5 Discussion

5.1 Overview

The importance of alloreactive donor $CD8^+$ T cells and their cytolytic activity for tissue damage during GVHD and for the beneficial GVT effect is well described (3, 4, 11). Here, it is shown in murine bone marrow transplantation models that $CD8^+$ T cells deficient in the inflammatory chemokine receptor CCR2 induce less GVHD morbidity and mortality than WT $CD8^+$ T cells, while their GVT activity is preserved. This results from an intrinsic migratory defect of $CCR2^{-/-}$ $CD8^+$ T cells to the gut and liver, which leads to a subsequent reduction in histopathological damage to these organs. The present study is the first to demonstrate a relevance of CCR2 for $CD8^+$ T cell migration in a pre-clinical disease model. It is also the first report, which shows that interference with chemokine-chemokine receptor signaling can reduce GVHD without interfering with GVT activity. This study establishes the rationale for the application of CCR2 inhibitors possibly in combination with other chemokine receptor inhibitors in a clinical setting for GVHD prophylaxis and therapy.

5.2 Survival

The central role of CCR2 for the control of monocyte migration has been documented *in vitro* (104) and in various models of monocyte-mediated inflammatory disorders such as thioglycollate-induced peritonitis (116, 117), atherosclerosis (122) or pulmonary fibrosis (123). Recently, an additional role of CCR2 for the control of T cell migration has been suggested by *in vitro* studies but convincing *in vivo* evidence for T cell-mediated disorders is missing so far. Accordingly, it was demonstrated *in vitro* that activated $CD4^+$ and $CD8^+$ T cells express CCR2 (108, 154), that CCR2 expression can be increased after exposure to IL-2 (155), that CCR2 expression levels correlate with chemotactic responsiveness (98, 155) and that CCR2-deficiency leads to a migratory defect (115). *In vivo*, it was shown that CCR2 is highly expressed on activated $CD8^+$ T cells in the spleens of mice with lymphocytic choriomeningitis virus infection (156), on $CD8^+$ T cells, which infiltrate the brains of mice with experimental cerebral malaria (157), on lung-infiltrating $CD4^+$ and $CD8^+$ T cells in mice infected with Mycobacterium tuberculosis (121) and on T cells, which infiltrate the synovium of rheumatoid arthritis patients (158). However, despite the strong correlation of CCR2 expression on T cells and inflammatory reactions, recent adoptive transfer studies with CCR2-deficient T cells found that CCR2 expression on T cells does not correlate with function and is somewhat redundant. Accordingly, Fife et al. showed in an experimental autoimmune encephalitis model that $CCR2^{-/-}$ mice develop

reduced cellular infiltrates in the spinal cord and develop less disease, but they also showed that sorted antigen-restimulated T cells taken from $CCR2^{-/-}$ mice are able to induce disease in WT mice and are able to form infiltrates (125). Similarly, Peters et al. found that $CCR2^{-/-}$ mice have an increased susceptibility to M. tuberculosis and develop less T cell infiltrates in the lung (120) but they later clarified that the observed T cell migration defect was not intrinsic but most likely secondary to CCR2-dependent macrophage and DC recruitment deficiencies (121).

In GVHD, a role of CCR2 in the control of T cell migration is suggested by the ubiquitous expression of the CCR2 ligand CCL2 in GVHD target organs early after transplantation. In mouse models, it was shown that CCL2 is highly expressed in the serum on day 0 after BMT (75), in the skin on day 7 (70), in the liver on days 7 and 35 (69), in the lung on days 3 and 7 (75) and in the gut on day 14 but not days 7 and 28 (M.R.M. van den Brink, unpublished data). CCL2 is mainly produced by host cells and high levels of CCL2 expression depend on coinfusion of allogeneic T cells with the BM inoculum (75). The time points of CCL2 upregulation in the skin, liver and gut correlate with the time points of the major donor T cell influx into these organs. Convincing evidence for a role of CCL2 in the control of T cell migration after allogeneic BMT was given by Hildebrandt et al., who found that administration of an anti-CCL2 polyclonal antibody to allogeneic HSCT recipients leads to a reduced T cell infiltration into the lung and to a subsequent reduction of IPS severity (73).

