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Brief communication

Adequate control of primary EBV infection and subsequent reactivations after cardiac transplantation in an EBV seronegative patient

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ABSTRACT

EBV seronegative recipients of cardiac transplantation are at risk for development of post transplant lymphoproliferative disease following primary EBV infection due to the ongoing treatment with immunosuppressive drugs. Here we present detailed kinetics of the EBV-specific T-cell response following cardiac transplantation in an EBV seronegative recipient who developed a primary EBV infection 15 weeks post transplantation and subsequent viral reactivations throughout follow up. The patient developed an EBV-specific $CD8^+$ T-cell response within 24 days after first detection of the primary infection. Subsequently, an increased EBV-specific $CD8^+$ T-cell response developed upon viral reactivation, indicated by a threefold increase of EBV-specific $CD8^+$ T cells and increased IFNy production after stimulation with EBV-specific peptide pools. These data indicate that an EBV-specific T-cell response capable of adequate control of a primary EBV-infection and subsequent viral reactivations can develop in an EBV seronegative cardiac transplant recipient in the presence of severe immunosuppression.

Epstein–Barr virus is a widespread gamma herpes virus which infects over 90% of the human population during life. Infection usually occurs during childhood where it has an asymptomatic course, however when infection is delayed until adolescence it can induce infectious mononucleosis [1,2]. After primary infection EBV persists as a lifelong latent infection in the memory B cell compartment [3]. The common understanding is that EBV infection is mainly controlled by cytotoxic $CD8^+$ T cells [1,4] establishing a lifelong equilibrium between virus-infected cells and the immune response. Disturbance of this equilibrium in immunocompromised hosts (i.e. cardiac transplant recipients) can lead to uncontrolled lymphoproliferation and subsequent post transplant lymphoproliferative disease (PTLD). The primary risk factor for development of PTLD after cardiac transplantation (HTx) is EBV seronegativity of the patient prior to transplantation [5,6]. Monitoring of EBV DNA in transplant recipients is a widespread tool for detecting patients at risk of PTLD [7–11]. However, EBV DNA levels have shown to be insufficient as a sole prognostic factor for PTLD. Since PTLD arises as a result of an imbalance between T-cell control and infection, the number of EBV-specific T cells, which indicate control of infection, could contribute to prediction of PTLD [11–15].

Here we present the EBV-specific T cell kinetics of a primary EBV infection and subsequent reactivations in an EBV seronegative cardiac transplant recipient in whom a T-cell response against EBV developed during primary infection resulting in adequate control of EBV infection throughout follow up.

Objective: to analyze whether an effective EBV-specific immune response can be initiated after primary EBV-infection in a EBV-seronegative cardiac transplantation.

A 60 year old male (HLA A1, A30, B51, B57) underwent a cardiac transplantation in June 2007 for ischemic cardiac failure. Immunosuppressive therapy following transplantation consisted of Tacrolimus, Mycophenolate mofetil and Prednisolone. Prednisolone was tapered and withdrawn at 9 months post HTx, according to clinical guidelines. He was routinely monitored for the presence of EBV viral DNA, based on a real-time TaqMan™ EBV DNA PCR assay in ethylenediaminetetra acetic acid (EDTA)-plasma as described previously [16–18]. EBV seroconversion was determined based on the presence of IgM and IgG antibodies against the viral capsid antigen (VCA) or the EBV nuclear antigen (EBNA). During episodes of positive EBV-DNA PCR, antiviral therapy (Valaciclovir), was given at a dose of 3×1000 mg daily as well as reduction of the immunosuppression.

