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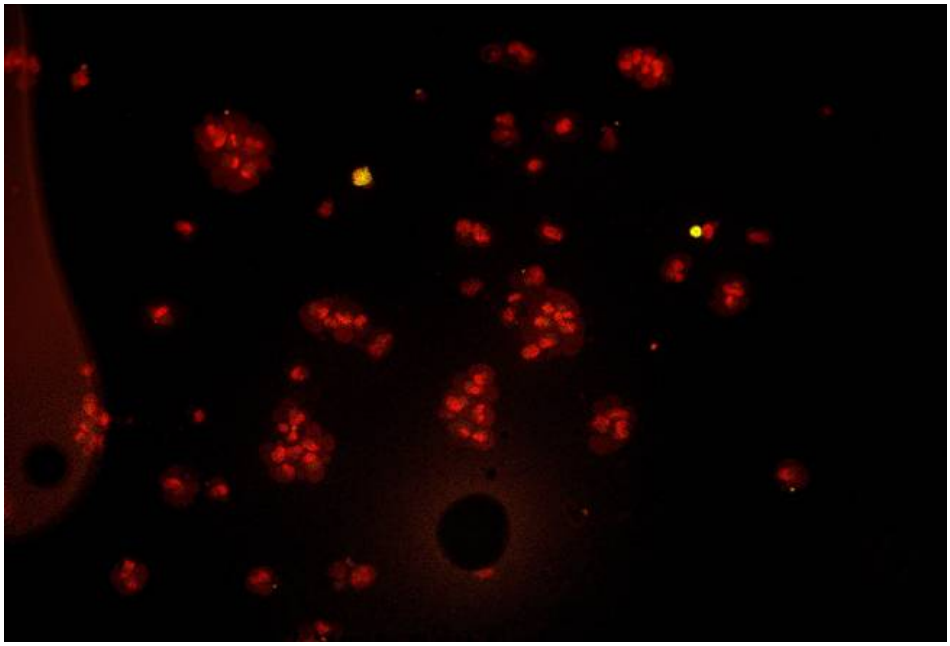
ALLOREACTIVITY IN STEM CELL TRANSPLANTATION



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To my late father

CONTENTS

1 SUMMARY

Acute graft-versus-host disease (GVHD), relapse and graft rejection are the main complications after allogeneic hematopoietic stem cell transplantation (HSCT).

The aim of this thesis was to achieve a better understanding of the alloreactivity seen after HSCT, focusing on graft rejection, but also including the graft-versus-leukemia (GVL) effect and GVHD.

We prospectively evaluated the GVL effect seen in 199 patients with acute lymphoblastic leukemia (ALL) after HSCT. Independent risk factors for relapse were the absence of chronic GVHD, absence of herpes simplex virus (HSV) infection after HSCT, GVHD prophylaxis with methotrexate (MTX) and cyclosporine A (CsA) and >6 weeks from the diagnosis to complete remission (CR). The association between HSV infection and a low relapse is a new observation and may indicate that viral antigens play a role in the induction of an antileukemic effect.

We also studied whether certain cytokine gene polymorphism were associated with severe GVHD after HSCT. We analyzed 196 patients and their corresponding donors for TNF-308, TNFd, IL-10(-1064) and IL-10(-1082) gene polymorphism. Serum analysis of TNF and IL-10 levels during conditioning therapy was also performed. Our results showed that among patients with sibling donors, the TNFd allele 4 was significantly correlated with acute GVHD grades II-IV. Acute GVHD grades II-IV were more common among patients homozygous for the IL-10-1064 allele 13. Patients homozygous for the TNF-308 allele (AA) correlated with higher TNF-alpha serum levels during conditioning.

We analyzed whether antibody-mediated rejection with antibodies against an important subpopulation of hematopoietic stem cells may cause rejection after HSCT. Between year 2000 and January 2006 20 patients rejected their grafts and we were able to analyze 11 patients with rejection. These 11 patients underwent totally 20 transplantations. In the study we also included 30 patients without rejection and 20 non-transplanted healthy individuals as controls. Ninety-three sera taken pre and post-transplantation from patients receiving HSCT were studied for the presence of donor CD34+/VEGFR-2+ cell-specific antibodies. Patients with rejection and controls were analyzed with FACS and microcytotoxicity assay.
We provide evidence that significantly higher numbers of patients with rejections 9/11 while 1/30 ($p=0.001$) without rejections had antibodies against donor CD34+/VEGFR-2+ cells, but not CD34-/VEGFR-2- cells. In eight transplantations, antibodies against donor CD34+/VEGFR-2+ cells were detected prior to transplantation.

We treated three patients with antibody-mediated rejection with immune modulation; i.e. plasmapheresis, intra venous immunoglobulin (IVIG) and rituximab prior to re-transplantation. With FACS and microcytotoxicity assay we could follow the pattern of antibodies of concern in sera during the immune modulatory treatment. Two patients had antibodies against donor CD34+/VEGFR-2+ cells and the third patient had anti-HLA-antibodies due to massive blood transfusions before HSCT. The immune modulatory regimen was well tolerated without any major side effects. In one patient with antibodies
against CD34+/VEGFR-2+ cells, plasmapheresis resulted in elimination of the antibodies according to microcytotoxicity assay but the patient did not have complete donor engraftment until after development of severe acute GVHD. In the patient

with high levels of anti-HLA-antibodies, receiving cord blood HSCT, plasmapheresis decreased the levels of anti-HLA-antibodies. Following cessation of plasmapheresis, the antibody titers increased again after HSCT and the patient never engrafted.

We also evaluated the value of cytotoxic T- and B-cell crossmatch testing before HSCT in 157 patients receiving grafts from unrelated donors. Eleven patients rejected their grafts. One of 11 patients with rejection was positive in a T-cell crossmatch before HSCT and 4/11 in B-cell crossmatches. This method showed a low sensitivity but high specificity concerning rejection. A positive T- and/or B -crossmatch before SCT had no predictive value for survival in this study as compared to patients with a negative crossmatch

To conclude, HSV infection may decrease leukemic relapse after HSCT, the TNFd4 allele and IL-10 (-1064) allele 13 in patients was correlated to acute GVHD grades II-IV. Antibodies to donor CD34+/VEGFR-2+ cells were correlated to rejection. Immune modulation including plasma exchange, IVIG and rituximab may eliminate CD34+/VEGFR-2+ antibodies and pave the way for engraftment. The cytotoxic crossmatch analysis did not predict graft rejection.

2 LIST OF PUBLICATIONS

I Graft-versus-host disease is associated with a lower relapse incidence after hematopoietic stem cell transplantation in patients with acute lymphoblastic leukemia.
Nordlander,A, Mattsson,J, Ringden,O, LeBlanc,K, Gustafsson, B, Ljungman, P, Svenberg, P, Svennilson, J, Remberger, M
Biol Blood Marrow Transplant. 2004 Mar;10(3):195-203.

II The TNFd4 allele is correlated to moderate-to-severe acute graft-versus-host disease after allogeneic stem cell transplantation.
Nordlander, A, Uzunel, M, Mattsson, J, Remberger, M
British Journal of Heamatology, Dec; 119(4):1133-6, 2002

III Novel antibodies to the donor stem cell population CD34+/VEGFR-2+ are associated with rejection after haematopoietic stem cell transplantation.
Anna Nordlander, Jonas Mattsson, Berit Sundberg, Suchitra Sumitran-Holgersson
Transplantation. 2008 Sep 15; 86(5):686-96.

IV Immune modulation to prevent antibody-mediated rejection after allogeneic hematopoietic stem cell transplantation.
Anna Nordlander, Olle Ringdén, Dan Hauzenberger, Jonas Mattsson Submitted to Transplantation

V Cytotoxic crossmatch analysis before allogeneic stem cell transplantation is a poor diagnostic tool for prediction of rejection
Jonas Mattson, **Anna Nordlander,** Mats Remberger, Michael Uhlin, Jan Holgersson, Olle Ringdén, Dan Hauzenberger
Submitted to Bone Marrow Transplantation

3 LIST OF ABBREVIATIONS

ACV	Acyklovir
ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
APC	Antigen presenting cell
ATG	Anti-thymocyte globulin
BMT	Bone marrow transplantation
BM	Bone marrow
Bu	Busulfan
CD	Cluster of differentiation
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CP	Chronic phase, CP1=1st chronic phase
CR	Complete remission
Cy	Cyclophosphamide
CsA	Cyclosporine A
DC	Donor chimerism
DLA	Dog leukocyte antigen
DLI	Donor lymphocyte infusion
EBMT	European group for Blood and Marrow Transplantation
EBV	Epstain Barr Virus
ELISA	Enzyme-linked immunosorbent assay
Flu	Fludarbin
G-CSF	Granulocyte colony-stimulating factor
GVHD	Graft-versus-host disease
GVL	Graft-versus-leukemia
GVT	Graft-versus-tumor
Gy	Gray
HLA	Human leukocyte antigens
HHV-6	Human herpes virus 6
HSCT	Hematopoietic stem cell transplantation
HSV	Herpes simplex virus
IBMTR	International Bone Marrow Transplantation Registry
IFN	Interferon
IL	Interleukin
i.v.	Intravenous
IVIG	Intravenus immunoglobulin
KIR	Killer cell Ig-like receptor
MC	Mixed chimerism
MDS	Myelodysplastic syndrome
MHC	Major histocompatibility complex
MiHA	Minor histocompatibility antigens
MMF	Mycophenolate mofetil
MRD	Minimal residual disease
MSC	Mesenchymal stem cell

MTX	Methotrexate
MUD	Matched unrelated donor
NK	Natural killer
OKT-3	Orthoclone, monoclonal antibody against CD3
PB	Peripheral blood
PBSC	Peripheral blood stem cell
PCR	Polymerase chain reaction
Ph	Philadelphia chromosome
PRA	Panel reactive antibody
PTLD	Post-transplant lymphoproliferative disease
PUVA	Psoralen and ultraviolet light A
SAA	Severe aplastic anemia
SCID	Severe combined immunodeficiency
SOS	Sinusoidal obstruction syndrome
RIC	Reduced intensity conditioning
TAM	Transplant associated microangiopathy
TBI	Total body irradiation
TCR	T-cell receptor
TLI	Total lymphoid irradiation
TRM	Transplantation-related mortality
VNTRs	Variable number of tandem repeats
VOD	Veno-occlusive disease of the liver
WBC	White blood cells

4 INTRODUCTION

Hematopoetic stem cell transplantation (HSCT) replaces abnormal hematopoiesis with a normal one, making it an effective therapy for nonmalignant diseases such as severe combined immunodeficiency disease (SCID), aplastic anemia, thalassemia, and sickle cell disease. Since hematopoietic toxicity is dose-limiting for many types of chemotherapy and systemic radiotherapy, HSCT can treat a variety of malignant diseases by delivering higher and potentially more effective doses of therapy. HSCT may also cure malignant diseases by itself through an immunologic attack on cancer cells.

4.1 ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

At the end of 1950s, E. Donnall Thomas et al. pioneered clinical HSCT studies[1]. In 1957, they performed the first allogeneic bone marrow transplantation in humans. However, the results in the early era of HSCT were poor[2]. In some patients with engraftment, a lethal immunologic reaction of the graft against the host was observed. This reaction, graft-versus-host disease (GVHD), was first described in mice and resulted in diarrhea, weight loss, and skin pathology[3-6]. Identifying the major histocompatibility complex (MHC) and the human leukocyte antigens (HLA) was necessary for development of rational strategies for HSCT. Early studies using dogs established that recipients of dog leukocyte antigen (DLA)-matched grafts could survive in good condition for several years after the procedure[7-10]. These studies also showed the necessity for suppressing the immune system of the recipient to achieve engraftment.

