Chapter 1

Introduction

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Immune reconstitution in recipients of allogeneic stem cell transplants

After stem cell transplantation (SCT) preceded by a myeloablative-conditioning regimen most haematological lineages regain their normal counts and function rapidly. This is not the case for B and T cell lineages, resulting in a high incidence of opportunistic infections in SCT recipients. Especially CD4+ T cell counts remain severely depressed during the first 6 months post-transplant, thereafter numbers gradually rise[1].

By 3 months post-transplant, recovery of B cell counts is rapidly established, except for patients with chronic graft-versus-host disease (GVHD)[1-5]. The in vitro response of B cells to T cell independent B cell mitogens such as rabbit anti IgM and Staphylococcus aureus Cowen strain, is significantly correlated to total B cell counts and normalizes within the same time period[2,3]. However, the in vivo antibody response to capsular polysaccharide vaccines, which are also T cell independent antigens, is severely impaired in SCT recipients, even when immunization is delayed until 12 months post-transplant. This decreased antibody response to polysaccharide antigens does exist in healthy children of 0-5 years as well[6]. Small et al[2] showed that immunoglobulin (Ig) M production normalized in conjunction with the return of circulating B cells. However, IgG production did not occur until the second year post-transplant, which is a pattern similar to that seen in healthy infants of 0-2 years. Furthermore, during the first year post-transplant B cells showed a similar phenotype and function as cord blood B cells and B cells from healthy neonates. These data support the hypothesis that B cell differentiation post-transplant is recapitulating normal B cell ontogeny[2]. In addition to recapitulation of normal B cell ontogeny, the recovery of B cell function is dependent on T helper cell recovery.

Reconstitution of T cells may result from two different pathways: 1) a thymus dependent production of naive T cells and 2) peripheral expansion of mature T cells[7,8]. Due to thymic involution in adults, the precise role of thymic dependent production of naive T cells in immune reconstitution after SCT is still a matter of debate. T cell immune reconstitution, especially the production of naive T cells, has been studied by different methods. When CD4+ or CD8+ T cells emigrate the thymus they co-express a specific isotype of the CD45 family (CD45RA+). CD45RA+ CD4+ T cells are considered naive CD4+ T cells, however, this marker is not reliable to evaluate naive CD8+ T cell production[9]. After challenge with

an antigen in the periphery these CD4+ T cells convert to a CD45RA- phenotype and become memory T cells[10,11].

Among recipients of fully T cell depleted (TCD) grafts no CD45RA+ CD4+ T cells were detected during the first 200 days post-transplant[12,13]. In these studies, T cell receptor (TCR) diversity was studied by TCR spectratyping as well[14]. TCR diversity was only observed when CD45RA+ CD4+ T cells appeared, which suggests that these cells are thymic emigrants. Furthermore, reconstitution of the TCR repertoire was due solely to the appearance of donor T cells with a random TCR diversity[12,13]. One thymectomized SCT recipient has been studied and results were compared with those of thymus-bearing allogeneic SCT recipients. The regeneration of CD4+ CD45RA+ T cells was strongly impaired in the thymectomized patient compared to the other patients, while CD8+ CD45RA+ T cells regenerated similarly[15].

Whether the abovementioned cell surface marker is an accurate marker for thymic function, has been debated, since research in nude rats showed that T cells may bi-directionally switch between the two isotypes (CD45RA+ and CD45RA-)[16,17]. Furthermore, in HIV-infected thymectomized patients, CD4+ CD45RA+ T cells rose after initiation of highly active antiretroviral therapy, indicating that T cells with this phenotype are not exclusively of recent thymic origin[18]. Recently, Douek et al[19] developed a new method to measure the production of naive T cells. During thymocyte development rearrangement of the TCR gene leads to the excision of circular DNA fragments from genomic DNA[20]. An assay was developed to measure the number of TCR-rearrangement excision circles (TRECs) in peripheral blood lymphocytes. These products are stable, unique to T cells and not duplicated during mitosis[19,20]. Therefore, their concentration in peripheral blood can be used to estimate thymic output[19].

