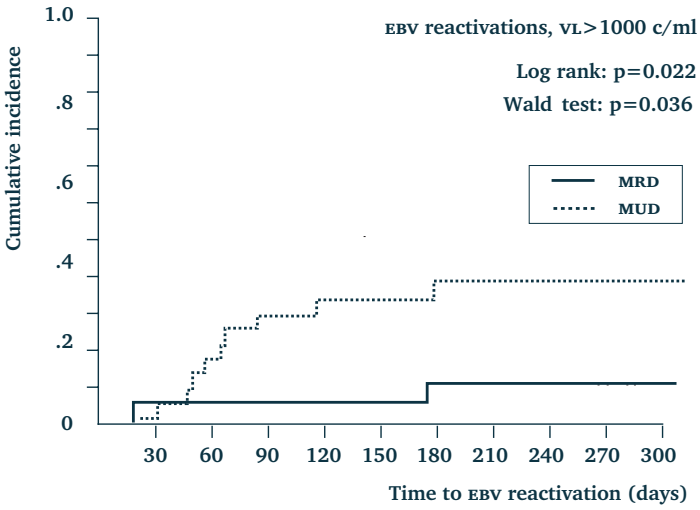
twice. Donor lymphocytes were infused (1 x 10^5 T cells/kg), resulting in a clearance of plasma EBV DNA in both patients.

Median time to EBV reactivation and median maximum VL showed a trend to be shorter and higher, respectively, in MUD compared to MRD recipients (57 vs 109 days and 1788 vs 190 c/ml, respectively, ns). Seven patients had complete responses after rituximab therapy. In 6 patients rituximab was given once, in the seventh patient twice. Median time to clearance of plasma EBV DNA after rituximab infusion was 10 days (range: 7-34). No recurrent EBV reactivations were diagnosed.

After multivariate analysis of prognostic factors for EBV reactivation (VL > 1000 c/ml) only CMV reactivation and a lower CD34+ cell count in the graft affected the incidence of EBV reactivation ($p<0.001$ and $p=0.072$, respectively). Multivariate analysis of only pre-transplant factors revealed that 'donor type' was the most important factor influencing the incidence of EBV reactivation ($p=0.036$, Figure 1). Since 6 of 7 CMV reactivations occurred in MUD recipients (see Table I), it is obvious there is an interaction between 'CMV reactivation' and 'donor type'.

Figure 1

EBV reactivations in recipients of MUD or MRD grafts.



EBV reactivations in plasma and PBMC samples (Table 3). In 17 patients (MRD: n=9; MUD: n=8) EBV monitoring was performed in plasma and PBMCs as well. Only 4 of 17 (23%) patients showed a reactivation in plasma samples compared to 15 of 17 (88%) in PBMC samples ($p<0.001$). Median time to EBV reactivation and median maximum VL showed a trend to be shorter and higher, respectively, in PBMC samples compared to plasma samples (49 vs 95 days and 5568 vs 2470 c/ml, respectively, ns). None of the patients with a reactivation in PBMCs only received rituximab and none progressed to EBV-LPD.

LMP-1 sequences of EBV in mouth washes and plasma or PBMC samples (Table 4)**.** In six patients pre-transplant EBV DNA from mouth washes and post-transplant EBV-DNA from plasma or PBMCs were successfully obtained and could be used for sequence analysis. In two of these, patient 5 and 6, also post-transplant mouth washes were analysed. Patient 1 and 2 were recipients of related grafts, only from donor 1 an EBV DNA positive mouth wash was obtained. Patients 3-6 were recipients of unrelated grafts, no donor mouth