However, little is known about the role of CCR2 in T cell migration after allogeneic HSCT. Panoskaltsis-Mortari et al. found increased CCR2 mRNA in the lungs of mice with IPS, but it was not shown if this was due to infiltration of CCR2 expressing leukocytes or local upregulation (75). Hildebrandt et al. also showed an increase of pulmonary CCR2 mRNA early after allogeneic BMT and this coincided with increasing broncho-alveolar lavage fluid cellularity and induction of lung pathology (73). Irradiated mice reconstituted with $CCR2^{-/-}$ BM and $CCR2^{-/-}$ T cells developed less IPS, but hepatic and intestinal damage and overall GVHD mortality were not reduced. The authors noted that their model for IPS was CD8-dependent, but that overall GVHD mortality was CD4-dependent, which suggests that CCR2 might be required for $CD8^+$ but not $CD4^+$ T cell function. The study also described a significant reduction of bronchoalveolar lavage $CD8^+$ T cell but not $CD4^+$ T cell numbers for recipients of $CCR2^{-/-}$ T cells, which again suggests a CD8-bias for CCR2-dependency. Another recent study supports this conclusion by demonstrating that recipients of $CCR2^{-/-}$ $CD4^+$ T cells do not have a survival advantage over recipients of WT $CD4^+$ T cells (130).

A very interesting clinical study on chemokine receptor gene expression in the peripheral blood of patients after allogeneic HSCT found that CCR2 was upregulated prior to the development of

GVHD and might therefore be a valuable molecular marker to diagnose or monitor the disease (87). Other studies that analyzed the role of CCR2 in allogeneic HSCT found that CCR2 expression was relevant for the function of a donor non-T-cell population with regulatory properties (130) but not relevant for the function of host cells (102).

In the present study, the role of CCR2 signaling in $CD8^+$ T cell migration and GVHD induction after allogeneic HSCT was analyzed in well-established murine bone marrow transplantation models where lethally irradiated recipients were transplanted with TCD WT bone marrow and WT or $CCR2^{-/-}$ $CD8^+$ T cells. This experimental design allowed the selective analysis of the effects of CCR2-deficiency on $CD8^+$ T cells without any confounding effects of CCR2-deficiency on other cell types. Three independent survival analyses revealed that adoptively transferred CCR2-deficient $CD8^+$ T cells induce significantly less GVHD morbidity and mortality than WT $CD8^+$ T cells, which is the first direct evidence of a requirement of CCR2 for T cell function in a pre-clinical disease model. Additional BMT experiments with unselected splenic T cells (i.e. containing both $CD4^+$ and $CD8^+$ T cells) revealed only a less prominent, non-significant survival advantage of $CCR2^{-/-}$ T cell recipients (data not shown), suggesting that an additional role of CCR2 for $CD4^+$ T cell function is unlikely. Yet, it is also possible that CCR2 is required for the function of $CD4^+CD25^+$ regulatory T cells (as recently suggested in a murine arthritis model (159)) and this might account for the observed CD8-bias. In this context it would be interesting to compare the potency of WT and $CCR2^{-/-}$ donor $CD4^+CD25^+$ regulatory T cells in modulating the immuneresponse after allogeneic BMT.

It is not totally clear why the present allogeneic BMT study found intrinsic functional defects of $CCR2^{-/-}$ $CD8^+$ T cells, while the EAE and M. tuberculosis studies by Fife and Peters mentioned above did not. However, fundamental differences in the nature and intensity of applied inflammatory stimuli (irradiation versus peptide immunization and infection), the antigenic targets (MHC/mHAg versus single peptide and bacterial antigens), the differential involvement of T cell subtypes ($CD8^+$ T cells versus $CD4^+$/$CD8^+$ T cells) and the specific chemokine environments as wells as the timing of analysis might have been important factors influencing the results.

Future experiments in other HLA-mismatched models, in models with a single HLA I or HLA II mismatch or in models with mismatches only for minor antigens (the most common situation in human HSCT) might provide further insights into the physiology of CCR2 signaling after HSCT.

