Control of cytomegalovirus viremia after T cell depleted allogeneic stem cell transplantation

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Chapter 1

General introduction and aim of the thesis

Allogeneic stem cell transplantation

Allogeneic stem cell transplantation (alloSCT) is a potentially curative treatment for various malignant and non-malignant hematological diseases[1]. The goal of alloSCT is to replace the recipient hematopoietic cells with hematopoietic cells derived from stem cells from a human-leukocyte-antigen (HLA) matched donor. Immune suppressive conditioning of the recipient prior to the alloSCT is necessary to allow engraftment of the donor stem cells. This conditioning leads to a period of profound pancytopenia prior to engraftment of donor hematopoietic cells. The duration of this pre-engraftment period is correlated with the occurrence of infectious complications[2]. Conventional myeloablative conditioning (MAC) regimens aim to fully eradicate the hematopoietic cells of the recipient. Reduced intensity conditioning (RIC) regimens aim to allow engraftment of donor hematopoietic cells without full elimination of recipient derived hematopoietic stem cells. RIC regimens are less toxic, but additional immune suppression is necessary to allow engraftment of donor hematopoietic cells leading to additional immune deficiency[3,4]. The mere ablation of recipient hematopoietic cells by chemotherapy and/or irradiation in the conditioning regimen is not sufficient to prevent disease relapse after transplantation, as demonstrated by the high risk of disease relapse after autologous stem cell transplantation in acute leukemia. Long-term control of the disease can be achieved by donor derived alloreactive T cells. These alloreactive T cells can eradicate residual malignant cells by inducing a graft versus leukemia (GVL) response when the immune response is directed against the hematopoietic cells of the patient[5,6]. However, when alloreactive T cells also target non-hematopoietic cells in the tissues and organs of the patient, potentially fatal graft versus host disease (GVHD) can occur. GVL is part of a spectrum of GVHD, as illustrated by the increased risk of disease relapse in the absence of GVHD in alloSCT using stem cells from an HLA identical syngeneic twin[7].

Prevention of GVHD

GVHD can be prevented or reduced by long-term immune suppression or by depleting donor T cells from the graft (T cell depletion, TCD)[8-10]. In non-TCD alloSCT, recipients are treated with long-term immune suppression, which may be tapered in the months or years after alloSCT. Immune suppression is not selective and suppresses not only alloreactive immune responses causing GVHD, but also potentially beneficial immune responses causing GVL or immune responses needed for protection against infectious diseases. In TCD alloSCT strategies, donor T cells are depleted from the stem cell graft or depleted in-vivo by infusion of T cell specific antibodies (e.g. ATG, alemtuzumab). Various methods are used to deplete T cells from the graft such as $CD34^+$ selection or the use of lymphocyte-depleting antibodies such as alemtuzumab (anti CD52)[11,12]. TCD strategies are effective in preventing GVHD and long-term post-transplant immune suppression is generally not required. The absence of immune suppression makes TCD alloSCT suitable as a platform for cellular therapy such as donor lymphocyte infusion (DLI) or adoptive cell transfer (ACT) of selected or manipulated T cell populations. Postponed application of DLI several months after TCD alloSCT to induce

a GVL effect is associated with an acceptable risk of GVHD[13-15]. In ACT strategies, in-vitro selected T cell populations are adoptively transferred to restore (anti-viral) immunity. By selecting specific T cells with a defined antigen specificity (e.g. targeting viral epitopes), the risk for inducing GVHD is lower compared to unmodified DLI containing T cells of unknown specificity[16, 17].

Inherently to the effect of TCD on the prevention of GVHD, a period of profound and prolonged T cell deficiency follows TCD. During this period patients are at risk for developing infectious complications, especially for reactivations of endogenous herpes viruses.