By the mid-1970s, researchers realized that successful outcome depended on the matching of donor and recipient[11]. When HLA matching could be performed, survival rates increased. In 1975, Thomas et al. reported their results on bone marrow transplantation (BMT) with HLA-identical sibling donors. In 1980, the first successful BMT with a matched unrelated donor (MUD) was reported[12]. The incidence and severity of GVHD is correlated with the degree of HLA incompatibility[13]. These results started the interest in establishing volunteer donor registries that identified HLA-matched unrelated marrow donors for patients in need of HSCT. Today more than eleven million volunteer donors are available worldwide in different registries (www.bmdw.org). The results using matched unrelated donors (MUD) have also been improved and are today nearly as good as using HLA-identical sibling donors[14,15].

Continuous research and evaluation of new methods and drugs have improved the results in treatment with HSCT. As a result of better survival rates and less severe complications after HSCT, the indications for HSCT have broadened. Today, HSCT is an established therapy for hematological malignancies, severe aplastic anemia (SAA), severe combined immunodeficiency (SCID), and some inherited metabolic disorders. It is also an experimental therapy in patients with solid tumors and in severe cases of autoimmune disease[16].

4.2 CONDITIONING

Conditioning therapy is administered before HSCT to eradicate malignant cells and to provide adequate immunosuppression to avoid graft rejection. Conditioning regimens differ depending on the diagnosis. In the case of a malignant disease, the main goal is to eradicate as many malignant cells as possible to diminish the tumor load and prevent relapse. In non-malignant diseases, the main purpose of the conditioning is to avoid graft rejection.

Today, there are two major principles for conditioning regimens. Myeloablative conditioning consists of high doses of chemotherapy with or without irradiation. This treatment is highly toxic and without the treatment the subsequent HSCT the patient would die. The most commonly used myeloblative conditioning treatment, developed by the Seattle group in the early 1970s, consisting of total body irradiation (TBI) of 10 gray (Gy) combined with cyclophosphamide (Cy) 60 mg/kg on two consecutive days [17,18]. Several variations in the dose and fractionation of TBI and the dose of Cy have also been applied[19]. The Cy/TBI conditioning regimen can be replaced with a combination of busulfan (Bu) and Cy. This regimen is used especially in children because they are more sensitive to the toxic effects of irradiation[20-22]. Busulfan is usually administered 4 mg/kg on four consecutive days combined with Cy 60 mg/kg on two consecutive days[21]. In a randomized trial in allogeneic marrow recipients with leukemia by the Nordic Bone Marrow Transplantation Group, long-term results showed that Bu/Cy, compared to TBI/Cy, increases the risk of chronic graft-versus-host disease, obstructive bronchiolitis, and alopecia[24]. However blood monitoring of Bu may reduce the regimen associated toxicity [25]. In addition, liposomal Bu may improve the results of the Bu/Cy regimen[23].

Reduced intensity conditioning (RIC) relies less on chemoradiation therapy and shifts the burden of tumor-cell killing to graft-versus-leukemia (GVL) effects[26-29]. The GVL effect is considered to be the main requirement for long-term disease control in patients undergoing HSCT for hematological malignancies[30]. Since RIC is less toxic compared to myloablative conditioning, older patients and patients with poor medical conditions can receive allogeneic HSCT[27] with fewer side effects. However, since the introduction of RIC, the frequency of patients with rejections after HSCT has increased. Reduced intensity regimens either use combinations of chemotherapy drugs, such as fludarabin (Flu), together with ATG, busulfan, Cy, or a low dose TBI[31].

4.3 SOURCES OF HEMATOPOIETIC STEM CELLS

Since the beginning of HSCT research, bone marrow has been used as the source of hematopoietic stem cells. Bone marrow cells are usually obtained from the donor's posterior iliaca crest under general or spinal anesthesia[18]. Reconstitution of hematopoiesis after HSCT is achieved by a constant replenishment from primitive, quiescent hematopoietic stem cells. The phenotype of HSC in human beings has been thoroughly characterized by flow cytometry and BM transplantation[32]. In humans, HSC are primarily defined by the expression of CD34, although there is evidence for CD34-negative HSC precursors. Recently, CD34+/VEGFR-2+ cells from adult bone marrow or cord blood have been identified, cells that can generate both haematopoietic and endothelial cells in vitro[33] . This cell population also seems to be of importance for engraftment after HSCT [34].

In adults, most hematopoietic stem cells are found in BM (1-2%) compared to 0.2% in blood [32]. However, stem cells may be mobilized in peripheral blood in higher amounts than in bone marrow after administration of the hematopoietic growth factor G-CSF [35]. The first reports using peripheral blood stem cells (PBSC) for allogeneic HSCT were published 1994[36,37,38]. The advantages with PBSCT are that the donor does not need any anesthesia and more stem cells may be collected, advantages that may speed up engraftment after HSCT. Patients receiving PBSCT showed a faster engraftment of neutrophils and platelets compared to patients given BM, but no difference in acute GVHD incidence and survival[39]. However, there is an increase in chronic GVHD events in patients undergoing HSCT with PBSC [40,41].

Umbilical cord blood, which contains hematopoietic stem cells, has also been used as source for HSCT [42,43]. Early studies noted that the frequency of hematopoetic stem and progenitor cells is

higher in cord blood grafts than in adult BM or PBSC [44] and stem cells from cord blood are more tolerant to one or two HLA mismatches. Unfortunately, cord blood has been associated with slower engraftment and an increased risk of graft failure[45,46] mainly due to the limited volume of cells that can be collected.

4.4 TISSUE TYPING AND CROSSMATCHING

Among the oldest immunological rules in transplantation is the requirement that both the donor and the recipient express identical cell surface antigens, ensuring "histocompatability" that may prevent or rather decrease the risk of graft-versus-host and host-versus-graft responses. The three classes MHC (I, II, and III) play a central role in both cell-mediated and humoral immune responses. MHC classes I and II are cell surface molecules controlling T-cell recognition and histocompatibility[47,48]. MHC class III is involved in immunity by expression complement proteins and cytokines. MHC class I antigens (HLA-A, HLA-B, and HLA-C) are widely distributed and are found on all nucleated cells. HLA class II antigens (DP, DQ, and DR) are found on antigen presenting cells (APC) such as B-cells, dendritic cells, and macrophages. There are hundreds of variant forms of each class I and class II molecule, and even small differences between them can provoke alloreactive T-cell responses. Hence, matching HSCT recipients with sibling donors sharing identical HLA antigens has significantly improved engraftment kinetics and decreased GVHD severity[49-51]. Although individuals are HLA-matched, there are still differences in many of the endogenous proteins presented by HLA, and T-cells from one person will react to the "minor" antigens of another person. Minor histocompatibility antigens (miH) are due to polymorphisms of other non-HLA proteins, differences in the levels of expression of proteins or genome differences between males and females[52,53]. The miH are critical in matched sibling allogeneic bone marrow graft.

Matching donors and recipients for MHC class I and II molecules not only prevents GVHD and graft rejection, but is also necessary for the recipient to recover a working adaptive immune system. After transplantation, most patients hopefully become stable chimeras in which hematopoietic cells are of donor HLA type, but all other cells are of recipient HLA type.

The first crossmatch techniques to be introduced used complement-dependent cytotoxicity with donor T- and/or B-lymphocytes as target cells, a technique that allowed for the detection of donor-reactive HLA class I and II-specific antibodies[54] . By applying flow cytometry to the crossmatch tests, increased sensitivity with regard to detection of donor-reactive antibodies has been accomplished[55]. In addition, to assess the panel-reactive antibodies (PRA), more sensitive solid phase techniques using single HLA antigens in ELISA, flow cytometry, or the Luminex formats have been introduced.

4.4.1 Immunosuppression

To prevent severe GVHD, immunosuppressive treatment is given after HSCT. Because tolerance is achieved in the majority of patients, immunosuppression is temporary and usually discontinued

within one year for malignant diseases and within two years for non-malignant diseases[56]. In siblings, the duration of immunosuppressive treatment is shorter compared to unrelated donor transplantations. Today, the immunosuppressive treatment of choice is the combination of methotrexate (MTX) and CsA[57-60]. Other drugs used are tacrolimus[61,62], mycophenolate mofetil (MMF)[63], and rapamycin[64]. Patients with hematological malignancy gain from mild GVHD since

GVHD is correlated to GVL. In these patients, immunosuppressive treatment is discontinued as soon as possible since prolonged immunosuppression increases the risk of relapse[65]. It was shown that low dose CsA compared to high dose decreased the risk of leukemic relapse in patients with acute myeloid leukemia, receiving grafts from HLA-identical sibling donors[66]. Patients with non-malignancies do not gain from GVHD but need to be protected against graft rejection. Another immunosuppressive treatment is the use of T-cells antibodies like ATG and OKT-3. Depletion of T-cells in the graft *ex* and *in vivo* has been shown to decrease the incidence of GVHD but also increase the risk of relapse and graft rejection[67-73]. After non-myeloablative HSCT, different immunosuppressive regimens have been tried including CsA alone[27,74], CsA combined with MMF[75], and CsA combined with methylprednisolon[29]. At our unit, we have used CsA and MTX in patients with hematological diseases and CsA combined with MMF ot MTX in patients with solid tumors[76].

4.5 COMPLICATIONS AFTER HSCT

4.5.1 Infections

The combination of an immature immune system and immunosuppressive treatment gives rise to numerous infectious complications that are a major cause of morbidity and mortality after HSCT[77,78]. Different pathogens are more common during different phases of the immune reconstitution. Immune recovery is often divided into three phases: the pre-engraftment phase, the post-engraftment phase, and the late phase.

During the pre-engraftment neutropenic phase, day 0-30 after HSCT, gram-positive bacteria from the skin, gastro intestinal tract and mouth are the most common cause of bacteremia[77,79,80]. Gastrointestinal gram-negative bacteria can cause more severe infections, but due to prophylactic treatment with for instance ciprofloxacin and/or early intervention with broadspectrum antibiotics in the case of fever, the incidence of these infections has decreased[81]. Among viruses, herpes simplex viruses (HSV) are frequently reactivated during the aplastic phase. Prophylactic treatment should be given in HSV-seropositive recipients with high titers after HSCT[82]. Fungal infections with candida species often occur during the aplastic phase with an incidence of around 10%[83,84]. Most common is oro-esopheagal candida, but invasive fungal infection with candida or aspergillus might occur. Prophylaxis with fluconazol is given at some centers during the aplastic phase[85].

During the post-engraftment phase, day 30-100 after HSCT, the cellular immunity is depressed but recovering. Cytomegalovirus (CMV) is the most common infection during this phase and is often associated with GVHD[86-88]. Pre-emptive treatment strategies based on PCR-surveillance of CMV have greatly reduced the risk of fatal disease[89-91]. Other viral infections such as EBV and adenovirus may occur. EBV may cause a lymphoma-like condition called post-transplant lympho-proliferative disease (PTLD)[90,92]. After the aplastic phase, the risk of fungal infections is decreased, but late aspergillus infection may occur and is associated with high mortality[93]. Due to prophylactic treatment with co-trimoxazole, opportunistic infections with toxoplasmosis and pneumocystis carinii are rarely seen.

During the late phase, the period beyond 100 days post HSCT, both humoral and cellular immunity are still impaired. Reactivation of varicella zoster virus, CMV, and infections with encapsulated bacteria are common during this period, especially in patients suffering from chronic GVHD[94,95].