5.3 Histopathology

The finding that CCR2-deficient alloreactive donor $CD8^+$ T cells cause less GVHD morbidity and mortality than WT $CD8^+$ T cells led to a more detailed analysis of damage to individual GVHD target organs. Gut, liver and skin histopathology from BMT recipients were analyzed in a blinded fashion by experts in the field at a different institution for well-defined criteria of GVHD histopathology. Damage to the thymus was analyzed by determination of thymic cellularity, which is a well-established parameter for thymic GVHD. In these analyses, it was found that organ damage was selectively reduced for the liver, the small and the large bowel but not for the skin and the thymus, suggesting that CCR2-deficient $CD8^+$ T cells have organ dependent functional defects but most likely no reduction in overall alloreactivity.

The reduction of damage to the intestinal tract is of particular interest because the intestinal tract is not only a primary GVHD target organ, but also plays a role in the amplification of systemic GVHD through the translocation of LPS and other inflammatory stimuli and the subsequent propagation of the "cytokine storm" phase of acute GVHD (21). Strategies to selectively reduce intestinal damage have been tested and were in some cases effective in reducing overall GVHD severity (160-162). A role of CCR2 for the induction of intestinal damage had already been suggested by a previous study in a dextrane-sodium sulfate mediated colitis model (which closely resembles GVHD pathophysiology), which demonstrated that $CCR2^{-/-}$ mice are protected from mucosal ulcerations and adhesions and develop less T cell infiltrates (127).

The importance of chemokine receptor-driven $CD8^+$ T cell migration into the liver and the importance of this organ for the development of systemic GVHD have been demonstrated by previous studies closely resembling the data presented here (76, 90, 144).

The fact that recipients of $CCR2^{-/-}$ $CD8^+$ T cells did not show a significant reduction of histopathological damage to the skin despite expression of CCL2 might be due to the large number of chemokines upregulated in this organ early after transplantation (including CXCL-1, -2, -9, -10, -11; CCL-2, -3, -5, -6, -7, -8, -9, -11, -19, -20) (70, 72), which might lead to a major redundancy in chemokine-triggered T cell migration. In this context, a central role of CCL20-CCR6 signaling for T cell migration into the skin after allogeneic BMT has recently been discovered (72). Yet, the overall levels of observed skin damage were very low compared with usual levels in experiments with unselected T cells and it is therefore difficult to draw final conclusions for this organ.

It is important to note that CCR2-deficiency on donor $CD8^+$ T cells did not have any effects on thymic cellularity and peripheral white blood cell counts, suggesting that blockade of CCR2

signaling does not impair reconstitution of the immune system after allogeneic HSCT and might therefore be a safe approach in the prophylaxis and treatment of GVHD.

5.4 Migration

A competitive *in vivo* migration assay was employed to determine whether CCR2-deficient $CD8^+$ T cells have a migratory defect for specific GVHD target organs. Lethally irradiated C3FeB6F1 mice received TCD WT BM and a mix of equal numbers of WT and $CCR2^{-/-}$ $CD8^+$ T cells. The percentage of WT and $CCR2^{-/-}$ $CD8^+$ T cells which infiltrated GVHD target organs and secondary lymphoid organs was analyzed by FACS and significant deviations from the 1:1-input ratio were interpreted as migratory deficiency. A comparable assay has been used by Weninger et al. to study the migration of naïve, effector and memory $CD8^+$ T cells into secondary lymphoid organs (139). This type of migration analysis has major advantages over the standard analysis where two separate animal groups receive either WT or $CCR2^{-/-}$ $CD8^+$ T cells and where absolute donor cell counts in GVHD target organs are determined. First, assessment of absolute donor cell counts in target organs such as gut and liver can be very inaccurate due to the fact that the cell separation process consists of many incubation and wash steps and a Percoll density centrifugation step. And second, in a competitive migration assay, both donor cell types are subjected to the same inflammatory environment and to the same spectrum of chemokines. This can largely reduce inter-animal variability. However, the sensitivity of this assay demands extreme care to ensure accurate input cell counts and accuracy of FACS staining procedures.

