1 Introduction

1.1 Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HSCT) is a well-established therapy for hematologic malignancies (e.g. leukemias, lymphomas), certain solid tumors (e.g. breast cancer) and non-malignant disorders (e.g. sickle cell disease or severe combined immunodeficiency disease) (1). HSCT relies on a conditioning regimen (chemotherapy with or without irradiation), which is able to reduce or destroy malignant or dysfunctional cells, and the subsequent transfer of a hematopoietic stem cell (HSC) graft from a healthy donor. Transfer of a HSC graft allows high intensity conditioning regimens, which would be otherwise limited by their hematologic toxicity. HSCs can be obtained from the donor by aspiration of bone marrow from the pelvis or by apheresis after their mobilization into the peripheral blood. In recent years, peripheral blood has become the major source of HSCs because of the relative ease of collection, faster engraftment and reduced costs (2). After intravenous infusion, HSCs home to the marrow of the conditioned recipient (host) and then differentiate into all hematological cell types. According to the relationship of donor and recipient, HSCT can be described as allogeneic (where the donor is genetically different), syngeneic (where the donor is an identical twin) or autologous (where donor and recipient are one and the same person).

In allogeneic HSCT, immunological differences between donor and host have three main consequences. First, immunocompetent cells in or derived from the graft can react against antigens of normal host tissues, which can result in the development of graft-versus-host-disease (GVHD) (3). Second, immunocompetent cells in the graft can also react against tissue antigens and specific tumor antigens of residual tumor cells leading to the so-called graft-versus-tumor (GVT) effect (4). Third, an immunocompetent host may mount an immunological attack against the graft, leading to graft rejection (5) (Figure 1A-C).

The major predisposing factor for donor-host incompatibility and therefore the intensity of these three events is the disparity of human leukocyte antigens (HLA) on donor and host cells. HLAs are cell surface proteins, genetically encoded by the major histocompatibility complex (MHC) region located on the short arm of chromosome 6, which are able to bind differentiation antigens derived from exogenous or endogenous proteins and are responsible for their presentation to T cells (6). If two individuals do not share the same HLA molecules (major transplantation antigen mismatch), T cells from one individual will elicit a strong immune response to cells from the other individual. Hence, matching HSCT recipients with donors sharing identical HLA antigens significantly improves engraftment kinetics and decreases GVHD severity (7). However, even if

major antigens are shared, T cells from one individual can mount responses to differentiation antigens presented by the HLA molecules (minor transplantation antigen mismatch) and can cause severe GVHD (8).

The two classes of HLA, which are critical for transplantation, are HLA class I, which is expressed on all nucleated cells (HLA-A, HLA-B, HLA-C) and HLA class II, which is expressed on B cells, dendritic cells and macrophages and can be induced on many other cell types (HLA-DP, HLA-DQ, HLA-DR). Both classes are highly polymorphic with currently 1245 alleles described for HLA I genes and 744 alleles described for HLA II genes (9). Differences in HLA class I lead to the alloresponse of $CD8^+$ T cells, and differences in HLA class II lead to the alloresponse of $CD4^+$ T cells (10). In HLA-identical allogeneic HSCTs, the alloresponse is induced by either or both subsets in response to minor histocompatibility antigens (mHAg) (8, 10).

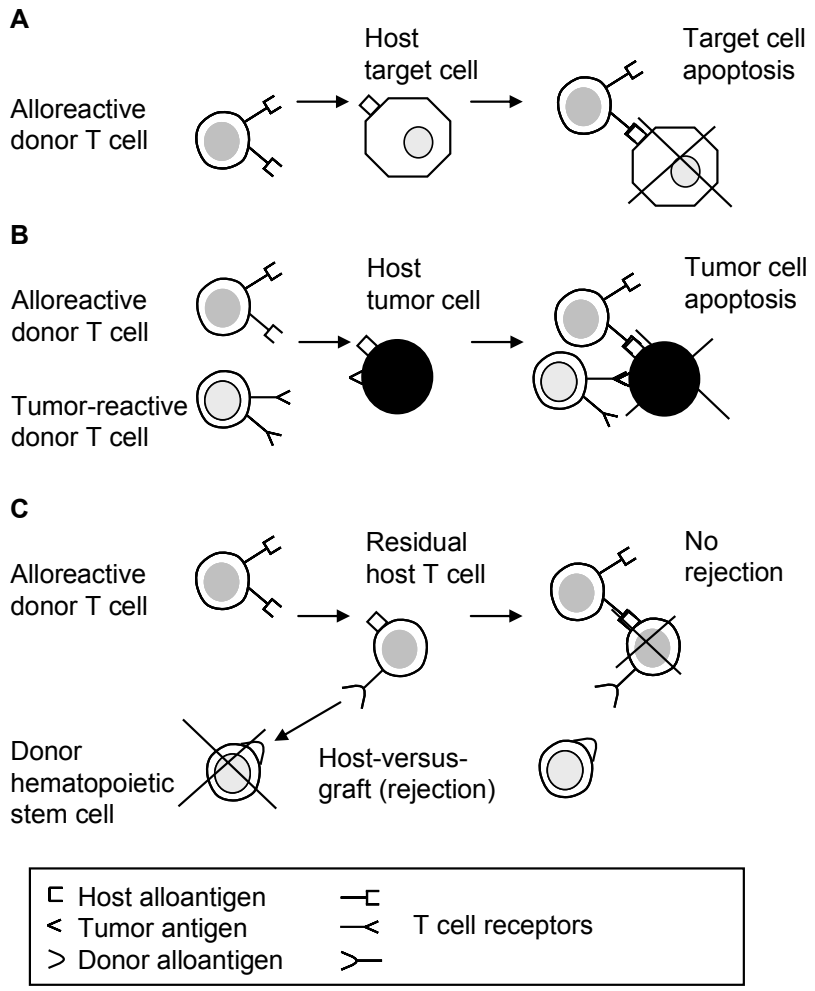

Figure 1: Immunological differences between donor and host result in GVHD, GVT and graft rejection.