Herpes virus reactivations after alloSCT

Infections with herpes viruses are common in the general population. These infections usually occur during childhood and the clinical symptoms are often mild or even absent. The infection is controlled by virus-specific memory T cells, which develop following a primary immune response. Although virus-specific T cells control these viruses, herpes viruses are not completely cleared and lead to latent infections in their hosts. This latency results in an equilibrium between these viruses and the virus-specific T cells. The most common herpes viruses complicating alloSCT are cytomegalovirus (CMV), Epstein-Barr virus (EBV) and varicella zoster virus (VZV)[18-20]. These viruses share the ability for lifelong persistence and reactivation when T cell immunity fades. T cell immunity is provided by $CD4^+$ and $CD8^+$ T cells, where $CD4^+$ T cells regulate immune responses and $CD8^+$ T cells eliminate the pathogens. $CD4^+$ T cells recognize peptides presented in HLA class II molecules, that are primarily expressed by antigen presenting cells (APCs), whereas $CD8^+$ T cells recognize peptides presented in HLA class I molecules that are ubiquitous expressed on all human tissues. Professional APCs are required for the induction of a primary T cell response leading to a rapid increase of effector T cells and the formation of memory T cells that can react directly upon re-encounter with the pathogen. In CMV and EBV infections, repeated stimulation of memory T cells by reactivation of the virus can result in frequencies of up to 40% of these virus-specific T cells within the T cell compartment in peripheral blood in immune competent individual[21, 22]. VZV resides in an immune privileged site and does not reactivate as often as CMV and EBV. VZV-specific memory T cells are therefore not stimulated repeatedly leading to decreasing frequencies of circulating VZV-specific memory T cells in time[23].

In the period of profound and prolonged T cell deficiency after (TCD) alloSCT the equilibrium between the T cells and the virus is lost and control of reactivation of CMV and EBV infection is impaired. The impaired control may lead to potentially fatal CMV disease in case of CMV reactivation or Post Transplantation Lymphoproliferative Disease (PTLD) after EBV reactivation, caused by uncontrolled proliferation of EBV infected B cells. The decline in VZV-specific memory T cells is accelerated by the conditioning and/or TCD leading to an increased risk for reactivation of VZV leading to herpes zoster. Uncontrolled herpes zoster due to insufficient VZV-specific T cells may lead to potentially fatal disseminated herpes zoster.

Cytomegalovirus

Cytomegalovirus, a double stranded DNA virus, can infect a broad range of cell types upon primary infection. Primary infection is followed by a lifelong persistence with monocytes and vascular endothelial cells as important sites for latency[24]. The clinical course of CMV infection in immune competent individuals is generally asymptomatic or mild and self-limiting with the exemption of congenital neurological disease by maternal transfer of the virus in primary CMV infection during pregnancy. In immune competent individuals CMV reactivation is controlled by CMV-specific memory T cells. In immune compromised patients, lack of CMV-specific T cells and consequential absence of immune control of CMV reactivation can lead to potentially fatal CMV disease, such as CMV pneumonitis, CMV colitis or CMV encephalitis following CMV infection or reactivation[25]. Reactivation of endogenous CMV is the most frequently occurring herpes virus reactivation following alloSCT with an incidence of 80% in CMV seropositive recipients[19]. Approximately 60% of alloSCT recipients are seropositive for CMV and are therefore at risk for endogenous reactivation of latent CMV virus[26]. CMV infection of a CMV seronegative recipient via a stem cell graft from a CMV seropositive donor occurs, but less frequently because endothelial cells and monocytes, the most important sites for CMV latency and persistence, are not an elementary components of the stem cell graft[27]. $CD8^+$ T cells can be analyzed and monitored using artificial HLA class I constructs loaded with a specific antigen. These constructs consist of multiple HLA molecules (tetramers or pentamers depending on the number of HLA molecules used) combined with a fluorescent label, allowing direct detection using flow cytometry. For CMV several HLA constructs have been developed and studies have demonstrated that presence of CMV-specific $tetramer^+$ $CD8^+$ T cells is directly related with control of CMV reactivation[28, 29].