Three independent analyses at three different time points (days 6, 14, and 28) were performed and revealed that $CCR2^{-/-}$ $CD8^+$ T cells have a migratory defect to the gut and liver, which correlates well with the reduction of histopathological damage to these organs. The fact that $CCR2^{-/-}$ $CD8^+$ T cells were found less frequently in the liver at all three time points might relate to the fact that CCL2 is highly expressed in this organ throughout the first 35 days after transplantation (69). Similarly, the fact that $CCR2^{-/-}$ $CD8^+$ T cells were found less frequently in the gut only on day 14 might relate to the fact that after transplantation CCL2 is highly expressed only at this time point (M.R.M. van den Brink, unpublished data). Migration of $CCR2^{-/-}$ $CD8^+$ T cells to the skin was not analyzed because no differences in skin histopathology could be observed for recipients of WT and $CCR2^{-/-}$ $CD8^+$ T cells. In addition, the process of T cell separation from mouse skin is very tedious and yields only very low cell numbers, which would require pooling of multiple samples.

Importantly, $CCR2^{-/-}$ $CD8^+$ T cells were able to enter the secondary lymphoid organs early after transplantation showing that the early alloresponse of $CCR2^{-/-}$ $CD8^+$ T cells is intact. Naïve T cells employ other chemokine receptors (such as CCR7) for entry into lymph nodes and CCR2 seems to become relevant only after activation and acquisition of an effector cell type (50). Interestingly, it was found that $CCR2^{-/-}$ $CD8^+$ T cells strongly accumulated in the secondary lymphoid organs on day 28. Therefore, it might also be possible that CCR2 is required for the egress from lymphoid structures into the bloodstream. However, it is more likely that the increased numbers of $CCR2^{-/-}$ $CD8^+$ T cells at this time point represent "trapping" and correlate with the reduced numbers in the peripheral GVHD target organs.

The key lymphoid compartment where donor-derived CTLs start to differentiate has not been defined yet, but some investigators believe that PP play an essential role (89). In this context it is interesting to note that $CCR2^{-/-}$ $CD8^+$ T cells were able to migrate into PP but still caused less GVHD morbidity and mortality.

In GVHD, two chemokine receptors that control $CD8^+$ T cell migration during experimental GVHD have been previously identified. A study in a parent-into-F1 model without irradiation (graft-versus-host reaction (GVHR)) demonstrated that CCR5 and its main ligand CCL3 are expressed at high levels in the liver during GVHD and that blockade of CCR5 with a monoclonal antibody leads to reduced liver infiltration with $CD8^+$ T cells (76). This study also found that liver damage was mainly mediated by $CD8^+$ T cells, which is in agreement with earlier reports showing that, despite Fas ligand upregulation on both donor $CD4^+$ and $CD8^+$ T cells, all anti-host CTL activity is accounted for by donor $CD8^+$ CTLs (163, 164). The same group showed in another study that CCR5 is critical for the migration of donor $CD8^+$ T cells into Peyer's patches and that this migration is critical for the subsequent induction of GVHD (89). Another study described CXCR3 as an important molecule for donor $CD8^+$ T cell infiltration into the small bowel and also documented a role for the development of histopathological damage in liver and gut and overall morbidity and mortality, closely resembling the data presented here (90).

However, the incomplete inhibition of GVHD by interference with CCR5 and CXCR3 and the redundancy of the chemokine system (65) suggest that $CD8^+$ T cell migration into GVHD target organs is under the influence of more than one molecule.

5.5 Functional assays

The reduced fraction of $CCR2^{-/-}$ $CD8^+$ T cells in the gut and liver could be due to a true migration defect but could also be due to decreased allo-activation, proliferation or increased

apoptosis at these sites. However, these possibilities seem unlikely because CCR2$^{-/-}$ CD8^{+} T cells showed intact accumulation in secondary lymphoid organs early after transplantation and showed intact development of an effector-memory cell type. The clonal expansion in response to alloantigen of CCR2$^{-/-}$ CD8^{+} T cells was intact as assessed *in vitro* with MLR assays and *in vivo* with CFSE assays. This agrees with previously published *in vitro* data where whole T cells or CD4^{+} T cells were analyzed (73, 102). Apoptosis was not analyzed in this study but a previous study found, at least for CCR2$^{-/-}$ CD4^{+} T cells, that Annexin V expression (which is upregulated in apoptotic cells) was not decreased after CD3/CD28 stimulation (102).