A) Alloreactive donor T cells recognize host tissue antigens and induce apoptosis. B) Alloreactive and tumor-reactive donor T cells recognize tissue antigens and tumor antigens and induce apoptosis. C) Residual host T cells recognize alloantigens on donor hematopoietic stem cells and cause graft rejection. This can be prevented by the alloreaction of donor T cells against host T cells. (Adapted from (11)).

1.2 Graft-versus-host disease

1.2.1 Clinical presentation

GVHD is generally divided into two pathophysiologically and clinically distinct syndromes: acute GVHD, which mainly occurs within three months of transplantation and which comprises dermatitis, hepatitis and enteritis (3, 12), and chronic GVHD, which usually develops more than 100 days after transplantation and which resembles a collagen vascular disease with dermatitis, sicca syndrome, arthritis, cholestasis and bronchiolitis obliterans (13).

Acute GVHD can occur in 25-70% of HLA-identical HSCTs (14) and in more than 70% of HSCTs across major HLA barriers (15). Acute GVHD is, apart from infections and relapse, the most important complication of an allogeneic HSCT and is therefore the focus of this study. Clinically, acute GVHD most commonly presents with an erythematous maculopapular rash on the nape of the neck and the palms and soles which often spreads to the rest of the body. Severe cases can include the formation of bullae and desquamation. Involvement of the intestinal tract presents with symptoms such as nausea, vomiting and diarrhea, which are often accompanied by abdominal pain and ileus. Involvement of the liver presents with painless jaundice and rises in transaminases and alkaline phosphatase. Acute GVHD can be complicated by delayed immunologic recovery and profound immunodeficiency with increased susceptibility to infections. Predisposing factors for the development of acute GVHD besides HLA-disparity include older age of donor or host, sex mismatch, a high number of T cells in the graft, intensive conditioning regimens, differences in minor histocompatibility antigens and cytokine gene polymorphisms. Acute GVHD is graded by the combination of semiquantitative scores for each organ system (Table 1 and 2). While mild GVHD (grade I or II) is associated with little morbidity and almost no mortality, higher grades (III and IV) are associated with mortality rates of greater than 90% (3, 12).

Organ	Clinical manifestation	Histology	Grading
Skin	Maculopapular rash often on palms and soles, severe disease: bullae	Keratinocyte apoptosis, dyskeratosis, exocytosis of lymphocytes, dermal-epidermal separation	Rash: 1: <25%, 2: 25%-50%, 3: generalized, 4: bullae
Liver	Conjugated hyperbili-rubinemia and increased alkaline phosphatase	Lymphocytic infiltration of ducts, epithelial bile duct damage, cholestasis	Bilirubin: 1: 2-3 mg/dl, 2: 3.1-6 mg/dl, 3: 6.1-15 mg/dl, 4: >15 mg/dl

Gut	Nausea, vomiting, diarrhea, abdominal pain, ileus, gastro-intestinal bleeding	Villus blunting, lamina propria inflammation, crypt destruction, mucosal atrophy	Diarrhea: 1: >500 ml/day, 2: >1000 ml/day, 3: >1500 ml/day, 4: ileus, bleeding

Table 1: Acute GVHD. Clinical manifestation, histology and organ grading. *(Adapted from (16)).*

Overall grade	Skin	Liver	Gut
I	1–2	0	0
II	1–3	1	and/or 1
III	2–3	2–3	and/or 2–3
IV	2–4	2–4	and/or 2–4

Table 2: Acute GVHD. Overall grading. *(Adapted from (16)).*

1.2.2 Pathophysiology

In 1966, Billingham defined three criteria that have to be met for the development of GHVD. Donor and recipient must be immunologically different, immunocompetent cells must be within the graft, and the recipient must be unable to reject the graft (17). The original Billingham criteria are still valid today, but a lot more detail about GVHD pathophysiology has been revealed (3, 18). The current concept describes three sequential phases (Figure 2). Phase 1: the conditioning regimen causes tissue damage and the generation of “danger signals”. Phase 2: donor T cells migrate into the secondary lymphoid tissues where they recognize histoincompatible host antigens, become activated and start to proliferate. Phase 3: differentiated effector T cells migrate into GVHD target organs (such as skin, gut and liver) and cause further injury. Phases 1 and 2 constitute the afferent (sensitizing) phase of GVHD, phase 3 constitutes the efferent (effector) phase.

Despite the complexity of GVHD pathophysiology and the involvement of a variety of cells and cytokines, T cell depletion of the donor graft remains the most effective way to prevent the development of disease illustrating the pivotal role of alloreactive donor T cells in GVHD (19).