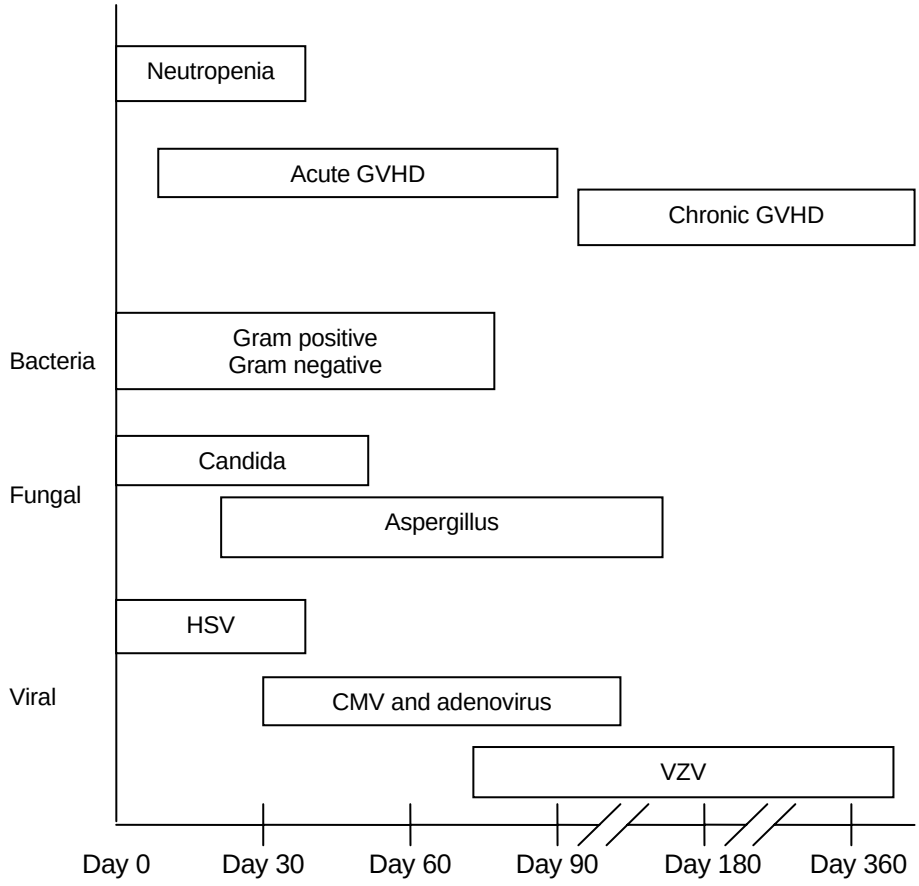

Figure 1. The major syndromes that complicate hematopoietic stem cell transplantation and the approximate periods in which they develop. Abbreviations: GVHD, graft-versus-host disease; HSV, herpes simplex virus; CMV, cytomegalovirus; VZV, varicella zoster virus.

4.5.2 Relapse

Relapse of the underlying disease is a major cause of treatment failure after HSCT. Generally, leukemia relapses occur in recipient-derived cells, indicating that the original malignant clone survived chemoradiotherapy and escaped from the immunological anti-tumor effects of the graft. However, in a few patients evidence for recurrent leukemia in the donor cells has been found[96-100], indicating the persistence of a leukaemogenic hazard. Most of the latter cases have occurred late after HSCT and have been associated with the use of TBI. Relapses are usually systemic; many cases exhibit a drop in platelet counts and sometimes improvement of GVHD.

Relapse incidence mainly depends on the disease, stage of the disease, and type of donor. Despite the improvements in HSCT, the risk of relapse has stayed at relatively constant level[101,102]. In patients with leukemia, relapse is the most common cause of treatment failure after HSCT. The lowest relapse rates are seen in patients transplanted in first complete remission[103]. T-cell depletion and absence of GVHD are factors associated with higher relapse incidences[60,67,104,105]. The outcome for patients who relapse early after HSCT is usually poor[102,103,106]. Diagnosis of relapse is traditionally made by morphological analyses of the bone marrow, laboratory findings in peripheral blood, and clinical symptoms and findings. Today, however, as more refined diagnostic methods have been developed to detect minimal residual disease, relapses can be detected at earlier stages than before .

One way to treat relapse is to use donor lymphocyte infusions (DLI). The first attempts to augment the immunological effects of a bone marrow graft were done in the 1980s. The initial purpose was

to prevent graft rejection in patients with aplastic anemia[108,109] and to reduce the risk of relapse in leukemic patients following HSCT[110]. As DLI was given early after HSCT, the transplant related mortality (TRM) was as high as 64%, mainly due to severe GVHD[110]. In animal experiments, Kolb et al. found that delayed donor cells were better tolerated and they were the first to induce lasting, complete remissions by DLI in patients with relapsed CML[111]. The potent GVL effects of DLI have since then been confirmed in many studies[106-120], establishing DLI as one of the most important treatment options for leukemias relapsing after HSCT.

4.5.3 Toxic side effects

The intensity of the conditioning is limited by toxicity on vital organs like the heart, liver, kidneys, lungs, gastrointestinal tract, and the central nervous system. The risk of cardiac damage increases after conditioning with cyclophosphamide in doses above 120mg/kg or if it is given in combination with TBI[112]. Early onset of hemorrhagic cystits is related to the conditioning regimen[22,113,114], but later development seems to be associated with BK virus[115,116], adenovirus[117], cytomegalovirus[118], and to GVHD[114,119,120]. Prevention and treatment are based on hyperhydration. Sinusoidal obstruction syndrome (SOS), previously called veno-occlusive disease (VOD), usually develops within a month after HSCT and is characterized by hepatomegaly, ascites, jaundice, and abdominal pain[121-123]. The incidence of SOS is about 5%[124]. Both busulphan and irradiation have been associated with SOS. Anticoagulants and thrombolysis along with symptomatic treatment have been used as therapy[122,125]. Transplant associated microangiopathy (TAM) is characterized by anemia, presence of schiztocytes, elevated lactate dehydrogenase, thrombocytopenia, fever, and renal insufficiency. Discontinuation of immunosuppressant drugs may resolve the syndrome. Plasmapheresis and thrombolytics have been used with varying results[126,127].

4.5.4 Late complications

Recipients of HSCT are at an increased risk of secondary malignancies[128-132]. This incidence is at least four times higher than in the general population and increases with time after HSCT[133]. Lymphoproliferative disorders are the most frequent. In solid tumors, squamous cell carcinoma, malignant melanoma, glioblastoma, and adenocarcinoma are seen[134]. Other late complications seen are cataract[135] and endocrinological dysfunction resulting in growth retardation or infertility[136].

4.6 ALLOREACTIONS POST-HSCT

Alloreactivity seen after HSCT is a complex process that involves donor T-cells, B-cells, and NK-cells interacting with specific recipient target tissue. This immune response is mediated by both direct lymphocyte target cell interaction and by cytokines. The alloimmune responses seen after HSCT are responsible for the three major transplant events – GVHD, GVL effects, and graft rejection – that determine success or failure of the transplant:

4.7 GRAFT-VERSUS-HOST DISEASE

Graft-versus-host disease is a major complication after HSCT. The frequency varies, and can be as high as 85%[50,59,137-139] depending on the type of donor and HLA-matching. GVHD exist in an acute form that often occurs during the first 100 days after HSCT. However, patients that have received RIC may develop acute GVHD beyond three months. Chronic GVHD often appears more than three months after HSCT and may be a continuation of acute GVHD[140]. Acute and chronic GVHD differ in clinical manifestations and are considered to be two different conditions[139]. Although the molecular pathogenesis of GVHD remains uncovered, there is general agreement

that infiltrating T-lymphocytes play a central role in both acute and chronic GVHD[141]. However, the relationship between chronic and acute GVHD is complex and incompletely understood.

Matching of MHC antigens speed engraftment and reduces the severity of GVHD[51], but despite HLA identity between a patient and donor, substantial numbers of patient still develop GVHD due to differences in miH antigens[53,142]. The clinical expression of acute GVHD mainly affects the skin, gastrointestinal tract, and the liver. Acute GVHD is classified on a 1-4 scale according to criteria published by Glucksberg in 1974[143]. Severe acute GVHD (grades III-IV) has a profound impact on the prognosis after HSCT[144].

Today, the pathophysiology of acute GVHD is divided in three phases[145]. Phase one involves tissue damage caused by the conditioning regimen, infections, and the underlying disease and/or its treatment[146]. The damaged tissue will secrete cytokines, chemokines, and upregulate expression of adhesion molecules, inducing an inflammatory response including activation of dendritic cells. In the second phase, activation of donor T lymphocytes occurs. Recipient APCs that have migrated to the lymph nodes will present peptides from recipient antigens to donor T lymphocytes inducing activation, proliferation and finally differentiation into effector T lymphocytes[147]. The third phase of acute GVHD involves the inflammation and cellular damage caused by the mature effector T lymphocytes. It includes the secretion of cytokines, specific cytotoxic T lymphocyte activity, directed against the recipient cells using Fas and perforin pathways, large granular lymphocytes or NK cells, and the release of nitric oxid[146]. The result of this phase of acute GVHD is further tissue damage and sustained inflammation.

Various treatments are used for acute GVHD including prednisolone, methylprednisolone, ATG, CsA, MTX, Tacrolimus, Psoralen and ultraviolet light (PUVA), thalidomide, anti-IL-2 antibodies, and other agents[50,137,148]. However, in severe acute GVHD no treatment is as yet satisfactory[50]. Recently, multipotent mesenchymal stromal cells (MSC) have been tried in therapy resistant GVHD with some encouragement[149]. Prospective randomized studies are under way to evaluate this treatment in steroid refractory acute GVHD.

Chronic GVHD is one of the most frequent late complications after HSCT, affecting 30 –50% of long-term survivors[85,140,150,151]. Chronic GVHD differs from acute GVHD not only in the timing after HSCT but also in clinical manifestation. Chronic GVHD develops with manifestations like dermatitis, keratoconjunctivitis, oral mucositis, and hepatic dysfunction. It is graded as limited or extensive[152]. In a study performed at our center, significant risk factors for chronic GVHD were advanced recipient age, acute GVHD grades I- IV, CML, and alloimmunized female donor to male recipient[85].

Treatment of chronic GVHD mainly consists of prednisolone, CsA, thalidomide, and PUVA[50,148,153]. The best prophylaxis of chronic GVHD is the prevention of acute GVHD since only 15-20% of the patients without acute GVHD will develop chronic GVHD compared to 40-100% with acute GVHD[151]. However, chronic GVHD and its associated morbidity and mortality remain a major obstacle after HSCT.

4.7.1 Cytokine gene polymorphisms

Allogeneic stimulation or an infection may increase the amount of cytokine production. Many of the reported cytokine gene polymorphisms occur within regulatory regions of the gene. In the normal population, high or low producers of cytokines naturally exist due to the inherited gene

polymorphisms. It has been shown that patients with high producer tumor necrosis factor (TNF) and low producer interleukin 10 (IL-10) genotypes were more likely to reject their solid organ graft. Although the role of donor T-cell activation in the induction of GVHD has been confirmed, it seems that several cytokines are also involved[154]. In the development of acute GVHD, cytokines are released as a result of conditioning regimen toxicity and infection initiates the synthesis of other cytokines, which increases target organ injury. It has been suggested that the tissue damage of GVHD might be mediated by the associated inflammatory response rather than a direct cellular immunologic attack[155].