Table 3 Results of double monitoring in plasma and PBMC samples of 17 patients

	Plasma	PBMC	P-value
No. of patients with EBV VL>50 c/ml (%)	4 (23)	15 (88)	<0.001
Median time to EBV reactivation	95	49	0.079
(d; range)	(47-159)	(17-131)	
Median maximum viral load	2470	5568	ns
(c/ml; range)	(135-12.175)	(190-937.500)	

washes were available. In 5 of 6 patients EBV serostatus was positive in both patients and donors. Patient 5 had a positive EBV serostatus, whereas donor serostatus was unknown.
In all 15 samples tested, a sequence pattern could be determined. Eight of these 15 patterns were unique. In 3 of 6 patients (patient 2, 3, and 5) the post-transplant EBV sequence pattern differed from the pre-transplant pattern, indicating a re-infection post-transplant with an exogenous strain rather than a reactivation of the original endogenous EBV strain. In patient 1 it is not possible to differentiate since pre-transplant, post-transplant and donor sequence patterns were identical. In patient 4 pre- and post-transplant sequence patterns were identical too, which indicates that a reactivation of the endogenous EBV strain is likely in this patient. Post-transplant EBV strains of patient 6 differed by only one nucleotide from the pre-transplant strain. This might have been the result of a point mutation during viral replication, making a definite conclusion about reactivation or re-infection impossible although it is more likely that this patient suffered a reactivation.
Finally, 3 of 53 recipients were EBV-seronegative pre-transplant. In 2 of these patients (1 MRD recipient with an EBV-seropositive donor; 1 MUD recipient with an EBV-seronegative donor) an EBV primo-infection was documented post-transplant.

Table 4 LMP-1 sequences of EBV in mouth washes and plasma or PBMC samples

Position	B95-8	Pat.1 MW Pre-SCT	Donor 1 MW	Pat.1 PBMC Post-SCT	Pat.2 MW Pre-SCT	Pat.2 PBMC Post-SCT	Pat.3 MW Pre-SCT	Pat.3 Plasma Post-SCT	Pat.4 MW Pre-SCT	Pat.4 PBMC Post-SCT	Pat.5 MW Pre-SCT	Pat.5 MW Post-SCT	Pat.5 PBMC Post-SCT	Pat.6 MW Pre-SCT	Pat.6 MW Post-SCT	Pat.6 PBMC Post-SCT
168357	C				G		A	G	A	A	G	G	G	A	A	A
168356	A						C					G	G			
168355	A								T	T				T	T	T
168352	G										T					
168341	T							A								
168339	G	C	C	C		C										
168337	A														C	C
168330	G						C									
168329	G						A									
168320	A				G			G	G	G	G	G	G	G	G	G
168309	T						C									
168308	T				C		C	C	C	C	C	C	C	C	C	C
168295	A				T		T	T	T	T	T	T	T	T	T	T
168293	G				A											
168279	C								-	-		A	A	-	-	-
168275	A								-	-	C			-	-	-
168269	G								-	-		C	C	-	-	-
168267	C						A		-	-				-	-	-
168266	A				G			G	-	-	G	G	G	-	-	-
168257	G				A				-	-				-	-	-
168253	T	C	C	C		C										
168248	A						C									
168238	G				A			A			A	A	A			
No. of differences		0			10		11		0		5			1		
Donor type		MRD			MRD		MUD		MUD		MUD			MUD		
SS R/D		+/+			+/+		+/+		+/+		+/?			+/+		

SS= EBV serostatus; R/D= recipient/donor.

Discussion

Our study shows that EBV reactivations in recipients of partially TCD SCT occur very frequently, especially among recipients of MUD transplants. When EBV DNA was monitored in PBMCs, significantly more patients showed EBV reactivations compared to plasma samples. In all patients with a PBMC EBV reactivation only, a spontaneous clearance of EBV DNA was observed and none progressed to EBV-LPD. Of 11 patients with a plasma EBV reactivation and a VL > 1000 c/ml, two developed EBV-LPD (18%) despite pre-emptive therapy with anti CD20 Mabs. In 6 patients, LMP-1 sequences could be determined in pre- and post-transplant samples, enabling EBV strain identification. In 3 of 6 patients the post-transplant EBV strain differed from the pre-transplant strain, indicating a re-infection with an exogenous strain.