1.2.2.1 Phase 1: Effects of the conditioning regimen

The conditioning regimen usually consists of chemotherapy with or without total body irradiation (TBI) and is performed prior to transplantation to cytoreduce the underlying disease

and to prevent graft rejection. Together with effects mediated by the underlying disease, the conditioning regimen leads to tissue damage and induces the secretion of proinflammatory cytokines such as TNF-α and IL-1 (20). These inflammatory cytokines can induce an increased expression of costimulatory molecules, adhesion molecules and MHC antigens on host cells, which represent "danger signals" critical for the activation of host antigen presenting cells (APCs) and the initiation of the donor T cell alloreaction (18). TBI-induced epithelial damage in the intestinal tract is an important pathophysiological factor in this step, as it allows microbial products such as lipopolysaccharide (LPS) to enter the circulation, leading to the propagation of cytokine production ("cytokine storm") and further amplification of GVHD (21, 22).

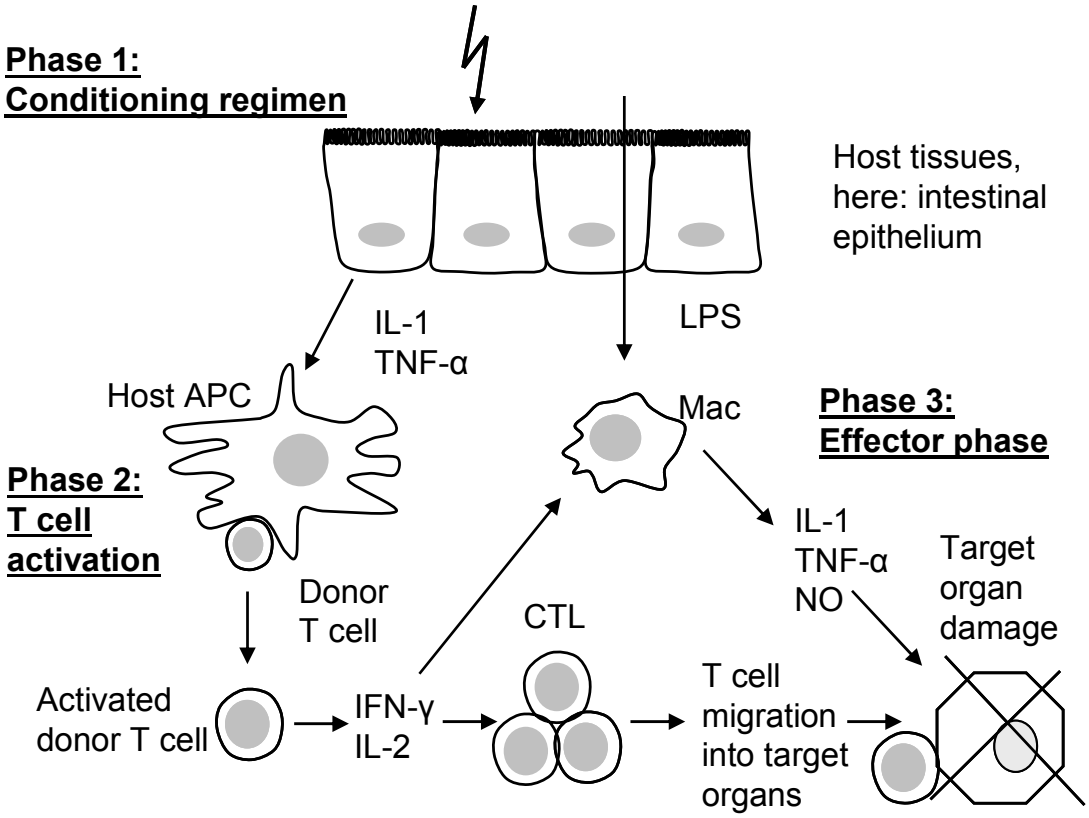

Figure 2: The pathophysiology of graft-versus-host disease.

LPS: Lipopolysaccharide, Mac: Macrophage, APC: Antigen presenting cell, CTL: Cytotoxic T lymphocyte.

1.2.2.2 Phase 2: Donor T cell activation

Naïve donor T cells contained within the HSC graft are infused into the pro-inflammatory environment, which develops in the host during phase 1, and subsequently migrate into secondary lymphoid organs where they come in direct contact with host APCs. Naïve donor T

cells then recognize allopeptide-MHC complexes on host APCs with their antigen-specific T cell receptors (TCR) (23) and become activated after they receive a second signal through costimulatory molecules such as CD40/CD40L or CD28/B27 (24). Although most interactions of donor T cells with host APCs take place in secondary lymphoid organs, some activation can also occur directly in GVHD target organs.

After activation, donor T cells start to produce inflammatory cytokines and upregulate the expression of cytokine receptors (25). Donor $CD4^+$ T cells secrete IL-2, which induces the clonal expansion of $CD4^+$ T cells in an autocrine fashion, but also of $CD8^+$ T cells, which upregulate the IL-2 receptor. IL-2 mediates the differentiation of $CD8^+$ donor T cells into cytotoxic T lymphocytes (CTL), which are the main cellular effectors mediating tissue damage during the effector phase (26). The importance of IL-2 in the development of GVHD is demonstrated by the effective GVHD prevention by administration of monoclonal IL-2-antibodies or the IL-2 production inhibitors cyclosporine and FK506 (12).