4.7.2 TNF gene

The TNF-alpha gene is located within the class III region of MHC on chromosome 6 near many polymorphisms; several of these are associated with inflammatory disease[156]. Therefore, in HLA-identical sibling transplantation, recipient, and donor genotype will be identical and may equally or additively affect TNF production and transplant outcome, including GVHD[157]. TNF-alpha is produced mainly by monocytes, or by T and B cells, and has a proinflammatory activity[158]. It can activate endothelial cells and induce expression of adhesion molecules, structures that are associated with leukocyte homing[159]. It also evokes expression of HLA molecules, which activate antigen specific T cells. Clinically, an increased level of TNF-alpha during conditioning before HSCT has been found to correlate to moderate-to-severe GVHD and transplant-related mortality (TRM)[160].

TNF d3/d3 has been shown to correlate with acute GVHD (grades III-IV) in patients receiving HLA-matched siblings transplants and CsA alone as immunosuppressive therapy[161]. A larger study among patients who received CsA and MTX prophylaxis showed an association of recipient TNFd3/d3 genotype with increased mortality[162]. A Japanese MUD transplant study described a correlation of the TNF-863 and -857 polymorphisms in donors and/or recipients with higher incidence of GVHD grades III-IV and a lower rate of relapse[163]. In individuals with the rare allele TNF2, the production of TNF-alpha is markedly increased by a high transcriptional activation[164].

4.7.3 IL-10 gene

The anti-inflammatory cytokine interleukin 10 (IL-10) inhibits monocyte production of proinflammatory cytokines[165], including TNF-alpha and decreases apoptosis induced by lipopolysaccharide and irradiation[166]. IL-10 also reduces MHC expression and attenuates recognition by cytotoxic lymphocytes[167]. In HSCT, IL-10 production before HSCT protects from TNF-alpha release, acute GVHD, and other transplant related complications[168]. Cells from patients with acute or chronic GVHD produce less IL-10 in vitro[169]. The IL-10 gene regulatory region includes two microsatellite polymorphisms, which are correlated to different in vitro IL-10 production[170]. One study showed a correlation between greater IL-10 (-1064) repeat number in the recipient and GVHD grades III-IV in CyA-treated matched sibling HSCT[161]. IL-10(-1082) A allele was associated with lower in vitro IL-10 production[171].

4.8 GRAFT-VERSUS-LEUKEMIA

By the end of 1950s, Barnes et al. noted that leukemic mice treated with syngeneic marrow were more likely to relapse than mice transplanted with an allogeneic graft[172] , and they therefore hypothesized that the allogeneic graft contained cells with immune reactivity. In 1979, evidence for a GVL effect in humans first emerged with the observation that relapses were markedly lower among patients who developed GVHD than among those who did not[173]. Today, it is well established that the relapse rate in leukemia's decreases with increasing severity of acute GVHD

and that chronic GVHD is associated with a stronger GVL effect than acute GVHD[60,174]. The best leukemia free survival is seen in patients with both grade I acute GVHD and chronic GVHD[104]. Patients receiving T-cell depleted grafts have less GVHD, but they also have a higher incidence of relapse[67,175,176], supporting the idea that the GVL effect is mediated by donor derived T-cells. On the other hand, it has also been shown that GVL can be present in the absence of GVHD[104,177]. This may indicate that GVL and GVHD have different underlying mechanisms[178]. Further proof for the GVL effect comes from the use of donor lymphocyte infusions (DLI) to treat relapse of the underlying disease after HSCT[179], demonstrating the immunotherapeutic effect of alloreactive T-cells. The effect of DLI, however, does not have to be immediate: remission has been seen as late as four to twelve months after infusion[180]. Unfortunately, DLI involves a risk of developing GVHD in up to 50% of patients; this can be reduced by using escalating doses or eliminating CD8 cells from the infusion[181].
GVHD may occur due to disparity between major and minor histocompatibility antigens[142,182]. The HLA disparity can trigger a powerful GVL effect but at the cost of increased TRM due to severe GVHD. In the HLA class I and II matched setting, targets for GVL effects are probably minor histocompatibility and/or tumor-associated antigens[53,183].

Another important effector cell involved in the GVL effect is the natural killer (NK) cell. NK cell activation is regulated by a balance between inhibitory and activating receptors – killer-cell Ig-like receptors (KIRs). NK cells are among the first immune cells to recover after SCT[184] and they mediate cytotoxic effects without prior sensitization. In vivo studies in murine models have shown that transplantation of grafts depleted of T cells, but retaining NK cells correlated with reduced relapse rates and minimal incidence of GVHD[185]. In HLA-mismatched haploidentical HSCT for AML, donor NK clones fail to encounter class I inhibitory KIR ligands, resulting in the killing of host leukemic cells. In clinical trials NK-cell-mediated anti-tumor reactivity in the context of KIR-ligand mismatched allo-HSCT shows promising results[186].

In 1995, Sakaguchi et al. described a small subset of CD4+ cells called regulatory T cells (CD4+CD25+), cells crucial for preventing autoreactivity. Mice depleted from these cells developed severe systemic autoimmune disease[187]. In humans, T-regs account for 1-2% of circulating CD4+ cells. Some studies have shown that co-infusion of allogeneic T-regs and effector cells prevented GVHD while preserving GVL, suggesting a distinctive pathway of cell killing for these populations[188]. The role of T-regs in clinical HSCT remains unclear and trials to investigate this are ongoing.

Cellular vaccines, using specific CTLs directed against tumor proteins (e.g., BCR-ABL, PR1, and WT1) or minor antigens are a way of enhancing the GVL-effect HSCT. The combination of GVL effect with a vaccine boost for leukemia-specific T cells would probably be a highly effective way to control refractory leukemias. Future perspectives are aiming at more specific responses against relapsed or persistent leukemias and tumors after HSCT and at inhibiting GVHD. These include depletion or infusion of selected cell populations, genomic modification, and vaccination.

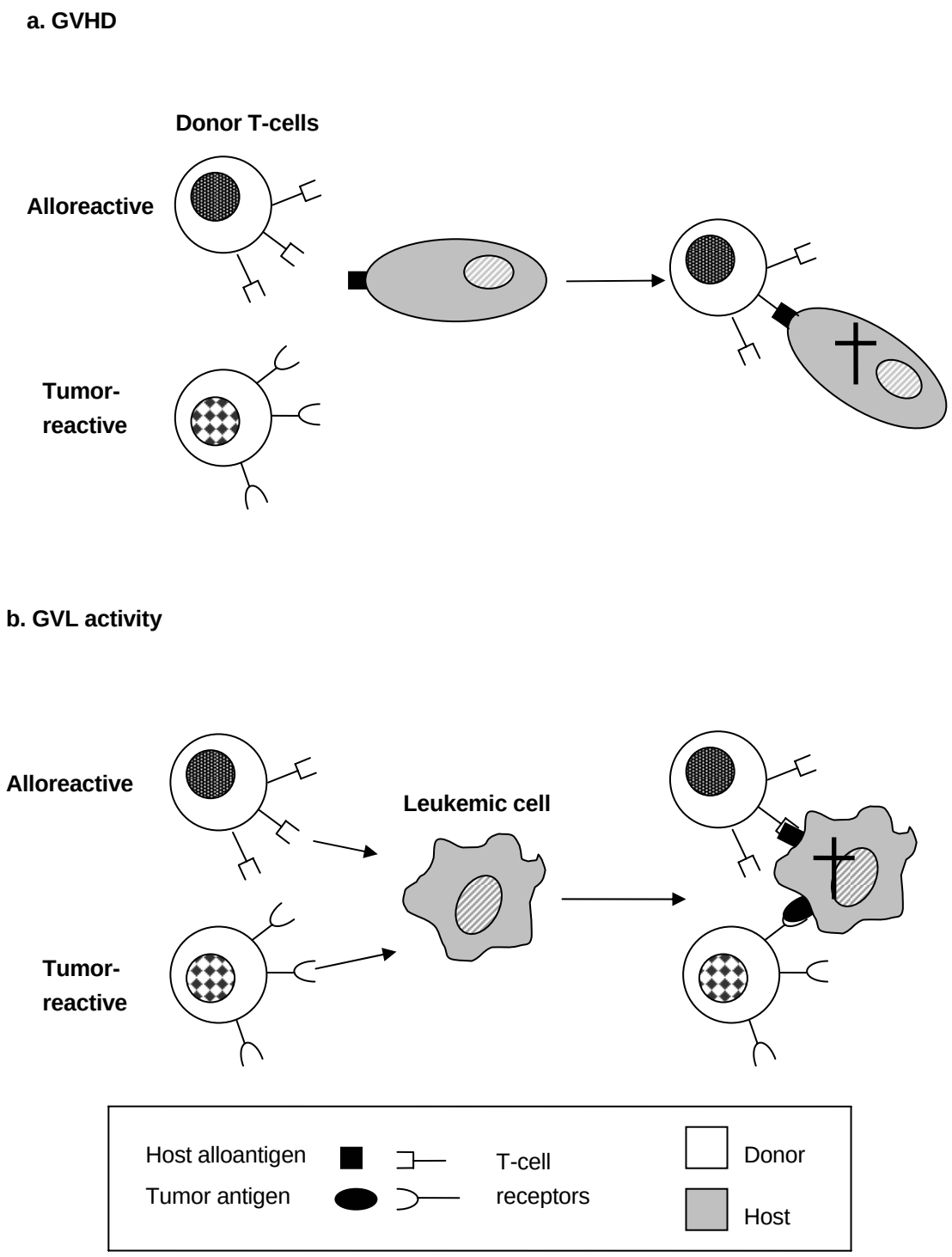

Figure 2. GVHD and GVL activity. a) Donor T-cell populations in the allograft contain alloreactive T-cells, which become activated and proliferate on recognition of specific MHC or minor histocompatibility antigen on host cells. These alloreactive T cells can have cytolytic activity against host cells, which contributes to the development of graft-versus-host disease (GVHD). **b)** Donor T-cell populations in the allograft can also contain tumor-reactive T cells, which can recognize tumor-associated antigens and exert cytolytic activity against leukemic cells, the graft-versus-leukemia (GVL) effect.

4.9 CHIMERISM ANALYSIS

In medicine, chimerism has been used to describe a body that contains cell populations from another individual of the same or a different species[189]. The term *mixed chimerism* (MC) describes a situation after HSCT when there is a mixture of recipient and donor hematopoietic cells after transplant, whereas the term *donor chimerism* (DC) or complete DC is used when all detected hematopoietic cells are of donor origin. In most cases, the aim with HSCT is to achieve donor chimerism. The basic use of the chimerism technique is to monitor the engraftment process.

Chimerism is usually analyzed in different cell lineages such as T cells (CD3+), B cells (CD19+), myeloid cells (CD33+), NK cells (CD3-/56+), and hematopoietic progenitor cells (CD34+)[190-193]. The clinical significance of the patient's chimeric status in different conditions – such as GVHD, rejection, and relapse – has been debated[194,195]. Early development of donor T-cell chimerism at day +7 after HSCT has been shown to be significantly correlated to acute GVHD grades II-IV[146]. Some studies have shown a correlation between increased numbers of recipient cells and relapse of acute leukemia, although some studies do not see a correlation of relapse to low numbers of remaining recipient cells[196-198]. However, detection of mixed chimerism in leukemia affected cell lineage is highly correlated to relapse[190,199,200]. Perhaps, T-cell MC is a risk factor for rejection[191,201]. T-cell MC is more common in RIC treated patients compared to patients treated with myeloblative conditioning[75]. Studies have also shown that patients with T-cell MC have a lower risk of developing GVHD[202,203].