TRECs have been measured in recipients of TCD and unmodified grafts and lower levels were found to be associated with older age, the presence of extensive chronic GVHD and the occurrence of opportunistic infections[21-23]. Earlier, also CD45RA+ CD4+ T cell counts were shown to be related to age in a similar way[13].

Apart from patient age and the occurrence of GVHD, other variables are considered adverse risk factors for T cell recovery post-transplant, such as use of 1) TCD grafts, 2) immunosuppressive therapy, especially serotherapy with Antithymocyteglobulin, to prevent graft rejection or to prevent or treat GVHD, 3) grafts from matched unrelated (MUD) or partially

matched related donors (PMRD) and 4) bone marrow grafts instead of peripheral blood stem cell grafts.

Influence of TCD of grafts on immune recovery Only a few studies have compared immune reconstitution in recipients of TCD grafts with immune reconstitution in recipients of unmanipulated grafts[2-4,24-26]. These studies were largely performed among recipients of related donor grafts and varying ex vivo TCD techniques were used. Recovery of B cell counts and proliferative responses of B cells to B cell mitogens (rabbit anti IgM, Staphylococcus aureus Cowen strain) were not influenced by TCD of grafts[2-4]. When T helper and B cell function was measured in a mitogen-stimulated Ig production assay, Ig production by B cells from recipients of non-manipulated grafts exceeded that of TCD SCT recipients in one study[3], however, no effect of TCD was found by others[2]. TCD did not influence recovery of EBV-specific cytotoxic T cell precursors nor their virus-specific cytotoxic activity[24]. Recovery of total T cell and subset counts was not influenced by TCD either[3,26], although in some studies CD4+ and CD45+ CD4+ T cell subsets showed a slower reconstitution in TCD SCT recipients[4,25]. Furthermore, the proliferative response of T cells to T cell mitogens (phytohemagglutinin, pokeweed mitogen) was found to be significantly lower during the first three months post-transplant in TCD SCT recipients[3]. Apart from one paper describing a higher incidence of CMV reactivations after TCD SCT[4], there is no increase in infectious complications or fatal infections after TCD SCT[3,25,27]. Overall, from these studies it can be concluded that ex vivo TCD of grafts from related donors does not have a major impact on immune reconstitution.

Influence of Antithymocyteglobulin on immune recovery Antithymocyteglobulin (ATG) is often used before SCT with grafts from MUDs or PMRDs to prevent graft rejection. Since ATG could be detected in sera at least during 2 months post-transplant with 25% of the initial peak concentration at day 28-48 [28,29], it is hypothesized that ATG may severely impair T cell recovery. Recently it was shown that total lymphocyte, CD3+ and CD4+ cell reconstitution was significantly lower in transplant recipients of unrelated grafts treated with high-dose ATG (15 mg/kg) as compared to low-dose ATG (7,5 mg/kg)[30]. Furthermore, the rate of viral infectious complications was significantly higher in the high-dose group. In adult recipients of MRD grafts, the use of ATG post-transplant to prevent graft failure resulted in an impaired recovery of CD45RA+ CD4+ T cells, a prolonged inversion of the CD4+/CD8+ T cell ratio, a delay in recovery of normal T cell mitogen responses and an increased incidence of opportunistic infections as compared to patients not treated

with ATG[31]. Patients given ATG showed relatively increased numbers of CD8+ CD28- CD57+ T cells. T cells with this phenotype respond poorly to T cell mitogens and can inhibit the generation and function of virus-specific cytotoxic lymphocytes[31], although this is controversial since others showed these cells to be cytotoxic effector T cells[9].

The results of both studies support the hypothesis that ATG interferes with T lymphocyte recovery[30,31], while B lymphocyte counts and immunoglobulin levels were not influenced by ATG[31].