The second important cytokine during the activation phase is IFN-γ (22). IFN-γ signaling upregulates adhesion molecules and MHC antigen expression and therefore facilitates antigen presentation and induces further T cell expansion. IFN-γ also causes direct tissue damage in the gastro-intestinal tract and skin and regulates the apoptotic death of activated donor T cells.

1.2.2.3 Phase 3: Effector phase

The effector phase of GVHD involves cytotoxic T cells as main cellular effectors but also involves natural killer (NK) cells and inflammatory cytokines (18). A central event during the effector phase is the migration of CTLs into GVHD target organs, where they exert their cytolytic activity in direct cell-to-cell contact (27). It was shown that blockade of CTL migration into GVHD target organs offers a new approach for GVHD treatment, and more and more molecules are being defined as potential therapeutic targets in this context (27).

The main pathways employed by CTLs to lyse their target cells are Fas/Fas ligand (FasL) and perforin/granzymes (11). The Fas receptor is expressed in many tissues, including the classic GVHD target organs, and can be upregulated during inflammation. The Fas ligand is expressed on activated T cells, macrophages and neutrophils, and the interaction of the Fas ligand with Fas induces apoptosis through activation of a caspase cascade (28). Perforin is a pore-forming molecule, which is stored in cytotoxic granules together with granzymes and other proteins. On recognition of a target cell, perforin is released and integrates into the target cell membrane. Perforin pores can cause osmotic lysis and allow granzymes to enter the target cell. Granzymes

then induce target cell apoptosis through various downstream effector pathways (29). Recent studies have shown that the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) also contributes to CTL-mediated cytotoxicity (30).

TNF-α is an inflammatory cytokine that plays a critical role in the afferent and efferent phase of GVHD (31). TNF-α is produced by macrophages in response to LPS and it enhances alloantigen presentation on APCs, induces chemokines, which direct the migration of T cells into target organs, and causes direct tissue damage. Other important inflammatory mediators that act in concert with TNF-α and CTLs to induce the target organ destruction during GHVD are IL-1 and nitric oxide (NO) (32, 33).

1.2.3 Prophylaxis and therapy

Without prophylaxis, GVHD occurs in the majority of allogeneic HSCT recipients even if HLAs are matched. However, the increasing knowledge about GVHD pathophysiology has led to the development of post-transplant immunosuppression regimens, which can significantly reduce incidence and severity of GVHD albeit at the cost of major adverse effects (Table 3) (12). Today most patients receive a combination of cyclosporine and methotrexate (34), sometimes supplemented by low-dose steroids (35). Randomized trials demonstrated that tacrolimus (FK-506) is an effective substitute for cyclosporine and may be better tolerated (36). Other drugs that are used for GVHD prophylaxis are antithymocyte globulin (37) and mycophenolate mofetil (38). T cell depletion of the stem cell inoculum is also effective in GVHD but is associated with an increased incidence of infections, graft rejection and post-transplant relapse (19). Established GVHD can be treated with high-dose steroids, antithymocyte globulin, or monoclonal antibodies against T cells (12).

Drug	Mechanism	Adverse effects
Corticosteroids	Lymphocyte toxicity, suppression of pro-inflammatory cytokines (IL-1, IL-2, TNF-α)	Hyperglycemia, acute psychosis, severe myopathy, neuropathy, osteoporosis, cataract
Methotrexate	Antimetabolite, induction of tolerance, inhibition of lymphocyte proliferation	Significant renal, hepatic and gastrointestinal toxicities

Cyclosporine A	IL-2 suppression, blockade of calcium-dependent signal transduction distal to T cell receptor	Renal and hepatic insufficiency, hypertension, hyperglycemia, nausea, vomiting, hirsutism, gum hypertrophy
Tacrolimus	IL-2 suppression, blockade of calcium-dependent signal transduction distal to T cell receptor	Similar to cyclosporine A
Mycofenolate mofetil	Inhibition of de novo purine synthesis	Body aches, abdominal pain, nausea and vomiting, diarrhea, neutropenia
Antithymocyte globulin	Polyclonal immunoglobulin against human T cells	Anaphylaxis, serum sickness

Table 3: Common drugs for GVHD prophylaxis and treatment. *(Adapted from (12)).*

1.3 Graft-versus-tumor activity

GVT activity of allogeneic HSCT was first demonstrated by Barnes et al. in 1956, who found that irradiated mice receiving allogeneic bone marrow were able to eradicate leukemia but that mice receiving syngeneic bone marrow were not (39). Later, clinical evidence for a GVT effect came from studies by Weiden et al., who observed an inverse association between GVHD intensity and tumor relapse after allogeneic HSCT (40). Other groups reported increased relapse rates after syngeneic and autologous HSCT and after T cell depletion of the bone marrow graft (41). Yet, the most convincing evidence for a GVT effect was the induction of long-lasting remissions achieved by administration of donor leukocyte infusion (DLI) in recipients with post-transplant leukemic relapse (42).