Epstein-Barr Virus

Epstein-Barr Virus (EBV) is a herpes virus, which infects more than 90% of the population. After primary infection, which may lead to the clinical syndrome of infectious mononucleosis, the virus latently resides in the B cell population[30]. Infectious mononucleosis is caused by a massive expansion of EBV specific T cells upon recognition of an EBV antigen presented in HLA molecules with the goal to control the EBV infection. After alloSCT, reactivation of EBV may occur in the absence of sufficient EBV specific T cell immunity. With failing T cell control, EBV infected B cells can expand massively leading to potentially fatal PTLD. Although the incidence of EBV associated PTLD is low following alloSCT (4%)[31], the risk correlates with the level of TCD. TCD strategies deleting only T cells, the risk increases because B cells, the principle site for EBV latency are not depleted. In TCD strategies using depleting antibodies targeting both T and B cells, such as alemtuzumab, the risk is not increased[32]. Analogous to CMV, also or EBV several HLA constructs have been developed and studies have also demonstrated increased control of EBV reactivation by EBV specific $tetramer^+$ $CD8^+$ T cells[28, 29, 33, 34].

Varicella zoster virus

Varicella zoster virus (VZV) is a herpes virus, which infects about 95% of the population. The primary infection with VZV leads to the clinical entity of varicella (chickenpox). After the primary infection VZV resides latently in neurons and reactivation leads to herpes zoster (shingles). Similar to CMV, cellular immunity is essential for preventing reactivation of VZV. After alloSCT, reactivation of the virus causes considerable morbidity and is potentially fatal in disseminated reactivation[18]. Most frequent complications are post-herpetic neuralgia and peripheral neuropathy. In contrast to CMV little is known about VZV-specific $CD8^+$ T cell immunity because validated VZV-derived immunodominant peptides for HLA class I are lacking[35]. Previous studies demonstrated VZV-specific memory $CD4^+$ T cells but VZV-specific $CD8^+$ T cells were only detectable after in-vitro expansion. The inability to directly detect VZV-specific $CD8^+$ T cells directly ex-vivo may be due to the low frequencies of VZV-specific $CD8^+$ T cells or to the low sensitivity of the screening methods used to detect $CD8^+$ T cells[36-38]. Identification of a VZV derived immunodominant peptide and the construction of VZV-specific peptide-HLA complexes is important to ex vivo analyze the role of $CD8^+$ T cells in the immune responses to VZV infection and reactivation after alloSCT.

Prevention of CMV disease by antiviral medication

In order to prevent CMV disease, a period of profound T cell deficiency after (TCD) alloSCT must be bridged to allow CMV-specific T cell immunity to restore and prevent CMV disease. Bridging this period is possible using antiviral medication. Ganciclovir is a synthetic nucleoside that inhibits DNA viruses, such as herpes viruses and especially CMV, by inhibiting viral DNA polymerase and viral DNA elongation. Ganciclovir is the golden standard for treating CMV disease but has considerable side effects, the most important being bone marrow suppression[39]. Furthermore ganciclovir has poor bioavailability, which precludes oral administration and often necessitates hospitalization for intravenous treatment[40]. Prophylactic use of ganciclovir to prevent CMV disease is therefore not feasible. However, because high viral loads precede the development of CMV disease when patients are still asymptomatic, prevention of CMV disease is possible by pre-emptive administration of ganciclovir[40, 41]. In a pre-emptive treatment strategy, antiviral therapy is initiated when the viral load is above a predetermined threshold. CMV viral load can be detected and monitored by using quantitative PCR[42]. Valganciclovir is an orally administered pre-drug of ganciclovir and suitable for pre-emptive outpatient clinical treatment to prevent CMV disease[43-45]. However, similar to ganciclovir, prolonged usage of valganciclovir is not appropriate for long-term prevention of CMV disease due to adverse effects and possible development of resistance[46].

CMV-specific T cell reconstitution

Restoration of immune control by reconstitution of CMV-specific T cells is required for long-term control of viral replication and prevention of CMV disease[28, 29, 47, 48]. Reconstitution of

CMV-specific T cells can be the result of expansion of recipient memory T cells that survived the conditioning regimen prior to alloSCT or donor memory T cells transferred with the graft. Various factors can influence CMV-specific T cell reconstitution. Immune suppression for prevention of GVHD after transplantation with an unrelated or partially matched donor or treatment of GVHD can impair T cell reconstitution. CMV-specific T cell reconstitution may also be impaired by more intensive conditioning regimens prior to alloSCT due to more profound eradication of residual recipient T cell immunity.