Several methods have been used to characterize chimerism after HSCT. The method of choice is PCR of variable number of tandem repeats (VNTR). After PCR, the different DNA sequences can be separated and visualized by electrophoresis. Recently Real time-PCR techniques have been introduced resulting in increased sensitivity[204].

4.10 REJECTION

Crucial for success after HSCT is persistent engraftment of the transplanted stem cells. The frequency of graft rejection varies depending on diagnosis and conditioning. Rejection is a major cause of graft failure and is caused by immunocompetent recipient cells acting against donor hematopoietic cells. Rejection is detected with chimerism analysis, showing only the presence of recipient cells (at Karolinska defined as < 1% donor cells). Meanwhile graft failure can also be caused by viral infections, (e.g. CMV, HHV 6 and parvovirus), drug toxicity and septicemia.

With the exception for multitransfused patients with severe aplastic anemia, graft rejection after HSCT has been a rather rare complication using myeloablative conditioning[205,206]. However, the use of T-cell depleted marrow[67], the introduction of haploidentical transplants[207] and since the introduction of RIC and cord blood transplants the incidence of graft rejection has increased[208,209].

4.10.1 Cellular and humoral immune response in HSCT rejection

Graft rejection is commonly due to recipient T- and NK-cells that survive the conditioning treatment[210-214]. Barao et al recently showed that T-reg cell depletion enhanced NK-cell mediated rejection[215].

Although, antibody-mediated rejection after HSCT is controversial[216-218], it has recently been shown by Taylor et al, in a mice model, that preformed antibodies resulted in rejection in allo-sensitized

recipients of MHC mismatched bone marrow[219]. Allosenstization can be a major barrier to successful engraftment after HSCT due to priming of the hosts both cellular and humoral immune responses[216,218,220-224].

4.10.2 Risk factors for rejection

Sensitization to MHC antigens because of multiple blood transfusions, pregnancy or previously failed grafts increases the risk of rejection. Patients with aplastic anemia, who have been treated with multiple transfusions prior to transplant, had rejection rates in the range of 5 – 60% in earlier transplant series[206,225]. Transfusion-induced sensitization can be largely avoided in the MHC-identical setting by leuko-depletion[226] and in vitro irradiation[227,228] of transfusion products.

It is well known that a low marrow cell dose is associated with an increased risk of graft failure[229]. This has also been seen among CB transplants[46,209], this could however be avoided by giving double CB[230]. A high cell dose may also avoid antibody-mediated rejection in allo-sensitized recipients[219].
One of the major risk factors for graft rejection is HLA incompatibility. Among patients with leukemia receiving HLA-mismatched grafts the rejection rate was 5% compared to HLA-identical siblings, receiving myeloablative conditioning, where the rejection rate was only 0.1% [13].
An increased risk of graft rejection is also seen in recipients of T-cell depleted grafts[67].
In RIC transplants where lower doses of chemo-radiation therapy is used; the host immune system may persist, resulting in an increased risk of allograft rejection[231].
It has also been shown that patients with major AB0 blood group mismatches had higher incidence of graft failure[232] . Red blood cell depletion of the graft might lead to losses of both stem cells and T lymphocytes, thought to be critical for sustained engraftment.

4.10.3 Prevention of graft rejection

To prevent rejection, more intensified chemoradiotherapy may be considered. For instance, increasing the dose by fractionated TBI, or adding total body lymphoid irradiation, or increase the doses of chemotherapy may reduce the risk of rejection[233,234].

In patients with an increased risk of graft rejection, one should give G-CSF mobilized PBSC instead of bone marrow in order to increase the donor cell dose [37,108].
The use of ATG in combination with Cy during conditioning in patients with aplastic anemia has resulted in a low incidence of graft rejection and impressive survival[235,236].
DLI is used to treat relapse in patients with CML, but could also be used to overcome rejection in the case of increasing recipient T-cell chimerism[111,237].
In patients with fulminant rejection, re-transplantation is necessary, using the same or another donor. However, re-transplantation is associated with increased TRM and poor survival[238]. Since there is an increased risk of rejection and GVHD with repeated transplants, ATG or Campath may be considered during conditioning.

4.10.4 Immune modulation for prevention of rejection

The combination of immune absorption, treatment with anti-B-cell antibodies, and immunoglobulin before transplantation might be able to remove ABO antibodies[239]. This method has successfully been employed in renal transplant recipients with ABO antibodies[239].

Therapeutic apheresis (TA) involves the removal of undesired blood components, such as toxins, lipids, or antibodies. Several TA techniques are used. For example, in renal ABO incompatible transplantation methods, the techniques include plasma exchange/plasmapheresis, double filtration

plasmapheresis, protein-A immunoadsoprtion, and antigen-specific immunoadsorption. The major difference between these techniques is their level of selectivity.

Plasma exchange/plasmapheresis is an unselective form of TA and the method that is still most commonly used. In the 1950s, plasma exchange was explored in humans[240]. Fifteen years later plasma exchange was used as a treatment for diseases caused by pathogenic blood components, e.g., hyperlipidemia, hepatic failure, acquired hemophilia, SLE, Goodpasture's syndrome, and cancer[241]. Plasma exchange has a proven benefit in the treatment of various diseases, but there are limitations. Plasma proteins–including albumin, coagulation factors, and immunoglobulins–are lost together with the pathogenic blood, limiting the clinical utility of the technique[242-245]. There are more selective methods for therapeutic apheresis developed, since plasma exchange removes all antibodies, regardless of specificity and decreases levels of complement[246].

Two different methods for selective antibody removal are double filtration plasmapheresis and protein A immunoadsorption. Both these methods are selective techniques for removal of immunoglobulins[247-249]. The major benefit with selective methods for antibody removal is that no coagulation factors are removed; larger plasma volumes can be processed[250,251].

In 1979, the first report on antigen-specific immunoadsorption in a patient with SLE, removing DNA antibodies[252]. This technique was also used in patients undergoing ABO incompatible bone marrow transplantation, eliminating anti-A/B antibodies[253].

Since it now seems like B cells play a role in rejection, therapies targeting the B-cell population could prevent rejection. Until recently, B-cell depletion could only be achieved by removal of the spleen or by using chemotherapeutic drugs. Today, there are a few drugs for B-cell suppression/depletion, but only rituximab is B-cell specific.
Rituximab is a chimeric mouse/human antibody of the IgG1 subtype directed at the transmembrane protein CD20[254]. CD20 is expressed on all mature B cells but not on hematopoetic stem cells or the plasma cell[255]. Rituximab treatment leads to a long-term depletion of B cells[256]. Rituximab is approved for the treatment of B-cell lymphoma and rheumatoid arthritis, but is also used for several autoimmune diseases and various indications in transplantation such as renal allograft rejection[257-259]. Rituximab is overall well tolerated with few serious side effects. There are several ongoing randomized trails evaluating rituximab in the treatment of graft rejection as a treatment to reduce HLA antibodies in kidney transplantation.

Intravenous immunoglobulin (IVIG) is used for desensitization[260-264]. The function is not fully understood but seems to bind alloantibodies, inhibit cytokines, inhibit T-cell response and antibody production and inhibit complement[261,265-268]. IVIG has a verified effect in many autoimmune diseases[269]. However, some serious side effects are associated with using IVIG, including thrombosis, myocardial infarction and anaphylactic reactions[269].

5 AIMS OF THE PRESENT STUDY

The overall aim of this thesis was to achieve a better understanding of risk factors and the components involved in the pathophysiology of GVHD, GVL and rejection after HSCT.
The specific goals were to evaluate:

1. Risk factors for relapse and the GVL effect in patients with ALL after HSCT.

2. Wheter the cytokine gene expression before HSCT may predict the risk of moderate to severe acute GVHD.

3. If antibodies against donor CD34+/VEGFR-2+ cells may be associated with rejection after HSCT.

4. Whether immune absorption could be used as a treatment for patients with allo-antibodies before HSCT to avoid rejection of the graft.

5. If cytotoxic crossmatch before HSCT is a useful method for prediction of rejection after HSCT

6 MATERIALS AND METHODS

6.1 PATIENTS

Patients included in the studies for this thesis were transplanted at Karolinska University Hospital, Huddinge, between 1981-2007. Patient characteristics are summarized in Table 1.

Table 1

	I	II	III	IV	V
No. Of patients (no. tx)	199	196	11 (20)	3 (7)	157
Diagnosis					
AML		61	2		58
ALL	199	46			
CML		49	3	1	27
Other hematological malignancies		23	3	2	38
Nonmalignant disorders		17			12
Solid tumors			3		22
Recipient age (median, range)	15 (1-60)	33 (0-57)	39 (3-64)	1, 11, 13	39(1-67)
Recipient sex (M/F)	123/76	101/95	8/3	F, F, M	88/69
Donor age (median, range)	25 (2-62)				
Donor sex (M/F)	103/96			1/3	99/58
Cell source (BM/PBSC/CB)	176/23	120/76	13/7	3/2/2	54/103
Donor (Sib/MUD/MMUD)	115/59/25	85/111	2/18	All UD	0/130/27
Conditioning					
TBI+Cy	181	140	1		37
Bu+Cy	18	52	4	1	50
RIC			15	6	70
GVHD prophylaxis					
MTX+CsA	126	187	13	5	130
MMF+CsA	1		7		13
Other	19	9		2	14
GVHD					
0	50	34		1	
I	98	105			41
II	30	36		1	41
III-IV	15	20		1	9

6.2 CYTOKINE ASSAY

In paper II TNF-alpha and IL-10 were analysed in the sera using Quantikine enzyme-linked immunosorbent assay (ELISA) kit from R&D (Minneapolis, MN, USA).

6.3 PCR

In paper II DNA was extracted from donor and recipient pretransplantation peripheral blood samples, using standard protocols (Qiagen, Hilden, Germany). Polymeras chain reactions were performed as describe previously[199]. The methodology to detect TNFd, IL-10(-1064), TNF2 and IL-10(-1082) have been described previously[164,170].

6.4 DETECTION OF GENE POLYMORPHISM

In paper II PCR amplified products were run on 10% polyacrylamide gels and visualized with silver staining.

6.5 SERUM SAMPLES

In paper III and IV, patient serum samples were separated from blood samples by centrifugation and stored at –20°C until use. Serum samples were collected from patients before HSCT and at several time points after HSCT.

6.6 ISOLATION OF CD34+/VEGFR-2+ CELLS

In paper III and IV, donor peripheral blood mononuclear cells (PBMCs) expressing VEGFR-2 and CD34 were isolated using antibodies against the specific molecules. To obtain CD34+/VEGFR-2+ cells, a two-step positive selection using magnetic particles (Dynal, Norway) coated with anti–CD34 (10ug/ml) and anti-VEGFR-2 (20μg/mL, RELIAtech) was used. The procedure was followed as described by the manufacturer. The negative fraction CD34-/VEGFR-2- was used as control. Fluorescence was used to phenotypically characterize the populations. A panel of CD34+/VEGFR-2+ cells from five healthy individuals were isolated and used to determine if the antibodies exhibit donor-specific reactivity.

