8. Summary / samenvatting

English Summary

Epstein-Barr virus is a γ-herpesvirus that infects more than 90% of the world population. Following primary infection in the oropharynx, EBV remains latently present in B-cells. Latent EBV infection is normally controlled by a cell-mediated immune response. EBV-infected B-cells may evolve to EBV-lymphoproliferative disease (LPD) in severely immunocompromised patients, due to inhibition of immunological control of latently infected B-cells. Recipients of allogeneic hematopoietic stem cell grafts are at risk for EBV-LPD during the time-period of insufficient T-cell recovery after transplantation. EBV-LPD is a rare but serious complication following allogeneic hematopoietic stem cell transplantation and is associated with considerable mortality. The lack of early and accurate markers of EBV reactivation has long hampered a timely diagnosis of EBV-LPD. The introduction of polymerase chain reaction (PCR)-based assays has allowed for sensitive and quantitative monitoring of EBV-DNA in peripheral blood samples. This thesis has addressed the question whether molecular monitoring of EBV-DNA would predict for EBV-LPD and whether preventive and therapeutic strategies could be developed based on viral load monitoring.

Chapter 2 describes the development of a real-time quantitative assay for detection of EBV-DNA in plasma. EBV-DNA encoding for the non-glycosylated membrane protein BNRF1-p143 was used as the target gene in this assay. EBV-DNA was assessed in plasma samples from healthy EBV-seropositive individuals, patients with infectious mononucleosis, asymptomatic immunosuppressed solid organ transplant recipients, and patients with a histologically confirmed diagnosis of EBV-LPD. EBV-DNA could not be detected in plasma of healthy EBV-seropositive individuals whereas it could be detected in plasma of 19 % (21/109) of solid organ transplant recipients, in 73 % (16/22) of patients with infectious mononucleosis, and in 100% (10/10) of patients with EBV-LPD. Quantitative values of patients with infectious mononucleosis and patients with EBV-LPD differed significantly from solid organ transplant recipients. Furthermore, the assay appeared to be rapid, sensitive, specific and highly reproducible over a range between 100 and 10^7 genome equivalents/ml (geq/ml).

We then wished to examine the clinical utility of the EBV-DNA test as an assay for diagnosing EBV reactivation and EBV-LPD following allogeneic hematopoietic stem cell transplantation. For this purpose, in chapter 3 we retrospectively evaluated 152 recipients of an allogeneic hematopoietic stem cell transplantation at weekly intervals for the presence of EBV-DNA in plasma during the first 180 days following stem cell infusion. Endpoints of this longitudinal study were: incidence of EBV reactivation, EBV-LPD, graft-versus-host disease and treatment-related mortality. The incidence of EBV reactivation did not differ between recipients of unmanipulated versus T-cell depleted grafts and measured approximately 50 % for the whole group. In multivariate analysis

patients pretreated with anti-thymocyte globulin in the conditioning regimen and patients receiving higher $CD34^+$ cell numbers in the graft were found to be at greater risk for EBV reactivation. EBV-LPD was not observed after unmanipulated stem cell transplantation but occurred only in recipients of a T-cell depleted allogeneic hematopoietic stem cell transplantation. Plasma EBV-DNA quantitatively predicted for EBV-LPD. The positive and negative predictive values of a viral load of 1,000 geq/ml were, respectively, 39% and 100% after T-cell depleted allogeneic hematopoietic stem cell transplantation. Thus, EBV reactivation appeared a frequent event after allogeneic hematopoietic stem cell transplantation and plasma viral load quantitatively predicted for EBV-LPD in recipients of a T-cell depleted allogeneic hematopoietic stem cell transplantation.