Altogether, these findings led to the hypothesis that immunocompetent donor cells in or derived from the graft not only cause GVHD but also lead to a potent graft-versus-tumor effect, which contributes to the elimination of residual tumor cells that survived the conditioning regimen (4). Most studies indicate that the GVT effect is primarily mediated by donor T cells, which either recognize leukemia-specific antigens or alloantigens expressed on normal and malignant cells (43, 44). However, recent studies have suggested that other effector cells (such as NK cells) could also display some GVT activity (45). As the potency of the GVT effect became increasingly recognized, a new model has emerged that sees HSCT as an immunotherapeutic strategy, rather than as a treatment for the hematologic toxicity of the conditioning regimen. In this context, non-myeloablative conditioning regimens with reduced toxicity but intact GVT effect have been explored and are increasingly employed in the clinical setting (46). These

regimens allow transplantation in older patients and in patients with comorbid conditions, who would otherwise be barred from receiving an allogeneic HSCT.

It is eminent that many pathophysiological mechanisms behind the development of GVHD and GVT effect are closely intertwined and therefore strategies to separate both have been difficult to establish. Recently, interference with T cell migration has been reported by two groups as a novel possible approach towards this goal. Kim et al. found that administration of the immunosuppressive drug FTY720 can block T cell migration into GVHD target organs while still allowing their access to the lymphohematopoietic system, where most hematopoietic malignancies mainly reside. Mice receiving FTY720 suffered markedly less GVHD morbidity and mortality, while antitumor effects against EL4 T cell lymphoma were intact (47). Similarly, Petrovic et al. reported that the $\alpha_4\beta_7$ integrin is required for the migration of alloreactive donor T cells into the gut and the subsequent development of intestinal GVHD and overall GVHD mortality, while it was not required for the induction of GVT activity (48).

1.4 T cell migration

One important functional characteristic of leukocytes is their ability to migrate. Excessive migration of leukocytes to sites of inflammation is a shared feature of inflammatory diseases, including T cell-mediated diseases such as GHVD (27, 49). Migration of naïve and antigen-experienced effector T cells depends on the expression of distinctive sets of adhesion molecules (50). Rare antigen-specific naïve T cells continuously patrol through secondary lymphoid organs such as spleen, lymph nodes or Peyer's Patches to increase the likelihood of encountering their cognate antigen (51). The set of adhesion molecules that drives the entry of naïve T cells into lymphoid organs is reprogrammed after encounter of antigen-MHC complexes on APCs and subsequently allows activated effector T cells to egress into the efferent lymph and migrate towards areas from where the antigen originated.

The current understanding of T cell migration is best represented by a multistep model (Figure 3) (49). Initially, circulating T cells slow down on the endothelial wall of postcapillary venules due to transient adhesive interactions of selectins and selectin ligands (tethering and rolling phase (tethers= microvillar contacts)) (Figure 3A). Slowly rolling cells then sample the endothelium for activating factors such as chemokines, which are constitutively expressed by endothelial cells in lymph nodes and Peyer's patches and can be upregulated in areas of inflammation. Chemokines bind to their respective receptors and are able to induce clustering and conformational change of integrins (activation phase) (Figure 3B). High-avidity integrins then mediate the firm adhesion of

T cells to the endothelial wall (firm adhesion phase) (Figure 3C). Adhering cells are able to enter target tissues by transmigration through interendothelial junctions (52) (Figure 3D). Many different selectin-chemokine-integrin combinations are possible and specific combinations of these molecules can be interpreted as molecular "area codes" for organ specific T cell migration (53). For example, T cells which were primed in skin-draining lymph nodes, express CC chemokine receptor 4 (CCR4) and the selectin cutaneous lymphocyte antigen (CLA) and home to the skin, and T cells which were primed in mesenteric lymph nodes or Peyer's patches, express CC chemokine receptor 9 (CCR9) and the integrin $\alpha_4\beta_7$ and preferentially home to the intestinal mucosa (54-57).

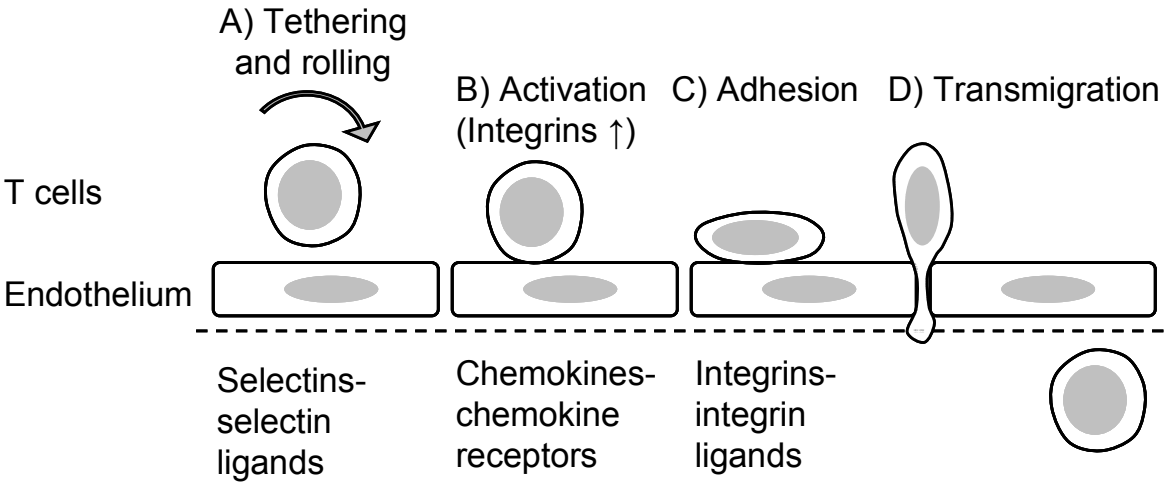

Figure 3: T cell migration is mediated by selectins, chemokines and integrins.
Selectins and selectin ligands induce a rolling and tethering phase, where T cells are enabled to sample the endothelium for activating factors such as chemokines. Chemokines bind to chemokine receptors and induce upregulation of integrin avidity, which leads to firm adhesion and subsequent transmigration into the tissues.