6.7 FACS FOR DETECTION OF DONOR ANTIBODIES TO CD34+/VEGFR-2+ CELLS

In paper III and IV, 5 ×105 CD34+/VEGFR-2+ enriched donor cells were incubated with 50 μl of patient serum for 30 minutes at room temperature and then washed twice with PBS. Ten microliters of 1:10 diluted fluoresceinated F(ab')2 fragments of goat anti-human IgG (Fc-specific) antibodies (Jackson Immuno Research) or IgM (Jackson Immuno Research) were added and incubated in the dark for 30 minutes. Heat-inactivated serum from a non-immunized male with blood group AB served as negative control. Cells were then analyzed on a 488nm laser flow cytometer (FACSorter, Becton Dickinson). A shift in the mean fluorescence of 20 channels in the test sample as compared to negative control was considered as positive, determined as described before270. All sera giving a positive reaction were further diluted (1:5, 1:10, 1:50) in PBS to determine the titre of the antibodies. In addition, ten sera from normal healthy non-transplanted patients were used as controls

6.8 MICROCYTOTOXICITY ASSAY

In paper III and IV, we studied the functional capacity of patients' antibodies directed against donor VEGFR2+/CD34+ cells, and we tested in vitro the ability of these antibodies to fix complement. For this purpose, we used the microcytotoxicity assay as described earlier. Briefly, cells and sera are incubated for 1 hour at room temperature in triplicates. Two microliters of rabbit complement containing the dyes acridine orange and ethidium bromide were added. After 45 minutes incubation at room temperature, the reactions were read in a fluorescence microscope. Reactions were considered positive when there was lysis of more than 10% above background as compared to the negative control. Negative controls consisted of heat-inactivated serum from normal healthy individuals.

6.9 HEMATOPOIETIC AND ENDOTHELIAL CELL COLONY FORMING UNITS

In paper III we studied whether antibodies against donor CD34+/VEGFR-2+ cells inhibit the formation of hematopoietic and endothelial cell colony forming units. After incubation with patient or control sera approximately 50x103 CD34+/VEGFR-2+ were mixed with 0.5 ml methylcellulose medium containing growth factors for human haematopoietic colony formation (Methocult GF H44334, Stemcell Technologies INC, Canada). Cells were seeded in a four well plate (Nunclone Surface, Nunc Brand, Denmark) and incubated at 370 C in 5% CO2 for 14 days. The resulting erythroid-, granulocytic-, and pluripotent colonies were examined using a phase-contrast light microscope. After 14 days, the plates were scored for colony-forming units (CFUs) according to standard criteria. Size of colonies was determined as number of cells/colony. For this purpose, colonies were picked from the methylcellulose medium and placed on glass slides with medium. Using a coverslip, the colonies were gently flattened and the cells were allowed to grow for three days, after which cell count was determined.

6.10 TUBULE FORMATION ASSAY

In paper III we studied whether antibodies against donor CD34+/VEGFR-2+ cells inhibit the formation of tubuli. The formation of tubule-like structures of untreated and purified IgG-treated CD34+/VEGFR-2+ was assessed in Matrigel-coated multi-well plates as described previously[271]. The resulting tube-like structures were examined using a phase-contrast light microscope.

6.11 ISOLATION OF SERUM IG

In paper III we isolated the immunoglobulin fraction by taking sera from patients with rejection and control patients were pooled and total IgG fractions were isolated using goat anti-human IgG (Fc-chain specific) agarose beads (Sigma Aldrich) according to standard procedure. Bound IgG was eluted by 0.1 M Glycine-HCl (pH 2.5) in fractions and neutralized with 1 M Tris-HCl (pH7.5). Protein concentration was measured spectrophotometrically at 280nm. IgG containing fractions were pooled, dialyzed against ddH_2O, and concentrated to a dry pellet by vacuum-freeze drying technique. Thereafter, the dry pellet was reconstituted in PBS. The total IgG concentration was determined by standard Mancini method on NOR-Partigen IgG Hc plates (Dade Behring AB, Skärholmen, Sweden).

6.12 DETECTION OF PANEL REACTIVE ANTIBODIES

Detection of panel reactive antibodies using flow cytometry was performed as described elsewhere272. In brief, 20 µl of fresh or frozen serum from the patient was incubated for 30 minutes at 22°C with 2,5 µl of HLA class I or II antigen coated beads (One Lambda). Following incubation, beads were washed twice according to the manufacturer's recommendation. The beads were subsequently incubated for 30 minutes at 22°C with 100 µl of FITC-conjugated goat-anti human IgG (One Lambda) as described by the manufacturer. Following incubation, the beads were washed twice as described above and resuspended using 250µL of phosphate-buffered saline added with formaldehyde (0.5%). Detection of possible bound panel-reactive antibodies was performed using the flowcytometer FACSCalibur from Becton Dickinson (BD Biosciences, Sweden), and the samples were analyzed using CELLQuest software (BD Biosciences, Sweden). Samples expressing <4.1 % reactivity for class I and < 2.9 % for class II were considered negative.

6.13 PLASMA EXCHANGE

In paper IV plasma exchange was conducted with Cobe Spectra. At each session, one plasma volume was drawn from the patient and replaced by the same amount of fresh plasma. The patient received Calcium-Sandoz 9mg/ml as continuous infusion during the whole process as prophylaxis against side effects due to citrate. Potassium was also administered if needed.

6.14 CHIMERISM ANALYSIS

For chimerism analysis, peripheral blood (PB) samples were collected from the donor and recipient before transplant and from the recipient on days +14, +21, +28, and usually every other week up to three months and monthly thereafter. DNA from donor and recipient pre-transplantation samples was extracted using standard protocols (MagNA Pure, Roche, Switzerland). To evaluate lineage specific chimerism, CD3, CD19, and CD33-positive cells were selected from PB using immunomagnetic beads (Dynal, Oslo, Norway). The methodology and sensitivity of chimerism analysis in the various cell lineages and definitions of rejection are described elsewhere [190,273].

6.15 CYTOTOXIC CROSSMATCH

T- and B-cell crossmatches were performed according to the standard complement-dependent method described earlier[271,274]. Briefly, after Ficoll density gradient centrifugation of a peripheral blood sample, donor T and B cells were isolated using antibody coated paramagnetic beads – anti-CD 8 coated beads for T cells and anti-CD 19 coated beads for B cells (Dynal, Norway). They were incubated with the recipient serum as well as rabbit complement. Presence or absence of complement fixing antidonor antibodies in the patient's serum was evaluated using a fluorescent dye (acridineorange/ethidiumbromide). A crossmatch is considered positive if more than 10% of the target cells are killed in excess of the number of dead cells incubated in a negative control serum.

6.16 STATISTICAL ANALYSIS

Statistical analysis was performed using Fisher's exact test for comparison of two proportions or the chi-square analysis with Yates' correction. A p-value <0.05 was considered to indicate a significant difference between the compared groups. In the uni- and multivariate risk factor

analysis for rejection after HSCT, the logistic regression model was used. Only factors at the 10% level from the univariate analysis were assessed in the multivariate (stepwise) analysis.

7 RESULTS AND DISCUSSION

7.1 GVL EFFECT IN ALL (PAPER I)

In paper I we aimed to study the risk factors for relapse and the GVL effect in patients with ALL. We retrospectively analyzed 199 patients with ALL, 114 (57%) were children younger than 18 years of age, and 85 were adults. Seventy-four patients were in first complete remission (CR1) and the remaining were in later stages of the disease. Conditioning consisted mainly of TBI and Cy. Most patients received CsA and MTX as GVHD prophylaxis. Acute GVHD developed in 143 patients and chronic GVHD in 67.

Leukemic relapse was defined as >30% blasts in BM or detection of extramedullary leukemic cells. The presence of 5% to 30% blasts in BM was regarded as an early relapse. Molecular relapse was considered as increasing levels of BCR/ABL transcript in peripheral blood.
Relapse was seen in 70 patients and 51 (73%) of the relapses occurred within 1 year of HSCT. The cumulative probability of relapse was 32% at 5 years in patients in CR1 and 53% in those with advanced disease ($P<0.01$).

In the Cox regression univariate analysis, 6 risk factors for relapse were significant at the 5% level. In the stepwise elimination multivariate analysis the absence of chronic GVHD, absence of HSV infection, GVHD prophylaxis with CsA and MTX and being a slow responder were independent risk factors for relapse in this study.

A herpes simplex virus (HSV) infection was defined as a positive HSV isolation or positive immunofluorescence from lesion or a positive PCR.
A total of 40 (20%) patients had a documented HSV infection after HSCT. Fifty-six patients were seronegative and 140 were seropositive for HSV prior to HSCT.
Among the seropositive patients, 53 had high titers (>10000) and 81 had low titers. The seropositive patients with high titers had significantly more HSV infections than the low-titer patients (43% verus 16%).

Since 1986 when we started to give ACV prophylaxis, the incidence of HSV infections have declined among patients with high pre-HSCT HSV titers. In this patient population, no correlation was found between HSV serostatus and relapse.
That absence of HSV infection was associated with an increased risk of relapse after HSCT in patients with ALL was a new and surprising finding. The correlation between HSV infection and a lower risk of relapse could be indirect and due to GVHD. However, in the multivariate analysis, the correlation between an HSV infection and a lower incidence of relapse was independent of GVHD. Another explanation may be that HSV infections have an antileukemia effect. Different studies have shown that[275-277] HSV-infected tumor cells could directly induce T-cell mediated immune reactions.

The association between an HSV infection and less risk of relapse in patients with ALL may indicate a role of viral antigens in the induction of an antileukemic effect. This is also in line with a study by Bostrom et al, who found a correlation between seropositivity to several herpes viruses and a reduced risk for leukemic relapse[278] In keeping with the results in this study we have decided to discontinue ACV prophylaxis early after engraftment in patients with ALL.

7.2 PREDICTION OF GVHD (PAPER II)

Certain cytokine gene polymorphisms may be associated with severe acute GVHD after HSCT. If this holds true a more individually tailored acute GVHD prophylaxis could be used.

We therefore evaluated the clinical importance of polymorphism associated with TNF-alpha and IL-10 in 196 patients and corresponding donors with regard to acute GVHD incidence after HSCT. We also performed a quantitative cytokine assay for TNF-alpha and IL-10 in order to determine cytokine production in relation to TNF-alpha and IL-10 genotypes.

In the patient group, 85 patients received stem cells from an HLA- identical sibling and 111 patients had a matched unrelated donor. The conditioning consisted mainly of TBI and Cy or BuCy. Most patients received CsA and MTX as GVHD prophylaxis.

Acute GVHD grade I was seen in 105 patients, grade II in 36 patients and grades III-IV in 20 patients.

We studied TNF -308, TNFd, IL-10(-1064) and IL-10(-1082) gene polymorphisms since these gene polymorphisms have been found to be associated with acute GVHD according to previous studies[161,162].

In our material we found a correlation between TNFd allele 4 among patients with sibling donor and acute GVHD grades II-IV. We also found an association between acute GVHD grades II-IV and patients homozygous for IL-10 (-1064) allele 13. The uncommon allele TNF2 (AA) genotype has previously been correlated with an increased in vitro TNF-alpha production[279]. This is in accordance with our findings where patients with TNF2 (AA) showed significantly higher levels of TNF-alpha during conditioning compared to patients not possessing this allele.

Studies by Middleton et al and Cavet et al have previously shown a correlation between TNFd3/d3 and IL-10(-1064) (alleles>12) and acute GVHD[161,162], this was not seen in our study. However the numbers are too small to make any definite conclusions and larger multicenter studies are needed.

7.3 ANTIBODY-MEDIATED REJECTION (PAPER III)

In paper III we hypothesized that antibodies against the donor cell population responsible for long-term engraftment, namely the hemangioblast defined as CD34+/VEGFR-2+ cells, could cause rejection in patients undergoing HSCT.