The post transplantation period remained uneventful. EBV load was measured weekly during 2 months post transplantation and

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approximately monthly until 12 months post transplantation, thereafter incidentally on out-patient visits until 3 years post transplantation. During the first year post transplantation, blood samples were drawn at each visit for which written informed consent was obtained from the patient in accordance with the declaration of Helsinki. These blood samples were used to perform EBV load measurements and isolation of peripheral blood mononuclear cells (PBMC) for EBV-specific T-cell analysis. HLA-B57 tetramer complexed with an EBV peptide derived from the latent antigen EBNA-3B (VSFIEFVGW) was used to identify EBV-specific $CD8^+$ T cells (Sanquin, Amsterdam, the Netherlands). At least 1×10^6 PBMC were stained with the HLA-B57 EBV tetramer, conjugated to PE, co-stained with CD3-Pacific Blue (eBioscience Inc., San Diego, California, USA), CD8-PerCP, CD45RO-FITC (Invitrogen, Carlsbad, California, USA) and CD27-APC Alexa Fluor 750 (Ebioscience) for phenotypical analysis or co-stained with HLA-DR-APC-Cy7 (BD Biosciences (BD), San José, California, USA), CD38 PE-Cy7, CD80-FITC and CD25 APC (eBioscience) for activation marker analysis. Perforin expressing $CD8^+$ T cells were measured directly on fresh whole blood samples as described previously [19]. In brief, erythrocytes were lysed and samples were washed and incubated with CD3-PerCP, CD8-APC-Cy7, CD56-APC (BD) and CD16 Pacific Blue (eBioscience). Cells were permeabilized and lysed and subsequently incubated with FITC conjugated perforin (BD). For all stainings at least 200000 events were acquired on a LSRII flowcytometer and analyzed using FACSdiva software (BD).

In addition, IFNγ producing EBV-specific T cells were enumerated using an IFNγ ELIspot assay as previously described [20]. In brief, 96 well multiscreen filter plates (Millipore, Volketswil, Switserland) were coated overnight with 50 µl of 15 µg/ml anti-IFNγ monoclonal antibody (MABTECH, Stockholm, Sweden) in PBS. PBMC were incubated in triplicate at 1×10^5 cells/well in the presence of 10 µg/ml of 15 mer peptide pools with 11 amino acid overlap. We used both the immunogenic C-terminal region of the latent protein EBNA-1 and the entire lytic protein BZLF-1 (JPT Peptide Technologies GmbH, Berlin, Germany). As a control the cells were cultured in the absence of stimuli or in the presence of phytohemoagglutinin (PHA) (Murex Diagnostics, Dartford UK). IFNγ production was detected using biotinylated anti-IFNγ monoclonal antibody (MABTECH). IFNγ producing cells were analyzed using an automated spot reader (AELVIS GmbH, Hannover, Germany). The number of EBV-specific IFNγ producing T cells was calculated per 1×10^6 PBMC after subtracting negative control values.

EBV serology and DNA load were monitored following cardiac transplantation in an EBV seronegative recipient. The patient had no detectable IgM or IgG antibodies against viral capsid antigen (VCA) or EBNA at 1 year prior to HTx and on the day of transplantation. The transplant donor was EBV seropositive (IgM VCA negative, IgG VCA, early antigen (EA) and EBNA positive). EBV DNA in plasma was detectable at day 102 post transplantation (50 copies/ml). This increased to 553 copies/ml on day 117 and decreased below detection limit on day 133 (Fig. 1A). An EBV reactivation subsequently occurred on day 249 post transplant with a maximum viral load of 107 copies/ml on day 256. EBV seroconversion was observed 270 days post transplantation, as evidenced by the appearance of IgM and IgG antibodies against VCA (Fig. 1A).

Kinetics of EBV-specific $CD8^+$ T cells were visualized using a HLA-B57 tetramer complexed with the EBV epitope VSFIEFVGW (derived from the latent antigen EBNA3B) throughout the first year post transplantation. In healthy individuals (HLA-B57 and EBV seropositive) the range of VSF specific $CD8^+$ T cells is 0.08–1.40% (data not shown). Representative tetramer stainings in our transplant patient are shown in Fig. 1B. EBV B57-VSF specific $CD8^+$ T cells were rapidly detectable after the onset of primary infection. Within 24 days after first detection of EBV DNA in plasma, we could detect EBV-specific $CD8^+$ T cells (0.38% of $CD8^+$ T cells)(Fig. 1A). This fits with a study performed in pediatric recipients of a renal transplant that showed appearance