1.4.1 Selectins and selectin ligands

Selectins and selectin ligands initiate the homing cascade by mediating contact of freely flowing leukocytes to endothelial cells of post-capillary venules (58). The selectin-mediated bonds are too transient to induce arrest of cells, but they are permanent enough to allow leukocytes to sample the endothelial wall for chemokines and other activating factors. Selectins are a family of C-type lectins expressed by leukocytes (L-selectin), endothelial cells (P- and E-selectin) and activated platelets (P-selectin) (58). P-selectin-glycoprotein ligand-1 (PSGL-1) is the main P-selectin and E-selectin ligand and is expressed in functional form on myeloid cells and effector T

cells. The main L-selectin ligands are the peripheral node addressins (PNAds) expressed on the luminal side of high endothelial venules in lymphoid tissues. L-selectin has been suggested as a relevant molecule for donor T cell migration during GVHD (59).

1.4.2 Chemokines and chemokine receptors

1.4.2.1 Chemokines

Chemokines (short for chemoattractant cytokines) are 6 to 14 kD secreted polypeptides which signal through G-protein-coupled heptahelical surface receptors (Table 4) (60). Chemokines are the major determinants of in situ upregulation of integrin avidity on rolling leukocytes leading to firm adhesion (61). In addition, transmigration of leukocytes through the endothelial wall and movement within secondary lymphoid organs or inflamed tissues can be directed by the spatio-temporal patterns of chemokine gradients (62). To date, 50 chemokines and 19 chemokine receptors have been discovered (63). *In vitro* data showing that most chemokine receptors bind to more than one ligand and most ligands bind to more than one receptor suggest that the chemokine system contains some redundancy. However, *in vivo*, the chemokine system acts through sequential temporal and spatial distinct events where blockade of one event can lead to an efficient blockade of the whole cascade. The chemokine system is therefore an interesting target for therapeutic blockade of leukocyte migration (64) and this has been increasingly recognized in the field of GVHD research (27).

Chemokines are structurally divided into four families according to the position of two cysteine residues near the amino(N)-termini of the proteins (65). CC chemokines comprise the largest chemokine family and characteristically the N-terminal cysteines are adjacent to each other (therefore CC). In the second largest family, CXC, the two cysteines are separated by another amino acid (therefore CXC). The two other families are the CX_3C chemokines (cysteines separated by three amino acids) and the XC chemokines (only one N-terminal cysteine). According to a systematic nomenclature (63), chemokine and chemokine receptor names consist of the respective family name plus L for ligand or R for receptor and a number (e.g. CCL4 for CC chemokine ligand 4 and CCR2 for CC chemokine receptor 2).

A functional classification categorizes chemokines as homeostatic or inflammatory. Inflammatory chemokines such as CCL2 or CXCL8 are induced by inflammatory stimuli (e.g. LPS, IL-1, TNF-α) and mediate leukocytes to inflammatory sites, whereas homeostatic chemokines such as CCL21 are constitutively expressed and mediate the homeostatic circulation of leukocytes through lymphatic and peripheral tissues (65).

Cell types that are able to produce chemokines include leukocytes, endothelial cells and stromal cells. After secretion, chemokines can be immobilized on matrix or endothelial cell surface heparan sulfate proteoglycans establishing a chemokine concentration gradient between the circulation and the target tissue (66). Soluble chemokines within the systemic circulation activate integrin avidity non-topically and transiently and are therefore unlikely to contribute to leukocyte trafficking to specific sites (67). Chemokines which have leaked into the circulation can be cleared by binding to non-signaling receptors such as DARC (Duffy Antigen Receptor for Chemokines) (68).

In GVHD, it was shown that a variety of inflammatory chemokines are expressed in different organs at different time points after transplantation and that some of them are required for the control of organ specific T cell migration. With Affymetrix microarrays it was found that CCL2, CCL5, CCL7, CXCL1, CXCL9 and CXCL10 are upregulated in the liver (69) and CCL2, CCL6, CCL7, CCL8, CCL9, CCL11, CCL19, CXCL1, CXCL9 and CXCL10 are upregulated in the skin (70), and comparable distribution patterns were observed for messenger ribonucleic acid (mRNA) and protein levels (71, 72). In the lung mRNA expression of CCL2, CCL3, CCL4 and CCL5 was increased (73-75), and in the gut mRNA expression of CCL3, CCL9, CXCL9, CXCL10 and CLXL11 was elevated (72, 74). A critical, non-redundant role of a chemokine for organ specific T cell migration in GHVD was so far demonstrated for the liver and CCL3 (76) and for the lung and CCL3 (74), CCL5 (77), CXCL9 and CXCL10 (78).