Following transplantation with a CMV *seronegative* donor, the CMV-specific T cells reconstituting after alloSCT are expected to be of recipient origin, because a primary immune response by donor T cells is not likely to occur shortly after alloSCT. Residual CMV-specific T cells of the recipient can be eradicated by alloreactive donor T cells when an immune response is induced after alloSCT and/or Donor Lymphocyte Infusion (DLI), leaving the patient at risk for developing CMV disease. In these patients, development of a primary donor derived CMV-specific T cell response from donor origin would be essential to prevent CMV disease. For a primary CMV-specific immune response, naive T cells recognizing CMV antigens are required. Naive T cells need thymic education, and because the function of the thymus is impaired in (adult) alloSCT patients[49], a primary donor derived CMV-specific T cell response is not expected shortly after alloSCT.

Following transplantation with a CMV *seropositive* donor, CMV-specific T cells can be of recipient and/or donor origin, possibly at the same time leading to a state of mixed CMV-specific T cell chimerism. CMV-specific T cell reconstitution can originate from donor memory T cells transferred with the graft from CMV seropositive donors. Manipulation of the graft by TCD may abrogate this transfer of CMV-specific T cells and increase the risk of developing CMV disease. Eradication of recipient lymphopoietic cells in patients with mixed CMV-specific T cell chimerism by an alloreactive donor T cell response is not expected to be harmful as protection by donor CMV-specific T cells is still present or transferred with the DLI.

Despite pre-emptive antiviral medication, persistent CMV reactivation or CMV disease can occur when CMV-specific T cell reconstitution is not sufficient. Adoptive transfer of donor T cells may be an elegant strategy to enhance T cell reconstitution after alloSCT. However, although this approach may be effective in reconstituting antiviral T cell immunity, it may induce potentially fatal GVHD. To enhance CMV-specific T cell reconstitution and to minimize the risk of inducing GVHD, donor derived CMV-specific T cells can be transferred to the recipient after alloSCT (CMV-specific adoptive cell transfer (ACT)) [50-52]. CMV-specific ACT can be used either as a prophylactic or pre-emptive treatment to prevent CMV disease or as treatment for overt CMV disease. Adoptive transfer of T cells is most effective in the absence of immune suppression, as is in general the case in TCD alloSCT. However, the use of adoptive transfer is not commonplace, as questions regarding safety and efficacy still need answering.

Aim of the thesis

Profound T cell deficiency can lead to reactivation of endogenous herpes viruses after TCD alloSCT. Inadequate control of these viruses by virus-specific T cells can lead to significant complications. Long-term immunity depends on virus-specific T cell reconstitution. In case of CMV reactivation, antiviral medication can bridge the period of T cell deficiency, at the expense of potential toxic side effects. The aim of this thesis is to evaluate several options for preventing CMV disease after T cell depleted (TCD) allogeneic stem cell transplantation (alloSCT). These options include a choice in conditioning regimen and in donor prior to alloSCT, pharmacological intervention following alloSCT and adoptive cell transfer in treatment of refractory CMV reactivation or CMV disease.