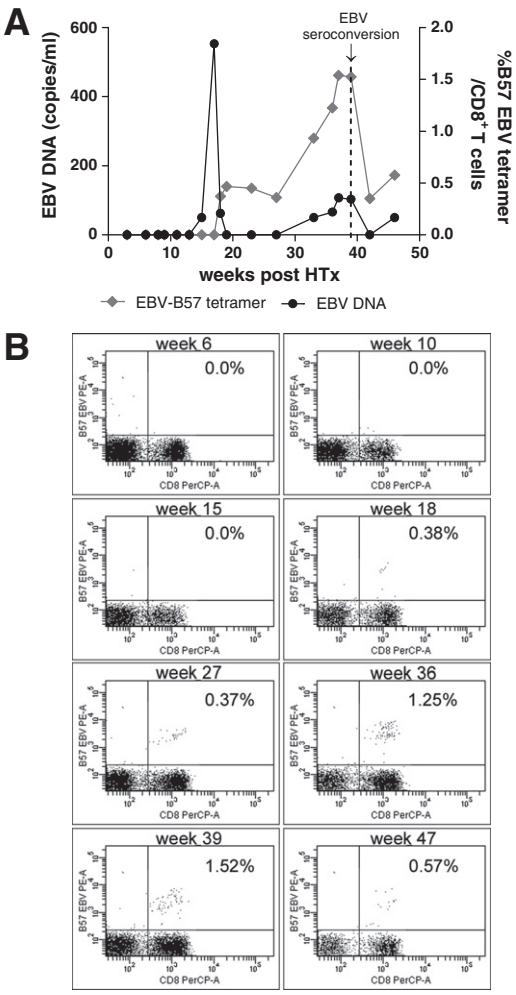

Fig. 1. A) EBV DNA load (copies/ml), depicted on the left y-axis (black line) and percentages of B57-VSF specific $CD8^+$ T cells (gray line) (right y-axis) throughout follow up. Thin dotted line indicates time point of seroconversion (detectable IgM and IgG antibodies against VCA) at 39 weeks post transplantation. B) Representative FACS plots of HLA B57-VSF specific $CD8^+$ T cells at different time points following cardiac transplantation. Percentages in upper right corner of each FACS plot indicate the percentage of B57-VSF specific cells/$CD8^+$ T cells.

of tetramer + T cells in the blood within one month after transplantation (irrespective of EBV serostatus) [21]. After the primary infection resolved (undetectable EBV DNA load at day 133), the B57-VSF specific $CD8^+$ T cells ranged from 0.38% to 0.47% of $CD8^+$ T cells until day 229. During the subsequent viral reactivation, the amount of B57-VSF specific $CD8^+$ T cells rapidly increased. On day 249, EBV DNA was detectable at 62 copies/ml and B57-VSF specific $CD8^+$ T cells accounted for 0.93% of total $CD8^+$ T cells. This increased to 1.5% (week 37) during viral reactivation and decreased again to 0.3% when the reactivation resolved (Fig. 1A). All B57-VSF specific $CD8^+$ T cells were of central memory phenotype both during primary infection and during reactivation ($CD27^+/CD45RO^+$) and were highly activated ($CD38^+/HLA\text{-}DR^+$). Percentages of $CD8^+$ T cells expressing effector phenotype ($CD27^-/CD8^+$) rapidly increased upon viral reactivation and peaked during onset of reactivation (53.7% of $CD8^+$ T cells). Percentages of activation markers on $CD8^+$ T cells ($CD38^+$ and/or $HLA\text{-}DR^+$) fluctuated throughout follow up, however, percentages of $CD38^+$ and $HLA\text{-}DR^+$ T cells increased during primary infection as well as during reactivation (Fig. 2A).

To analyze the potential cytotoxic capacity of the total $CD8^+$ T cell population present we measured intracellular perforin expression directly ex vivo on whole blood. In healthy individuals the