We studied twenty patients who (total number transplanted: 389) rejected their grafts between 2000 and January 2006. In 11/20 patients, recipient serum and donor cells were available for the study. Thirty patients without rejections after HSCT and 20 normal healthy individuals were included as controls.

Six patients were re-transplanted, two patients with the same donor, 3 patients with two different donors (two patients were transplanted three times) and one patient with three different donors. Among patients with rejection, thirteen transplants were from an HLA-A,-B,-C,-DR and DQ-identical donor. Four transplants had an antigen mismatch (mm) at HLA-C. Three patients had both an HLA-C antigen and an allele mm.

Among the controls, four patients had an allele mm, four patients had an antigen mm at HLA-C, two patients had both an allele and an HLA-C antigen mm. The remaining 20 controls received HLA 10/10 antigens matched grafts.

In the rejection group there were 13 transplants with a major AB0-mismatch, 2 had a minor mismatch and 5 transplants had the same blood group.

Among the controls 5 patients had a major AB0-mismatch, 8 had a minor mismatch and 17 patients had the same blood group.

Myeloablative conditioning was given before 5 transplants and 15 patients received RIC as preconditioning therapy. All patients with grafts from matched unrelated donors received ATG.
As prophylaxis against GVHD, patients received mainly CsA combined with MTX.

For chimerism analysis, peripheral blood (PB) samples were collected from the donor and recipient before transplant and from the recipient on days +14, +21, +28 and usually every other week up to 3 months and monthly thereafter.
Rejection was defined as either no detection of donor cells (< 1%) after HSCT or complete loss of donor cells after initial engraftment. In all patients with rejection, relapse of the underlying disease was excluded either by morphology examination of bone marrow aspirates or by RT-PCR of BCR-ABL or other relevant chromosomal aberrations.

We analyzed sera from patients and controls taken pre- and post-transplantation for the presence of antibodies against donor CD34+/VEGFR-2+ cells.
In this study, we showed that significantly higher numbers of patients with rejections (9/11) had antibodies against donor CD34+/VEGFR-2+ cells, but not CD34-/VEGFR-2- cells. Among controls only 1/30 had antibodies against donor CD34+/VEGFR-2+ cells. In eight transplantations, antibodies against donor CD34+/VEGFR-2+ cells were detected already prior to transplantation.
Purified IgG fractions from patients with rejections but not controls significantly decreased the ability of these cells to form hematopoietic and endothelial colonies. In multivariate analysis antibodies against CD34+/VEGFR-2+ cells proved to be the most significant risk factor for rejection. We concluded that these are alloantibodies since all patients with rejection had received blood transfusions prior to HSCT and therefore were alloimmunized. Determination whether these antibodies have various specificities need to be evaluated in future studies.

Graft rejection after HSCT is an increasing problem during the last years due to the use of RIC, T-cell depleted marrow and cord blood transplantation. Since graft rejection is correlated with high mortality new methods are needed to identify patients at risk before HSCT.
The findings in the present study may have important implications for treatment of HSCT patients. According to the results in this study, antibodies against CD34+/VEGFR-2+ cells may significantly and commonly conribute to rejection after HSCT. However, this is a retrospective study with its limitations and few patients with rejections were included. We have therefore recently started a prospective study to further evaluate whether this antibody population play an important role in graft rejection after HSCT

7.4 PREVENTION OF REJECTION (PAPER IV)

In paper III we showed that antibodies towards donor CD34+/VEGFR-2+ cells are correlated to rejection. Rejection of the graft was correlated with a high mortality rate. It is well known that anti-HLA-antibodies may cause rejection in patients receiving HLA-mismatched organ grafts.
The aim with paper IV was to remove alloantibodies in order to avoid rejection after HSCT using immune modulation. Such immune modulation has previously been successfully used in renal transplantation[258].

We included three patients with rejection and treated them all with plasma exchange, intravenous immunoglobulin (IVIG), and rituximab before re-transplantation. Two patients had antibodies against donor CD34+/VEGFR-2+ cells [280] and the third patient had anti-HLA antibodies due to

massive blood transfusions before transplantation. The first patient (1), a one-year-old girl with hemophagocytic lymphohistiocytosis (HLH), received an HLA, -A, -B, -C, -DRB1, -DQ, and -DP allele matched bone marrow from an unrelated donor with the same ABO blood group as the patient. Before the first HSCT, she was given myeloablative conditioning. The second patient (2), a 13-year old girl with Philadelphia positive CML, was given PBSC with an HLA-C antigen mismatch from an HLA, -A, -B, -DRB1, -DQ, and -DP allele matched unrelated donor with a major blood group mismatch as first transplant. Due to cardiomyopathy caused by carnitin deficiency, she received RIC pre-treatment. The third patient (3), an 11-year old boy with Fanconi anaemia, was first grafted with CB with a DRB1 antigen mismatch but had matched blood groups. The patient received RIC. All three patients rejected the grafts.

They all tolerated the immune modulatory regimen without side effects. In one patient with antibodies against CD34+/VEGFR-2 + cells, plasma exchange eliminated the antibodies according to microcytotoxicity assay and the patient had a complete donor engraftment after development of severe acute GVHD. The patient with high levels of anti-HLA antibodies received cord blood HSCT. Plasma exchange decreased the levels of anti-HLA antibodies but in spite of this the patient never engrafted.

The findings in this study and recent previous reports indicate that antibody-mediated rejection may occur after HSCT. According to experience from kidney transplantation and from this study, antibodies that may cause graft failure can be decreased using immune modulation. To avoid graft rejection one should, if possible, select a donor with a negative crossmatch in patients with alloantibodies [280]. If there is only one possible donor where alloantibodies are detected, immune modulatory treatment may be tried. Because rebound effect resulting in increasing antibody levels may occur after immune modulation, it is important to monitor antibody levels also after HSCT. Additional immune modulatory treatment may be given depending on antibody levels. Acute GVHD may be beneficial in patients with a threatening rejection in order to eliminate recipient cells that may be involved also in antibody-mediated rejection. The importance of a high celldose to avoid humoral rejection after HSCT has previously been shown [270]. To avoid rejection in CB transplantation, it may be beneficial to increase the cell dose, for example, by giving double CB. In our study, despite double CB, rejection occurred, indicating the power of anti-HLA antibodies.

To conclude, if a donor with a negative cross-match cannot be found for a patient with antibodies againstCD34+/VEGFR2+ or HLA-antigens, immune modulation including plasma exchange and rituximab may be tried to facilitate engraftment.

Graft rejection is primarily seen in patients receiving T-cell depleted grafts[67], in patients treated with RIC[208] and patients receiving CB-transplants[209]. Rejection is also more common in patients with non-malignant diseases and solid tumors, probably because these patients usually have not received any prior chemotherapy and most oftenly are given RIC as conditioning therapy. In all these riskgroups of patients mixed chimerism is much more common and higher levels of recipient cells remain after HSCT as compared to patients with leukemia and patients receiving myeloablative conditioning. In paper III and IV we demonstrate that antibodies against a subpopulation of hematopoietic stem cells – i.e., CD34+/VEGFR-2+ cells, may cause rejection. We believe that these antibodies may be involved in the rejection mechanism but in most patients these antibodies need remaining recipient cells to actually cause rejection. Rejection early after HSCT may be a combined effect of antibodies against CD34+/VEGFR-2+ cells and recipient NK-cells or

macrophages. In later rejections remaining recipient T-cells and antibodies against CD34+/VEGFR-2+ cells may be the cause of rejection. Possible mechanisms of antibody-mediated rejection after HSCT are illustrated in Figure 3.

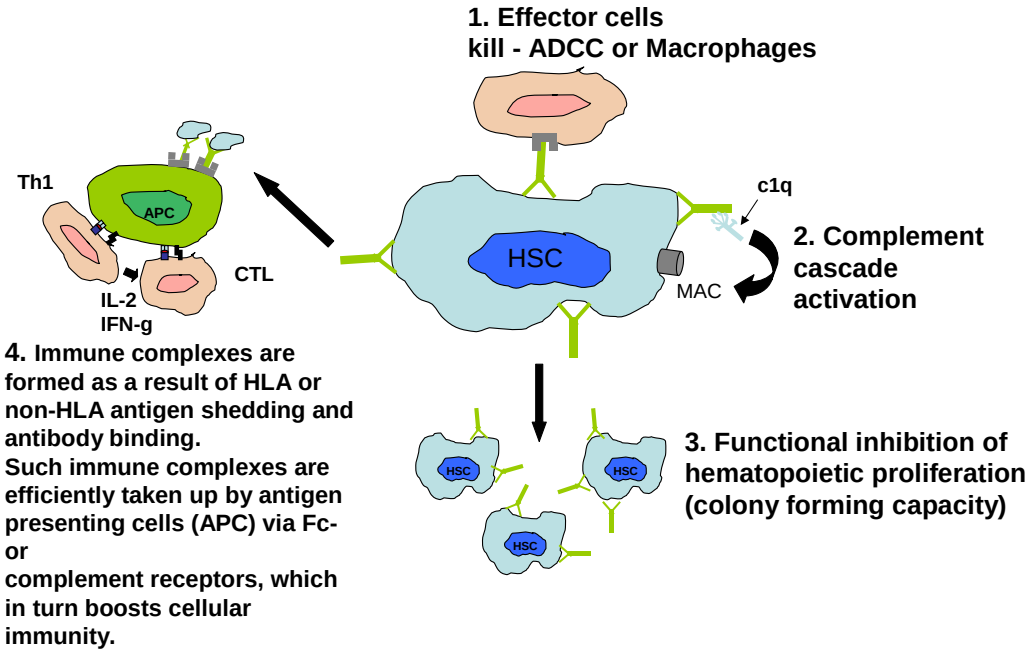

Figure 3. Possible mechanisms of antibody mediated rejection after HSCT

7.5 PREDICTION OF REJECTION (PAPER V)

Since cytotoxic crossmatch testing against T- and B-cells involves antibodies mainly directed against HLA antigens, the question arises whether this test is relevant when using an HLA matched unrelated donor.

In paper V we showed that cytotoxic crossmatch analysis before HSCT is a poor diagnostic tool for prediction of rejection. This was also seen in paper III were lymphocyte cross match was performed in 6 of 20 transplantations. One of 11 patients had a positive cross match before HSCT; the rest had a negative cross match or were not tested. Furthermore, 1/30 patients in the control group had a positive cross match before transplantation.

In paper V we retrospectively analysed the results of the cytotoxic T- and/or B-cell crossmatching performed prior to HSCT between January 2000 and June 2005. During this period we performed 230 MUD HSCT and cytotoxic crossmatch tests were performed before 157 of these transplants. Only patients receiving grafts from unrelated donors were crossmatch tested. Eighty-seven patients receivied myeloablative pre-treatment and 70 patients RIC before HSCT. All patients with HLA-A, -B and –DRB1 MUD received treatment with ATG at a total dose of 4 - 8 mg/kg . All patients and donors were typed using PCR-SSP high resolution typing for both HLA class I and II antigens . A

MUD graft with identity for HLA-A, -B and DRB1 was given to 130 patients and 27 patients received an HLA- A, -B, or DRB1 allele mismatched graft. Most patients received CsA combined with a short course of MTX as GVHD prophylaxis [58].