In chapter 4 we set out to assess the value of serial monitoring of EBV-DNA plasma levels for predicting response to treatment and subsequent survival in recipients of a T-cell depleted hematopoietic stem cell with established EBV-LPD. Fourteen patients were monitored frequently from the time of EBV-LPD diagnosis until clinical response or death. Seven patients obtained a complete response and 21% (3 out of 14) survived beyond 6 months from EBV-LPD diagnosis. Clinically responding patients showed a rapid decline of EBV-DNA plasma levels within 72 hours from the start of therapy. In contrast, all clinical non-responders showed an increase of EBV-DNA levels. Absolute EBV-DNA levels at the time of EBV-LPD diagnosis did not predict for response, but the pattern of EBV-DNA levels within 72 hours from the start of therapy strongly predicted for clinical response. In addition, lymphopenia at the time of EBV-LPD diagnosis was associated with non-responsiveness and poor outcome. It was concluded that the quantitative monitoring of viral load accurately predicts for response to therapy in patients with established EBV-LPD.

In chapter 5 we studied whether pre-emptive therapy with anti-CD20 B-cell antibody (rituximab) would prevent EBV-LPD, and EBV-LPD mortality. We monitored 49 recipients of a T-cell depleted allogeneic hematopoietic stem cell transplantation weekly for EBV reactivation by quantitative real-time PCR. Pre-emptive therapy by a single infusion of rituximab was given to patients as soon as EBV-DNA value exceeded 1,000 geq/ml as these values identify patients at high risk for EBV-LPD (chapter 3). Results were compared with a control group of patients retrospectively monitored for EBV reactivation at similar intervals. Seventeen prospectively monitored patients showed EBV reactivation $\geq$ 1,000 geq/ml and 15 of them received pre-emptive therapy. Fourteen patients had complete responses to therapy. One patient progressed to EBV-LPD despite pre-emptive therapy, but obtained complete remission after 2 infusions of rituximab and donor lymphocyte infusion. Two patients had already developed EBV-LPD prior to pre-emptive rituximab, but obtained complete remission following 2 rituximab infusions. Comparison of this prospective cohort of patients followed series to a historical cohort with a similar high-risk profile showed a reduction of EBV-LPD incidence and a complete abrogation of EBV-LPD mortality. These results indicated that frequent monitoring of EBV reactivation

and pre-emptive therapy by rituximab improves outcome in patients at high-risk for EBV-LPD.

It is assumed that cytotoxic T-cells play a critical role in the control of evolving EBV reactivation and the prevention of progression towards EBV-LPD. The aim of the study described in chapter 6 was to evaluate the recovery of EBV-specific $CD8^+$ T-cells after allogeneic hematopoietic stem cell transplantation and to study the relation between EBV-specific $CD8^+$ T-cells, EBV reactivation and EBV-LPD. EBV-specific immunity was studied using a panel of 11 HLA class I tetramers presenting peptides derived from 7 EBV proteins. Forty-five patients showed EBV reactivation, including 25 with high-level reactivation (≥ 1,000 geq/ml). Nine of these patients progressed to EBV-LPD. Repopulation of $CD8^+$ T-cells specific for latent and lytic EBV epitopes in the peripheral blood was similar. Absolute numbers of EBV-specific $CD8^+$ T-cells after allogeneic hematopoietic stem cell transplantation recovered to normal levels within 6 months in the majority of patients. Concurrently, the incidence of EBV reactivation strongly decreased. Patients with insufficient recovery were at higher risk for EBV reactivation in the first 6 months after stem cell transplantation. A profile of absent EBV-specific $CD8^+$ T-cells coupled to high-level EBV-DNA plasma levels was significantly associated with the subsequent development of EBV-LPD (P=0.048). Thus, the earlier defined positive predictive value of approximately 40%, based on high-level viral reactivation only, increased to 100% in patients without EBV-specific T-cells. These results show that the absence of EBV-specific $CD8^+$ T-cells in patients with high-level viral reactivation may identify a subgroup of patients at extremely high risk for EBV-LPD. This observation is consistent with the idea that EBV-specific $CD8^+$ T-cells protect recipients of an allogeneic hematopoietic stem cell graft against progressive EBV reactivation and EBV-LPD.

Finally, the results of the studies presented in chapters 2-6 are discussed in chapter 7 (General Discussion). Emerging questions with respect to diagnosis, prevention, and treatment of EBV-LPD based on molecular monitoring are addressed.