Available online at www.sciencedirect.com
ScienceDirect
Transplant Immunology 17 (2007) 203–210
Transplant Immunology
www.elsevier.com/locate/trim

Quantitative analysis of EBV-specific CD4/CD8 T cell numbers, absolute CD4/CD8 T cell numbers and EBV load in solid organ transplant recipients with PLTD

Kathrin Sebelin-Wulf [a], Tuan D. Nguyen [b], Stephan Oertel [a], Matthias Papp-Vary [a], Ralf Ulrich Trappe [a], Antje Schulzki [a], Antonio Pezzutto [a], Hanno Riess [a], Marion Subklewe [a,*]

[a] *Charité-Universitaetsmedizin Berlin, Campus Virchow Klinikum, Med. Klinik m. S. Haematologie/Onkologie, D-13353 Berlin, Germany*
[b] *Max-Delbrueck Center for Molecular Medicine, Berlin, Germany*

Received 24 July 2006; received in revised form 24 October 2006; accepted 31 October 2006

Abstract

Post transplant lymphoproliferative disease (PTLD) in solid organ transplant (SOT) recipients is assumed to be the result of impaired Epstein–Barr Virus (EBV)-specific cellular immunity. We analyzed the absolute CD4 and CD8 T cell counts as well as the EBV-specific CD4 and CD8 T cell responses in relation to EBV load in SOT recipients with PTLD. A prospective, single center study was initiated and 10 immunosuppressed patients with diagnosis of PTLD were analyzed and compared to 3 patients without PTLD (2 SOT recipients with EBV-reactivation, 1 patient with Infectious Mononucleosis) and 6 healthy EBV positive controls. EBV-specific CD8 T cells were enumerated using HLA class I tetramers and the IFN-γ cytokine secretion assay. EBNA1-specific CD4 T cells were analyzed after protein stimulation and EBV load was quantified by real-time PCR. Absolute CD8 T cell counts were highly variable in all 19 cases analyzed. In contrast, the absolute EBV-specific CD8 T cell count was found to be low in 7/9 patients with PTLD (<5/μl whole blood). These frequencies were similar to absolute EBV-specific CD8 T cell numbers observed in healthy EBV positive donors, but much lower compared to patients with EBV reactivation but no PTLD. Absolute CD4 T cell counts were significantly lower in PTLD patients (mean: 336/μl±161 vs. controls 1008/μl±424, $p=0.0001$), with EBNA1-specific CD4 T cell responses being also low, but highly variable. Moreover, low absolute CD4 T cell counts (<230/μl) were associated with an elevated EBV load (>1000 copies/μg DNA). We conclude that SOT recipients with PTLD have an inadequate functional EBV-specific T cell response. Our data suggest that the frequency and function of circulating EBV-specific CD8 T cells are dependent on absolute CD4 T cell counts. Further studies are needed to verify if a low absolute CD4 T cell count presents a risk factor for the development of PTLD in SOT recipients.

Keywords: EBV; PTLD; CD8 T cells; CD4 T cells

1. Introduction

Epstein–Barr virus (EBV) is an oncogenic γ-herpes virus that causes an asymptomatic life-long persistent infection in >90% of the world population [1,2]. Following primary infection in the oropharynx, EBV persists latently in B cells. The asymptomatic carrier state is maintained by a continuous T cell mediated immune control [1]. The importance of T cell surveillance is underlined by the increased incidence of post-transplant lymphoproliferative disease (PTLD) in immunosuppressed patients, e.g. marrow or organ transplant recipients who receive immunosuppressive therapy to prevent graft rejection [3,4]. Moreover, adoptive immunotherapy with EBV-specific cytotoxic T cells has been used successfully in the prophylaxis and treatment of PTLD after stem cell transplantation [5–7], and less successfully in recipients after solid organ transplantation (SOT) [8].

PTLD presents the most common malignancy after SOT [9] and has a high mortality rate approaching 50% at 1 year [10].

* Corresponding author. Tel.: +49 30 450 553 214; fax: +49 30 450 553 930.
E-mail address: marion.subklewe@charite.de (M. Subklewe).