Of the 157 patients evaluated with cytotoxic crossmatch before HSCT, 148 and 139 patients were tested with T- and B-cell crossmatching, respectively. Of the 148 patients tested with cytotoxic T-cell crossmatch, four patients received HSCT across a positive crossmatch out of which one rejected the graft. Twenty-two of 139 (16%) had a positive B-cell crossmatch before HSCT, but only four of these patients (18%) rejected their graft. Sensitivity was 9% in T-cell crossmatches and 36% in B-cell crossmatches. Specificity was 97% and 86 % for T- and B-cell crossmatches, respectively. There was no difference in survival between patients with a negative versus patients with a positive cytotoxic crossmatch.

In multivariate analysis a positive B-cell crossmatch was significantly correlated to graft failure but only four patients of 22 with a positive crossmatch rejected the graft. Although specificity was high in crossmatches, the sensitivity was very low indicating that this technique is a poor predictor of rejection after HSCT. In multivariate analysis other immunosuppression than CsA and MTX was correlated to rejection. The reason is probably because several of these patients receiving CsA and MMF as GVHD prophylaxis, were patients with solid tumors not receiving any prior chemotherapy before HSCT. These patients have an increased risk of rejection[76]. B-cell cytotoxic crossmatch was significantly correlated to rejection according to multivariate analysis but with no impact on survival. This may be due to that some of these relatively few patients survived re-transplantation.

According to the findings in this study new methods are needed to identify patients at risk for antibody-mediated rejection after HSCT. More sensitive and specific solid phase methods to detect anti-HLA antibodies may be used for patients receiving an HLA-mismatched graft . This may be of particular use in patients receiving cord blood transplants or haploidentical HSCT . In the situations where patients receive fully or HLA-A, B or DRB1 allele matched grafts, anti-HLA antibodies may prove to be less important. In paper III we showed that recipient antibodies towards donor CD34+/VEGFR-2+ cells are correlated to rejection . According to the findings in paper III and IV, we believe that these cells may prove to be a more suitable target for crossmatches as compared to lymphocytes before HSCT.

8 CONCLUSIONS

- Chronic GVHD and HSV infection are associated with a lower relapse rate in patients with ALL
- TNFd allele 4 and allele 13 of IL-10(-1064) may be correlated to severe acute GVHD, grades III-IV
- Antibodies against donor CD34+/VEGFR-2+ cells are associated with rejection after HSCT
- Immune-modulatory treatment may decrease antibody levels and prevent rejection after HSCT
- The pre-transplant cytotoxic T-and/or B-cell crossmatch is a poor predictor of rejection after HSCT

9 SUMMARY IN SWEDISH

Benmärgstransplantation kallas idag även hematopoetisk stamcellstransplantation (HSCT). Benmärgen bildar vita blodkropppar som bygger upp vårt immunförsvar, röda blodkroppar som transporterar syre till kroppens alla celler samt blodplättar som hjälper blodet att koagulera. HSCT är idag en etablerad behandlingsmetod vid en rad sjukdomar som drabbar kroppens stamceller som exempelvis leukemier, svåra blodbristsjukdomar, immundefekter samt vissa mer ovanliga enzymbristsjukdomar. Dessa sjukdomar kan innebära att patienten måste få sin benmärg ersatt av nya, friska blodstamceller.

Allogen stamcellstransplantation innebär att patienten får stamceller från någon annan individ, antingen ett syskon eller en obesläktad, frivillig givare. Idag beräknas ca 30 % av patienterna som är i behov av HSCT ha tillgång till ett syskon vars vävnadstyp passar. De övriga 70 procenten får förlita sig på att det finns en obesläktad, frivillig givare som passar. Idag finns det mer än 11 miljoner frivilliga givare i register runt om i världen. Det är viktigt att givarens och patientens celler stämmer överens vävnadstypmässigt. På ytan av en människas celler finns vävnadsmarkörer som är specifika för varje individ. Dessa kallas för MHC-molekyler och hjälper de vita blodkropparna att känna igen vad som är ”eget” och ”icke eget”. Celler med ”icke eget” MHC uppfattas som främmande och dödas.

Före själva transplantationen behandlas alla patienter med cellgifter och/eller strålning. Syftet med förbehandlingen är att avlägsna de sjuka cellerna i kroppen och att ta bort det egna immunförsvaret så att den nya, friska märgen inte stöts bort. Donatorns friska stamceller ges därefter till patienten som en blodtransfusion. Trots att givarens och patientens celler tycks vara lika vävnadstypmässigt, d.v.s. MHC-molekylerna är matchade, finns det ändå små skillnader mellan dessa individer som cellerna kan uppfatta som främmande. Detta gör att det nya immunförsvaret (vita blodkroppar från de nya stamcellerna) uppfattar den nya kroppen som ”främmande”, vilket framkallar en immunologisk attack. Denna reaktion, som kallas transplantat-kontra-värd-reaktion (på engelska graft-versus-host disease, GVHD), drabbar först och främst kvarvarande blodceller från patientens ”gamla” märg och dödar dessa. För leukemipatienter är detta en mycket önskvärd reaktion, eftersom den hjälper till att utplåna cancerceller som överlevt förbehandlingen. Tyvärr kan transplantat-kontra-värd-reaktionen även drabba kroppens övriga celler. Om reaktionen blir alltför kraftig kan den bli livshotande för patienten. Denna reaktion är alltså på både gott och ont, eftersom en viss reaktion från givarens celler mot patientens eftersträvas, men samtidigt får reaktionen inte bli allt för kraftfull. Det optimala vore att finna ett sätt att bli av med transplantat-kontra-värd-reaktionen samtidigt som den s.k. transplantat-kontra-leukemi-effekten (på engelska graft-versus-leukemia, GVL) bibehålls.

Alla patienter har efter HSCT en ökad risk att drabbas av infektioner p.g.a. avsaknad av vita blodkroppar, innan den nya benmärgen börjar fungera. Det tar tid för det nya immunförsvaret att mogna, varför många patienter har en ökad infektionsrisk lång tid efter transplantationen.
De mest allvarliga komplikationerna efter HSCT är transplantat-kontra-värd-reaktion, infektion, avstötning av den nya märgen samt återfall i grundsjukdomen.
Den här avhandlingen handlar om att förstå samspelet mellan de nya, donerade stamcellerna och de kvarvarande patientstamcellerna.

I delarbete I har vi studerat vilka faktorer som är viktiga för att minska risken att återfalla i grundsjukdomen, akut lymfatisk leukemi. Vi fann att de faktorer som minskar risken för återfall framför allt var kronisk transplantat-kontra-värd-reaktion samt herpesvirusinfektion. Det är sedan tidigare väl känt att kronisk transplantat-kontra-värd-reaktion ökar den s.k. transplantat-kontra-leukemi-effekten, d.v.s. att donatorns nya stamceller attackerar patientens kvarvarande leukemiceller. Däremot är kopplingen mellan herpesvirusinfektion och den minskade risken för återfall ett nytt fynd. En förklaring kan vara att herpesviruset framkallar en ökad reaktivitet hos immunförsvaret, som i sin tur ökar attacken även mot leukemiceller. Alternativt att herpesvirusinfektionen i sig har en anti-leukemisk effekt.

I delarbete II studerade vi möjligheten att förutbestämma före transplantationen vilka patienter som har en ökad risk att utveckla allvarlig form av transplantat-kontra-värd-reaktion. Syftet är att på detta sätt kunna individanpassa den förebyggande transplantat-kontra-värd-behandling som ges efter transplantationen. Tyvärr visade det sig i detta arbete inte möjligt att bara genom att studera genuttrycket hos inflammationsämnen kunna förutspå vilka patienter som har en ökad risk för allvarlig transplantat-kontra-värd-reaktion.

I del arbete III har vi studerat avstötningsfrekvensen hos patienter som genomgått HSCT samt möjlig orsak till denna. Avstötning av stamceller efter HSCT har tidigare inte varit ett stort kliniskt problem, men hos patienter som fått mindre förbehandling (cellgifter och strålning) före transplantationen har risken för avstötning ökat. Detta beror till stor del på att efter denna typ av behandling finns flera av patientens egna celler kvar som kan stöta bort de nya givarcellerna. Även de patienter som får navelsträngsblodceller har en ökad risk för avstötning. Avstötning av de transplanterade stamcellerna är förknippad med hög dödlighet. Vi fann i delarbete III att antikroppar mot en del av givarens stamceller troligen orsakade avstötning. Att antikroppar skulle kunna vara riktade mot en del av stamcellerna har aldrig tidigare visats. Dessa antikroppar kan patienter ha utvecklat i samband med blodtransfusioner eller graviditet före själva transplantationen. Det är därför förmodligen av stor vikt att kontrollera om patienten har dessa antikroppar redan före transplantationen för att undvika att givarens celler stöts bort. Om så är fallet bör man antingen försöka hitta en annan givare alternativt försöka ta bort dessa antikroppar före transplantationen.

I delarbete IV studerade vi om det är möjligt att ta bort de antikroppar som orsakat avstötning hos 3 olika patienter. Vi använde samma reningsmetod som man använder på njurtransplanterade patienter som har antikroppar riktade mot givarens njure. Denna metod går ut på att man tar patientenss blod och låter det gå igenom en maskin som tar bort antikroppar samt att patienten även får ett läkemedel som tar bort de celler som producerar antikroppar. Denna behandling ledde till att 2 av 3 patienter fick behålla sina stamceller.
Bägge dessa studier (III och IV) är s.k. pilotstudier, vilket innebär att de är de första studierna av detta, varför flera och större studier behöver genomföras.

Inom njurtransplantation använder man sig numera av en ny metod för att bestämma vilka patienter som har antikroppar mot donatorns njure före transplantationen. De patienter som har antikroppar får s.k. antikroppsrening. Detta har lett till att fler patienter får behålla sina njurar utan att avstötning sker. Förhoppningen är att man inom stamcellstransplantation skall kunna utnyttja samma typ av metod. Om patienten har dessa antikroppar är det bästa att försöka hitta en annan givare som patienten inte reagerar på. Annars är antikroppsrening enligt ovan också en möjlighet.

Vår förhoppning är att denna nya kunskap ska resultera i minskad avstötning i framtiden och därmed en ökad överlevnad efter HSCT.

Man har inom stamcellstransplantation använt sig av ett s.k. korstest för att se vilka patienter som riskerar att stöta bort sina stamceller. Korstestet har använts endast på de patienter som får stamceller från en obesläktad givare, dvs. inte hos patienter som får stamceller från ett syskon. Korstestet används för att se om patienten har antikroppar mot givarens vävnadsmarkörer, de s.k. MHC-molekylerna. I arbete V ville vi utvärdera nyttan av denna typ av korstest. Vi analyserade hur det gick för alla patienter som genomgått korstest sedan år 2000 med avseende på avstötning. Vi fann då en väldigt låg känslighet med detta test. Detta kan delvis förklaras av att patienter som genomgår HSCT är väl matchade med givarena avseende vävnadstyp. Vi föreslår att man endast bör korstesta, fast med nyare och modernare metoder, de patienter som erhåller navelsträngsstamceller och de patienter som erhåller stamceller från ena föräldern (vi är ju bara lika den ena föräldern till 50 %). I de flesta fall där patient och donator är vänadsmatchade anser vi att man inte behöver genomföra korstest. Resultatet av denna studie är att vi idag har slutat med denna typ av äldre korstest. Detta spar både tid och pengar till verksamheten. Istället kommer vi att utvärdera nya metoder.

Vår förhoppning är att resultaten från dessa studier har bidragit till ökad kunskap och ny behandling rörande några av de allvarliga komplikationer som kan uppträda i samband med HSCT. Framför allt hoppas vi med denna nya kunskap kunna undvika en del fall av avstötning i framtiden, vilket i sin tur ökar chansen för patienterna att överleva denna svåra behandling.

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