Chapter 11

Summary

Recipients of stem cell transplants (SCT) are severely immunocompromised at least during the first 6 months post-transplant. In the introduction (**chapter 1**) this immunodeficiency is further described. During the neutropenic period patients are at risk for developing bacterial and fungal infections. Once neutropenia has recovered, opportunistic viral infections are a major threat. Both the Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) are herpes viruses and share the phenomenon that primary infection is followed by latent infection. In immunocompetent individuals latent infection of these viruses is controlled by T cell immunity. However, in immunocompromised patients herpes viruses frequently show reactivations, regularly resulting in herpes virus related disease. In this thesis, T cell immunodeficiency and EBV and CMV infections after allogeneic SCT are studied.

The extent of the immunodeficiency is determined by several factors such as patient age, development of graft-versus-host disease (GVHD) and the institution of immunosuppressive therapy. Furthermore, ex vivo T cell depletion (TCD) of grafts, the use of alternative donors (partially matched related donors or matched unrelated donors) and the source of stem cells may play a role as well, although their contribution is less clear. Antithymocyteglobulin (ATG) is generally used in recipients of TCD grafts from unrelated donors to prevent graft failure. In **chapter 2** the effect of ATG on quantitative immune recovery and GVHD was analysed in recipients of MRD grafts compared to MUD recipients. Apart from ATG infusion in MUD recipients treatment of both patient groups was similar. Recovery of T cell subsets, NK cells and B cells was determined at 2, 3, 6 and 8 months post-transplant. In the MRD group markedly higher T cell and subset values were measured during this period. MRD recipients received significantly less T cells and suffered more acute and chronic GVHD compared to MUD recipients. These data suggest that ATG is an important negative factor in the reconstitution of T cells and their subsets in MUD recipients. On the other hand, ATG results in a lower incidence and severity of GVHD.
In **chapter 3** the effect of 3 different ATG doses (8 mg/kg, 6 mg/kg and 4 mg/kg) on graft failure and incidence of GVHD was evaluated. Lowering ATG dose from 8 mg/kg to 6 mg/kg did not influence the occurrence of graft failure nor the incidence of GVHD. However, when ATG dose was decreased to 4 mg/kg the incidence of severe acute GVHD grade III-IV and extensive chronic GVHD rose dramatically, without affecting the occurrence of graft failure.

EBV reactivations or primo/re-infections in severely immunocompromised patients may result in the development of EBV-associated lymphoproliferative disorders (EBV-LPD). In MUD recipients of TCD grafts reactivation of EBV is a frequent event, which is described in **chapter 4**. In this study it was shown that plasma EBV DNA quantitatively predicted EBV-LPD. The negative and positive predictive values of a viral load of 1000 c/ml were 100% and 39%, respectively. EBV-LPD was not diagnosed in recipients of non-TCD grafts, although EBV reactivations did occur in these patients. The use of ATG in MUD recipients, was an important risk factor for the occurrence of EBV reactivations.

Until now it is not known whether EBV reactivations result from a true reactivation of the endogenous patient strain or a re-infection with exogenous strains. In **chapter 5** preliminary data are presented of EBV strain typing in six patients in pre-transplant collected mouth-washes and post-transplant collected plasma samples, peripheral blood mononuclear cells (PBMCs) and mouth washes. Furthermore, prospective data of EBV DNA monitoring performed in PBMC as well as cell free plasma are given. 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 one MRD patient it was not possible to differentiate since pre-transplant, post-transplant and donor sequence patterns were identical. In a MUD recipient pre- and post-transplant sequence patterns were identical too, which makes a reactivation of the endogenous EBV strain likely in this patient, although a re-infection is not excluded. Post-transplant EBV strains of the sixth patient 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 this patient suffered a reactivation instead of a re-infection.

In 17 patients double EBV DNA monitoring was performed in PBMCs and plasma. Only 4 of 17 (23%) patients showed a reactivation in plasma samples compared to 15 of 17 (88%) in PBMC samples ($p<0.001$). None of the patients with a reactivation in PBMCs only progressed to EBV-LPD, compared to 18% of patients with EBV reactivation in plasma. Therefore, it can be concluded that monitoring in PBMC samples is less specific for predicting EBV-LPD compared to plasma samples.

The risk for EBV-LPD varies according to the techniques used for T cell depletion, being lowest (<2%) when methods are used which remove both T and B cells. In **chapter 6** the incidence of EBV-LPD among MRD and MUD recipients of TCD grafts is presented. All MUD

recipients were treated with ATG. When grafts from MUDs were both T and B cell depleted, 4 out of 31 patients (13%) developed EBV-LPD compared to 5 of 7 patients (71%) when no B cell depletion was performed. A T/B cell ratio in the graft of ≥ 0.25 seemed sufficient to significantly reduce the incidence of EBV-LPD after TCD SCT from MUDs. In contrast, among recipients of grafts from an HLA-identical sibling donor the incidence of EBV-LPD was not influenced by B cell depletion in addition to TCD (5% in T and B cell depleted group; 4% in TCD group).
In **chapter 7** an overview is given of the prevention and treatment of EBV-LPD in recipients of stem cell and solid organ transplants. Several risk factors for the development of EBV-LPD after solid organ transplantation (SOT) and SCT, respectively, have been identified: 1) primary EBV infection in EBV-seronegative patients, 2) type of transplanted allograft, 3) CMV serostatus mismatch (seronegative recipient/seropositive donor), 4) CMV disease 5) use of T cell antibodies in SOT recipients and 1) TCD of grafts, 2) use of unrelated or ≥ 2 HLA antigen mismatched related donors, 3) use of ATG or 4) anti CD3 monoclonal antibodies in SCT recipients. Guidelines for monitoring of EBV viral load are given (which patients should be monitored, at what frequency, for how long). Furthermore, the varying treatment modalities are reviewed and treatment recommendations are given.