In **chapter 2** we aimed to determine the efficacy and safety of oral valganciclovir compared to intravenous ganciclovir to prevent CMV disease after TCD alloSCT in a pre-emptive outpatient strategy. Ganciclovir is associated with hematological toxicity and intravenous administration necessitates hospital admission. Oral valganciclovir is considered to be less toxic compared to intravenous ganciclovir and does not necessitate hospital admission. Efficacy and safety of valganciclovir was already demonstrated in other high-risk populations such as renal- and heart-transplant patients. In this chapter we evaluated the use of oral valganciclovir in preventing CMV disease in 107 consecutive patients following TCD alloSCT. Reduced intensity conditioning (RIC) relatively spares residual recipient hematopoietic cells compared to conventional myeloablative conditioning (MAC). Therefore, reconstitution of CMV-specific T cells may be improved after RIC by sparing residual recipient CMV-specific T cell immunity. In **chapter 3** our aim was to determine whether the incidence and severity of CMV reactivation was affected by the intensity of the conditioning regimen. To determine whether a less toxic conditioning regimen would lead to differences in incidence of CMV reactivation and disease, we compared the frequency and severity of CMV reactivation and the incidence of CMV disease in 107 consecutive patients following RIC or MAC TCD alloSCT. Transplantation with a CMV seropositive donor implies that the donor graft may confer donor derived CMV-specific T cells in contrast to the graft from a CMV negative donor. CMV-specific T cells may be transferred with the graft from CMV seropositive donors and provide protection for CMV disease, but profound TCD can eradicate this transfer of CMV-specific T cells. In **chapter 4** our aim was to determine the effect of donor CMV serostatus on the incidence of CMV disease and T cell reconstitution after TCD alloSCT. We analyzed the incidence of CMV disease after TCD alloSCT in CMV positive recipients transplanted with either a CMV seropositive or seronegative donor. Furthermore we investigated if and when a primary donor derived CMV-specific T cell response could be detected following TCD alloSCT. Demonstrating CMV-specific T cells of donor origin after transplantation with a CMV seronegative donor who lacks CMV-specific memory T cells would be illustrative of the induction of a primary CMV specific T cell response. Therefore, we determined the origin of

CMV-specific T cells in CMV seropositive recipients transplanted with a CMV seronegative donor.

The risk for potentially fatal CMV disease increases if pre-emptive treatment fails to control CMV reactivation and rapid reconstitution of CMV-specific T cells is then pivotal for preventing CMV disease. Adoptive transfer of CMV-specific T cells may be a treatment option in patients failing preemptive anti-viral treatment, although routine application of adoptive cellular immunotherapy is hampered by questions regarding safety and efficacy. Therefore, in **chapter 5** we aimed to analyze the safety and efficacy of adoptive transfer of CMV pp65-specific $CD8^+$ T cell lines to restore CMV-specific T cell immunity in patients with persistent CMV reactivation failing anti-viral therapy. CMV-specific T cells from donor or patient were isolated using an IFNg-based isolation technique, cultured for 1–2 weeks to generate CMV-specific T cell lines, which were transferred to patients with refractory CMV reactivation. Adverse events, clinical effects and CMV-specific T cell reconstitution were monitored to assess the safety and efficacy of adoptive transfer of CMV-specific T cells.

In contrast to CMV-specific $CD8^+$ T cell reconstitution little is known about VZV-specific $CD8^+$ T cell reconstitution. Identification of VZV-derived immunodominant peptides binding in HLA class I and development of VZV-specific peptide-HLA complexes could facilitate analysis of VZV-specific T cell reconstitution. In **chapter 6** we searched for immunogenic antigens for VZV to develop VZV-specific pentamers using a new pentamer-based epitope discovery method. This method has the potential to quickly assess whether part of a protein can be immunogenic by determining the binding affinity with the HLA molecule. Development of VZV-specific peptide-HLA complexes is important to ex vivo analyze VZV-specific $CD8^+$ T cell reconstitution and the immune response to VZV infection, reactivation, and possibly VZV vaccination.

In **chapter 7** we summarized and reviewed recent studies on prevalence and treatment of CMV disease after alloSCT in the era of pre-emptive antiviral treatment. We reviewed literature on the influence of Graft versus Host Disease, unrelated or HLA mismatched donors and TCD on the prevalence of CMV disease. We reviewed studies on the influence of donor CMV status on CMV-specific T cell reconstitution and CMV disease. Recent studies on the safety and efficacy of adoptive transfer of donor CMV-specific T cells for the prevention and treatment of CMV disease following alloSCT are discussed, including studies on adoptive transfer of third-party CMV-specific T cells as a possible alternative when donor T cells are not available.

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