Influence of donor type on immune recovery It is speculated that T and B cell recovery is related to the degree of HLA matching between host and donor. HLA disparities may alter the capacity of lymphoid progenitors of donor origin to mature within the host thymus or bone marrow. On the other hand, HLA disparities stimulate the occurrence of GVHD. Therefore, patients receiving grafts from unrelated or mismatched related donors will always be treated with more intensive immunosuppression compared to recipients of MRD grafts. A well known characteristic of GVHD itself is a defect in the development of donor derived T cells, which leads to a long lasting T cell deficiency state[32,33]. Dulude et al[33] have demonstrated that GVHD impairs the production of new T cells by the thymus and disturbs the expansion of mature peripheral T cell pools in secondary lymphoid organs. This defective expansion was due to a restriction in the number of functional T cell niches. It is hypothesized that during the acute phase of GVHD, the thymus and secondary lymphoid organs are damaged to such a degree that a prolonged impairment in the development of donor derived T cells will occur. Furthermore, it has been demonstrated that patients with acute GVHD show an increase in CD3+ cell apoptosis, which further impairs T cell dependent immune reconstitution[34].

Altogether, this entanglement of factors makes it impossible to solely analyse the impact of donor type on immune recovery. Despite this, efforts to analyse the influence of donor type were made in several studies[21,23,35-38]. A more prolonged and profound CD3+, CD4+ and CD8+ lymphopenia was seen in MUD recipients compared to MRD recipients. However, the proliferative T cell response to PHA was comparable between the two groups. Furthermore, interference of GVHD with the observed impaired T cell subset recovery in MUD recipients could not be excluded[35]. Niehues et al[38] showed, in the setting of paediatric cord blood transplantation (CBT), that T cell recovery was favourable affected by use of a related donor. In the other studies, no effect of donor type was observed[21,23,36,37]. Recently, immune recovery in unrelated CBT recipients was analysed and compared to data from recipients of

MRD grafts. TREC levels were comparable between the two groups, while TCR diversity was normalized earlier in CBT recipients[39]. Two other studies described earlier[12,13] did study the patterns of CD4+ and CD4+ CD45RA+ T cell recovery and restoration of TCR diversity in recipients of TCD grafts from MUDs and MRDs, respectively. The results were highly comparable in these two reports. One might conclude from these results, that HLA disparities not necessarily result in an altered maturation of lymphoid progenitors of donor origin in the host thymus.

Influence of stem cell source on immune recovery Allogeneic unselected peripheral blood stem cell (PBSC) grafts contain about 10 times more CD4+ naive and memory T cells, CD8+ T cells and B cells[40,41]. Lymphocyte recovery has been shown faster in PBSC recipients[40-43], the difference being most striking for CD4+ naive and memory T cells[40,41,43]. Furthermore, the rate of severe infections after engraftment was significantly higher in bone marrow recipients[43]. However, when ex vivo TCD of grafts was performed with Campath-1 antibodies, the recovery of B and T cells and subsets was comparable among recipients of PBSC and BM grafts[44].

In conclusion, patient age, the occurrence of GVHD and the use of immunosuppressive therapy (especially ATG) are all adverse risk factors for T cell recovery post-transplant. However, the contribution of ex vivo TCD, the use of unrelated or partially matched related donors and the use of bone marrow instead of PBSC is less clear.

The aim of this thesis was:

1 to analyse the impact of high- and low-dose ATG on immune recovery and GVHD among MUD recipients (chapter 2, 3 and 9)

2 to study aspects of EBV and CMV reactivation/infection in recipients of allogeneic SCT:

- ***a*** the predictive value of the EBV DNA viral load for the development of EBV-LPD (chapter 4)
- ***b*** the origin of the EBV strain giving active EBV infection post-transplant (chapter 5)
- ***c*** the benefit of additional B cell depletion of T cell depleted grafts from matched unrelated donors regarding the incidence of EBV-LPD (chapter 6)
- ***d*** the role of patient and/or donor CMV-seropositivity as an adverse risk factor for survival post-transplant (chapter 8 and 9)

3 to review:

- ***a*** the prevention and treatment of EBV-LPD in recipients of bone marrow and solid organ transplants (chapter 7)
- ***b*** the prevention of Cytomegalovirus disease in recipients of allogeneic stem cell transplants (chapter 10)

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