Due to the high mortality rate of CMV disease, latent CMV infection has long been considered a negative risk factor for overall survival (OS) and TRM. In **chapter 8** the effect of CMV serostatus on OS and TRM in 253 consecutively treated patients receiving partial TCD stem cells from either matched related donors (n=205) or matched unrelated donors (n=48) was analysed. Transplantations were performed between July 1990 and May 2000. All patients were given leukocyte-depleted blood products. CMV monitoring was performed using the pp65 antigenemia assay. Pre-emptive therapy consisted of short-course (2 weeks) low-dose (2.5 mg/kg intravenously b.i.d.) ganciclovir treatment as soon as a positive antigenemia assay was obtained (≥ 1 positive staining granulocyte/150.000 cells). Ganciclovir prophylaxis, identical to pre-emptive therapy, was given to CMV-seropositive patients with acute GVHD grade II-IV who were treated with high-dose corticosteroids. This analysis showed that CMV-seropositivity was not an adverse risk factor for OS and TRM in MRD recipients of partial TCD SCT. However, in MUD recipients, patient CMV-seropositivity still had a high impact on OS and TRM.

The effect of CMV-seropositivity on outcome in MUD recipients is mostly studied in patients treated before 1999. In **chapter 9** it is evaluated whether CMV-seropositive MUD recipients transplanted after 1999, still showed inferior outcome compared to CMV-seronegative recipients. In our transplantation centre two important changes in transplantation procedure were introduced in that year. In April 1999 ATG dose was lowered from 20 mg/kg to 8 mg/kg. Furthermore, in January 1999 sequence based typing (SBT) of HLA-DRB1 was introduced. Both changes may result in an improved immune reconstitution post-transplant. Low-dose ATG by a direct effect on T lymphocyte counts and high resolution HLA-DRB1 typing by a decreased incidence of GVHD. Considering the immunosuppressive effect of (latent) CMV infection this may have a positive impact on outcome in CMV-seropositive SCT recipients. In total 80 patients received a partial TCD graft, 36 before 1999 and 44 after 1999. CMV-seropositive patients transplanted before 1999 showed a highly significant inferior outcome compared to seronegative recipients (see also chapter 8). In contrast, in patients transplanted after 1999 no difference in outcome was observed between the two groups.

In **chapter 10** the prevention of CMV disease in recipients of allogeneic stem cell transplants is reviewed. Before the introduction of ganciclovir, CMV infection and pneumonia developed in 38 and 17%, respectively, of SCT recipients, while mortality due to CMV pneumonia was 85 %. Currently, two antiviral strategies, prophylactic or pre-emptive treatment, are used for prevention of CMV disease in seropositive recipients. Prophylactic treatment usually consists of antiviral therapy started at engraftment until at least day 100 post-transplant. Pre-emptive therapy is defined as antiviral treatment based on the detection of reactivated CMV infection by positive CMV cultures, a positive antigenemia assay or positive molecular assays. In this chapter these antiviral strategies are reviewed and recommendations for prevention of CMV disease are given. Furthermore, several other aspects of prevention of CMV disease are reviewed, which are: 1) methods available for early detection of CMV reactivations, 2) monitoring of CMV-specific T cell responses, 3) the value of several antiviral drugs and 4) adoptive immunotherapy as prophylaxis or (pre-emptive) treatment of CMV reactivations/CMV disease.

In conclusion, recipients of stem cell transplants are severely immunocompromised at least during the first 6 months post-transplant. This is even more true for recipients of grafts from matched unrelated donors. However, several important changes in transplantation proce-

dure have resulted in a more favourable outcome among MUD recipients. B cell depletion - in addition to TCD- of grafts, a reduced dosage of ATG and high resolution DNA typing of HLA-antigens all have contributed to this better outcome. The reduced ATG dose and sequence based typing of HLA-antigens may have improved immune recovery in MUD recipients, thereby eliminating CMV-seropositivity as an adverse risk factor for overall survival and transplant related mortality. In MRD recipients pre-emptive treatment of CMV reactivations alone was sufficient to eliminate CMV-seropositivity as an adverse risk factor for outcome. The optimal dose of ATG is unknown, however, considering the immunosuppressive and direct toxic effects of ATG, the lowest possible dose should be used. In the setting of SCT with (partially) TCD grafts from MUDs, using limited post-transplant immunosuppression with cyclosporine A, a total dose of 6-8 mg/kg is recommended. A further decrease greatly increases the risk of severe GVHD. The future aim is to further improve immune reconstitution post-transplant and to implement new methods for monitoring, prevention and treatment of opportunistic infections. With respect to prevention of EBV-LPDs, it is important to know the most frequent transmission route of EBV re-infections. Further studies using LMP-1 fingerprinting for strain identification of patient, donor and partner samples are necessary.