0966-3274/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.trim.2006.10.006

Table 1
Patient characteristics

Pt#	Sex/ age	Transplanted organ	Tx to PTLD [mo]	HLA- (serological specificity)	Immunosuppressive drug regimen	Histology	Stage	EBV association		Response	Outcome
								EBNA2/ LMP1	EBER		
1	F/26	Kidney+ Pankreas	4	A3, A9, B27, B5, DR1, DR4, DQ1, DQ3	Prednison, Sirolimus Tacrolimus	Diffuse large B cell lymphoma (DLC-B-NHL)	IV ES	pos	nd	PD	Died under therapy
2	M/35	Heart	61	A2, A11, B15, B16	Cyclosporin A Prednisolon	Polymorphic B-cell lymphoproliferation with signs of a plasmacell differentiation and latent and lytic EBV-infection	III A	neg	pos	CR, Relapse	Died in progressive disease
3	F/63	Kidney	73	A2, B7, DR51, DR52 DQ1, DQ2	Cyclosporin A MMF, Prednisolon	Mantle cell lymphoma	IV A	neg	nd	PR, Relapse	Alive
4	M/51	Liver	45	A2, A3, B47, DR3, DR6, DR52	Sirolimus	Small cell, not further classifiable B-NHL	IV A	neg	nd	PD	Alive
5	F/23	Kidney	84	A2, A3 B18, B35, Bw6, DR4, DR8, DR53, DQ3, DQ4, DQ4	Azathioprine Tacrolimus	DLC-B-NHL	II AES	neg	nd	CR	Alive
6	M/60	Kidney	84	A2, B8	Dexamethasone Tacrolimus	DLC-B-NHL	III B E	neg	nd	PR	Died of other cause
7	M/51	Liver	16	A3, A9, B7, DR2, DR6, DQ1	MMF, Tacrolimus	Anaplastic plasmocytoma	I A	neg	pos	CR, relapse	Alive
8	M/74	Kidney	78	B7, DR5, 9, 52, 53 DQ3	Cyclosporin A Prednison	Follicular lymphoma, grade IIIB; transition into a DLC-B-NHL	II A E	neg	neg	CR, relapse	Died
9	F/62	Sharp Syndrome	84	A3, A19, B16, 17, DR1, 6, 52, DQ1	Azathioprine Prednisolon	Hodgkin's disease of the CNS	I A	pos	pos	CR	Alive
10	M/57	Heart	96	A1, 3, B5, 15, DR2, 51, DQ1	Cyclosporin A Everolimus Prednisone	DLC-B-NHL	II A	neg	nd	SD	Alive
11	F/9	Heart	36	A3, B7, B8	Cyclosporin A Everolimus	n/a	n/a	nd	nd	n/a	Alive
12	F/28	Heart	52	A3	Azathioprine Cyclosporin A	n/a	n/a	nd	nd	n/a	Alive
13	M/21	IM	n/a	A3	n/a	n/a	n/a	n/a	n/a	n/a	Alive

n/a: not applicable, IM: infectious mononucleosis, CR: complete remission, PR: partial remission, SD: stable disease, PD: progressive disease.

The majority of all PTLD cases is of recipient B cell origin and is EBV-associated, whereas in late-onset PTLD, EBV is often not detected [11,12]. In EBV positive PTLD the full spectrum of all nine EBV latent proteins are expressed [13]. Several papers have established the hierarchy of CD8 T cell reactivity to the EBV latent antigens in healthy donors. In all cases, the repertoire of reactive CD8 T cells is skewed toward EBNA3A, EBNA3B, EBNA3C and LMP2 recognition [14–16]. More recent work has investigated the CD4 T cell repertoire against the different latent EBV antigens and detected that CD4 T cells preferentially recognize EBNA1 and LMP1 [17,18]. Interestingly, EBNA1 is consistently recognized by CD4 T cells in healthy EBV carriers [19,20]. Still, the role of EBV-specific CD4 T cells in controlling EBV-associated disease is unclear [21–24]. Results from adoptive transfer studies in humans as well as animal data indicate that a failing CD4 T cell response can endanger a functional CD8 T cell response [25–29]. In addition, EBV-specific CD4 T cells have been shown to directly lyse EBV-infected target cells *ex vivo*, but the relevance of this finding *in vivo* is unclear [21,30].

So far, studies have enumerated CD8 T cell responses against dominant EBV-epitopes in pediatric SOT recipients and adult stem cell recipients at risk for the development of EBV-associated disease [31,32]. A prospective study in pediatric SOT recipients has shown that after primary infection a high viral load is indicative of the PTLD risk only if there is a low concomitant cellular immune response [33]. In adult stem cell transplant recipients, it was shown that EBV-reactivation was controlled only in patients who mounted a significant EBV-specific T cell response [34]. So far, no data on EBV-specific CD4 and CD8 T cell numbers and absolute CD4/CD8 T cell numbers in adult SOT recipients with PTLD have been reported.

Table 2
EBV tetramers

Tetramer	HLA	Sequence	Protein	Lytic/latent
A2-SVR	A2	SVRDRLARL	EBNA3A	Latent
A2-CLG	A2	CLGGLLTMV	LMP2A	Latent
A2-GLC	A2	GLCTLVAML	BMLF1	Lytic
A3-RVR	A3	RVRAYTYSK	BRLF1	Lytic
B7-RPP	B7	RPPIFIRRL	EBNA3A	Latent
B7-LPC	B7	LPCVLWPVL	BZLF1	Lytic
B8-FLR	B8	FLRGRAYGL	EBNA3A	Latent
B8-QAK	B8	QAKWRLQTL	EBNA3A	Latent
B8-RAK	B8	RAKFKQLL	BZLF1	Lytic