1.4.2.2 Chemokine receptors

As mentioned above, chemokines induce cell migration by binding to G-protein-coupled cell surface receptors on target cells (79). So far, eleven CC chemokine receptors (CCR1 to CCR11), six CXC chemokine receptors (CXCR1 to CXCR6), one CX_3C chemokine receptor (CX_3CR1) and one XC chemokine receptor (XCR1) have been identified in humans (Table 4). The expression patterns of chemokine receptors markedly depend on the cell type and activation state (79). For example, CCR3 is expressed on eosinophils, basophils, mast cells, platelets, airway epithelial cells and Th_2 cells, whereas CCR5 is expressed on Th_1 cells, dendritic cells, monocytes and NK cells. Naïve T cells express CCR7 and CXCR4, whereas antigen-experienced T cells express CCR2, CCR3, CCR4, CCR5, CCR8, CCR9 and CXCR5 (54, 80-83). This change in receptor profile is triggered after TCR stimulation and allows recently activated cells to leave lymphoid organs and to enter areas of inflammation in the periphery (84). Receptor activation results in the activation of several downstream pathways, including activation of phospholipase

C, increase in cytosolic calcium and activation of GTPases and MAP kinases (85), leading to an increase in integrin avidity and firm adhesion on the vascular endothelium.

As chemokine and chemokine receptor expression is specific for homeostatic or inflammatory conditions as well as for different leukocyte subsets and organs (e.g. CCR9 is a gut homing receptor (56), CCR4 is highly expressed on the CLA^+ subset of skin homing memory cells (54), the CCR4 ligand CCL17 is expressed in inflamed skin but not mucosal tissues), blockade of chemokine-chemokine receptor interactions could be a very specific treatment of inflammatory disorders without deleterious side effects (86).

In GVHD it was shown that CCR1, CCR2, CCR5 and CXCR3 are upregulated in the blood (87), CCR1, CCR4, CXCR3 and CXCR6 are upregulated in the liver (76, 88), CCR1 and CCR5 are upregulated in the skin (70), and CCR2, CCR5 and CXCR3 are upregulated in the lung (75, 78). A critical role of a specific chemokine receptor for organ specific T cell migration in GVHD was so far demonstrated for the liver and CCR5 and CXCR6 (76, 88), for the gut and CCR5 and CXCR3 (89, 90), for the skin and CCR6 (72) and for the lung and CXCR3 (78).

Receptor	Ligand	Receptor expressing cell	Disease
CCR1	CCL3, CCL5, CCL7, CCL8, CCL13, CCL14, CCL15, CCL23	Monocyte, dendritic cell, T cell, neutrophil, eosinophil, mesangial cell, platelet	MS, RA, transplant, asthma, nephrits
CCR2	CCL2, CCL7, CCL8, CCL13	Monocyte, dendritic cell, T cell, basophil, NK cell, fibroblast, endothelial cell	MS, RA, transplant, asthma, atherosclerosis
CCR3	CCL5, CCL7, CCL8, CCL11, CCL13, CCL14, CCL15, CCL24, CCL26	Eosinophil, basophil, mast cell, Th_2 cell, platelets, airway epithelial cell	Asthma, atopic dermatitis
CCR4	CCL17, CCL22	Dendritic cell, basophil, Th_2 cell, platelets	Asthma, atopic dermatitis
CCR5	CCL3, CCL4, CCL5, CCL8, CCL11, CCL13	Th_1 cell, dendritic cell, monocytes, NK cell	MS, RA, transplant, nephritis, IBD, AIDS, GVHD
CCR6	CCL20	Dendritic cell, T cell, B cell	Psoriasis, GVHD
CCR7	CCL19, CCL21	Dendritic cell, T cell, B cell, NK cell	Transplant

CCR8	CCL1, CCL6	Th_2 cell, monocyte, NK cell, B cell, endothelial cell	Asthma
CCR9	CCL25	T cell	IBD
CCR10	CCL27, CCL28	T cell, melanocyte, Langerhans cell, dermal endothelium, fibroblast	Psoriasis, atopic dermatitis
CCR11	CCL19, CCL21, CCL25	Astrocyte	
CXCR1	CXCL5, CXCL6, CXCL8	Neutrophil, monocyte, endothelial cell, astrocyte	Sepsis, atherosclerosis, COPD, psoriasis
CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL7, CXCL8	Neutrophil, monocyte, eosinophil, endothelial cell	Sepsis, atherosclerosis, COPD, psoriasis
CXCR3	CXCL9, CXCL10, CXCL11	Th_1 cell, B cell, mesangial cell, smooth muscle cell	MS, RA, transplant, COPD, GVHD
CXCR4	CXCL12	T cell, dendritic cell, monocyte, B cell, neutrophil, platelet, astrocyte	AIDS, cancer
CXCR5	CXCL13	B cell, T cell, astrocyte	Cancer
CXCR6	CXCL16	Th_1 cell	RA
CX3CR1	CX3CL1	Th_1 cell, NK cell, astrocyte	RA, atherosclerosis
XCR1	XCL1, XCL2	T cell	

Table 4: Chemokines and chemokine receptors.