In patient 1, who received a graft from a matched related donor, the pre-transplant, post-transplant and donor strains were identical. It is known from other studies[6,17] that intrafamilial spread does play an important role in EBV transmission. In these two studies, EBV DNA RFLP and size variation of the EBNA proteins expressed in EBV transformed lymphoblastoid cell lines (ebnotyping) were used to distinguish EBV strains. It was found that different members of the same family could carry the same EBV strain and that a given EBV strain was observed only within a single family. All unrelated individuals (apart from one husband and wife) did carry individually distinct viral strains[17]. In the setting of allogeneic SCT, in 4 of 6 related patient/donor pairs similar strains were isolated pre-transplant in patients and donors[18]. Therefore, our finding of identical recipient and sibling donor strains is not unusual and does not allow differentiation between reactivation and re-infection. Identical strains in pre- and post-transplant samples were also found in patient 4, a MUD recipient. These results also indicate the feasibility of LMP-1 sequence analysis for strain identification.

The technique we used to characterise EBV strains has also been used by others[9-11]. In those studies and the present report the EBV strain found in patient 1 and 4 is relatively common, making a definite exclusion of a re-infection impossible. All other identified strains showed unique sequences. The finding that a specific EBV strain was carried by several unrelated individuals, is different from data published by Gratama et al[17] and Katz et al[6]. These authors showed that all unrelated individuals carried individually distinct viral strains, when strain identification was performed by ebnotyping or RFLP.

Table 5 Results of studies performing EBV strain typing in patient/donor pairs

Study	No. of patients	Origin of EBV strain post-transplant	
		Endogenous	Exogenous
Gratama[4]*	2	0	2 (1 donor, 1 husband)
Gratama[18]*	1#	1	0
Van Kooij[11]**	1	1	0
Present study **	5#	2$	3

* = EBV strain typing with "Ebnotyping"; ** = EBV strain typing using LMP-1 sequence analysis; # = In both studies more patient/donor pairs (n=4 in Gratama et al; n=1 in the present study) were analysed. These pairs were related and pre-, post-transplant and donor strains were identical. Therefore, no differentiation was possible between endogenous reactivation or exogenous re-infection; $ = Exogenous re-infection is unlikely, however, has not been excluded.

The post-transplant EBV strains of patient 6 differed by only one nucleotide from the pre-transplant strain. This might have been the result of a point mutation acquired during viral replication, making it more likely that this patient suffered a reactivation instead of a re-infection.

In combination with previously published data, the present data show that the original EBV strain could no longer be detected post-transplant in 5 of 9 patients (see Table 5). We conclude that in these patients a re-infection with an exogenous strain has occurred. It is well known that EBV persistence requires the establishment of a latent infection in circulating B cells. As EBV replicates poorly in B cells in vitro, oropharyngeal epithelial cells have long been considered to be the site of EBV replication[19,20,21]. If the oropharyngeal epithelium is a permanent EBV reservoir, one would expect the pre-transplant EBV strain of the recipient to persist after SCT. The absence of the pre-transplant EBV strain in 5 of 9 patients, however, is inconsistent with that assumption. Furthermore, in patients with X-linked agammaglobulinemia, who are deficient in mature B cells, no evidence of past or present EBV infection in mouth washings and peripheral blood samples could be found[19,20]. However, our findings can not prove the eradication of endogenous strains, since multiple infections do occur in immunocompromised patients[22-25] and the original endogenous strain might have become non-dominant.

In 20 healthy asymptomatic EBV carriers, EBV strains present in mouth washes and peripheral blood were recently found to differ very often[26]. These authors used a very sensitive heteroduplex tracking assay. Their findings are not supported by other studies among a total of 267 healthy individuals[23,27]. We were able to compare EBV strains from mouth washes with strains from PBMCs in two patients. In both patients, EBV strains from the two compartments were identical. Therefore, current knowledge does not support the hypothesis of compartmentalisation of EBV infections.

Analysis of two solid organ transplant recipients with EBV-LPD, showed that EBV transmission from donor cells to host cells is possible. The EBV strain identified was of donor origin, while in both patients the lymphoma resulted from proliferation of B lymphocytes of recipient origin[7]. This finding suggests that, apart from EBV transmission from transplant B cells to host cells, other routes of transmission (oral or by transfusions) are possible as well, which is supported by the documented primo-infection of our EBV-seronegative (Recipient-/Donor-) patient.

In conclusion, EBV reactivation is a very frequent event, especially in recipients of TCD grafts from matched unrelated donors and it frequently results from a re-infection with an exogenous EBV strain instead of a true reactivation of the endogenous strain.

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