MS: Multiple sclerosis, RA: Rheumatoid arthritis, IBD: Inflammatory bowel disease, COPD: Chronic obstructive pulmonary disease. (Adapted from (91)).

1.4.3 Integrins and integrin ligands

The integrin family of homing receptors contains 18 different α subunits and 8 different β subunits which together form 24 different heterodimeric integrin αβ pairs (92). Relevant for the immune system are members in the β_1, β_2 and β_7 families. As for most other homing molecules, integrin expression differs in different leukocyte subsets and their state of activation. The integrin ligands are members of the immunoglobulin superfamily, which are expressed on the luminal side of endothelial cells. Integrins are able to mediate transient adhesive interactions of leukocytes with the endothelial wall without activation, but they require chemokine-triggering to

mediate firm adhesion (61). Chemokine-triggering increases the avidity of integrins within fractions of a second through induction of a conformational change and integrin clustering (61). In GVHD, the integrins $\alpha_L\beta_2$, $\alpha_4\beta_1$, $\alpha_4\beta_7$, and the integrin ligands ICAM-1 and MadCAM-1 have been suggested as important molecules for the control of donor T cell migration (48, 93-95).

1.4.4 CC chemokine ligand 2 and CC chemokine receptor 2

1.4.4.1 CC chemokine ligand 2

CCL2 (originally termed monocyte chemoattractant protein-1 (MCP-1)) belongs to the family of inflammatory CC chemokines and was one of the first chemoattractive proteins to be identified (96). Initially, CCL2 was thought to be relevant only for monocyte chemoattraction (97), but subsequently its relevance for T cell chemoattraction was demonstrated *in vitro* (98-100). CCL2 expression is induced in response to inflammatory stimuli such as IL-1 and TNF-α and cell types that produce CCL2 include macrophages, DCs, endothelial cells and fibroblasts (101). CCL2 is a critical mediator of leukocyte migration in many inflammatory disorders (97).
In GVHD, the expression of CCL2 in the liver (69), skin (70), lung (102) and gut (M.R.M. van den Brink, unpublished data) coincides with the increase in the inflammatory cytokines IL-1, TNF-α, IFN-γ early after transplant (71) and the time course of CCL2 levels correlates with the severity of the cellular infiltrates (102). CCL2 is mainly produced by host cells and induction of high levels of CCL2 depends on irradiation (103) and donor T cell infusion (102). Blockade of CCL2 with a monoclonal antibody (MAB) resulted in reduced severity of idiopathic pneumonia syndrome (IPS) after allogeneic HSCT but had no effect on mortality (73).

1.4.4.2 CC chemokine receptor 2

CCR2 belongs to the class of hepta-helical G-protein-coupled transmembrane CC chemokine receptors and is the dominant signaling receptor for CCL2 (other ligands are CCL7, CCL8, CCL12 and CCL13) (104). In humans, two isoforms of CCR2 derived from a single gene via alternative splicing have been identified (105), in mice only one CCR2 isoform exists (106, 107). CCR2 is expressed on monocytes, macrophages, effector, memory and activated T cells, NK cells and immature dendritic cells (108-112) and also on a variety of non-hematopoietic cell types such as endothelial cells, astrocytes and microglial cells (113, 114). The functional significance of CCR2 expression on non-hematopoietic cells is unknown, but CCR2 expression on hematopoietic cells is relevant for triggering the upregulation of integrin avidity. CCR2-deficient leukocytes show reduced migration towards CCL2 *in vitro* (115, 116), reduced

adhesion to the microvascular endothelium (117) and reduced extravasation into areas of inflammation (116-118). CCR2-deficient mice develop normally and are not severely immunocompromised (116-118), but they show a reduced susceptibility to a variety of inflammatory disorders (116-124) including T cell-mediated disorders such as experimental autoimmune encephalomyelitis (EAE) (125, 126) and dextran sodium sulfate-induced colitis (127). A reduction of T cell numbers within the inflammatory infiltrates of $CCR2^{-/-}$ mice has been described in these models, but an intrinsic migratory defect of $CCR2^{-/-}$ T cells without possible confounding effects of CCR2-deficiency in other cell types (such as defective migration of CCR2-deficient dendritic cells (DC) (128, 129)) has not been shown so far.

In an analysis of chemokine receptor gene expression in the peripheral blood in patients with GVHD after allogeneic HSCT it was found that CCR2 was upregulated prior to the development of clinical disease (87). Studies that analyzed the role of CCR2 in murine HSCT models concluded that CCR2 expression was relevant for the function of a $CD4^-$ donor cell population with regulatory properties (130) but irrelevant for host cells (102). In a study that analyzed the role of CCR2 expression on donor cells in murine idiopathic pneumonia syndrome and GVHD, it was shown that irradiated mice reconstituted with $CCR2^{-/-}$ BM and $CCR2^{-/-}$ T cells developed less IPS, but that hepatic and intestinal damage and overall mortality were not reduced (73). This study also described a significant reduction of bronchoalveolar lavage $CD8^+$ T cell numbers but not $CD4^+$ T cell numbers when $CCR2^{-/-}$ T cells were administered. Together with data from a recent study, which found WT and $CCR2^{-/-}$ $CD4^+$ T cells to be equally potent to induce GVHD (130), this suggests a more prominent role of CCR2 for $CD8^+$ donor T cells than for $CD4^+$ donor T cells.