The observed differences for organ damage and GVHD severity could be also due to defective effector functions of CCR2$^{-/-}$ CD8^{+} T cells and not only to decreased effector cell numbers in GVHD target organs. Therefore, the production of the inflammatory cytokine IFN-γ and the cytolytic ability of CCR2$^{-/-}$ CD8^{+} T cells were analyzed. No defects were found in serum IFN-γ concentration, in intracellular IFN-γ concentration and in *in vitro* cytotoxicity of *in vivo* activated CD8^{+} T cells. In addition, CCR2$^{-/-}$ CD8^{+} T cells separated from GVHD target organs showed similar activation status as WT CD8^{+} T cells as assessed by CD44 and CD62L expression levels. Earlier studies had found defects in T cell differentiation and Th_1 cytokine production in CCR2$^{-/-}$ mice during some inflammatory conditions (116, 152, 165), but others support the data presented here by demonstrating that the observed differences were not intrinsic to CCR2$^{-/-}$ T cells but rather secondary to DC recruitment deficiencies (73, 128, 129).

5.6 Graft-versus-tumor activity

A major goal for GVHD prevention and therapy is the specific blockade of T cell functions that mediate GVHD while maintaining the beneficial effects of T cell-mediated GVT activity. Previous attempts to reach this goal have focused on the identification of specific GVHD or tumor antigens, donor T cell dose, selective donor T cell depletion, delayed leukocyte infusion, *in vitro* polarization of donor T cells (Th_1/Tc_1 or Th_2/Tc_2), cytokines (such as IL-2) and cytolytic pathways (11, 166-170). In the present study, it was examined in detail whether interference with CCL2-CCR2 signaling can also selectively inhibit GVHD. Survival studies with P815 mastocytoma cells as tumor inoculum and analyses which employed *in vivo* bioluminescence imaging with the newly generated luciferase-expressing P815 TGL mastocytoma and A20 TGL lymphoma cell lines were performed.

With BLI, spatio-temporal analysis of tumor burden can be carried out in individual mice without the need to sacrifice mice at specific time points. Other advantages of BLI over imaging

modalities like fluorescence imaging, PET or CT include the absence of significant background signal in normal tissues and the very high sensitivity that allows as few as 1000 tumor cells to be visualized *in vivo* (132). In addition, BLI can be repeated frequently due to the short half life of luciferin. The development of integrated imaging systems such as the Xenogen IVIS bioluminescence imaging system has facilitated *in vivo* studies with larger group sizes and has led to a variety of studies in cancer research (133). Limitations of BLI are the smaller spatial resolution compared to PET or CT and the variability of the signal intensity, which largely depends upon thickness and variable optical characteristics of tissues (including fur color). In addition, current BLI systems are only able to record two-dimensional planar images as opposed to three-dimensional images obtained by PET or CT (132).

In two independent experiments, $CCR2^{-/-}$ $CD8^{+}$ T cells exhibited a potent GVT effect against unlabeled P815 cells, while they did not induce significant mortality due to GVHD. BLI signal intensity of P815 TGL growth was similar or slightly less in $CCR2^{-/-}$ $CD8^{+}$ T cell recipients compared to WT $CD8^{+}$ T cell recipients. P815 and A20 tumors tend to form infiltrates in lymphohematopoietic organs, including liver, spleen, lymph nodes and bone marrow (161, 171, 172), and it is tempting to speculate that the intact or even slightly increased GVT activity of $CCR2^{-/-}$ $CD8^{+}$ T cells is due to the increased accumulation of $CCR2^{-/-}$ $CD8^{+}$ T cells in the lymphohematopoietic system at later time points (day 28 analysis). Most hematopoietic malignancies mainly reside within the lymphoid organs and a mere increase of T cell numbers might be able to improve GVT activity. The fact that the reduced potential of $CCR2^{-/-}$ $CD8^{+}$ T cells to infiltrate the liver did not have a major impact on overall GVT activity suggests that the remaining cells were potent enough to target liver infiltrating tumor cells. Trapping of lymphocytes in secondary lymphoid organs, reduced organ infiltration and subsequent amelioration of GVHD with intact GVT were previously shown by others in studies investigating the immune-modulatory drug FTY720 or integrin a_4b_7-negative T cells (47, 48). Whether CCL2-CCR2 signaling is required for the trafficking of leukocytes into solid tumors is not known and it would be interesting to examine the GVT effect against solid tumors (173).

The fact that the GVT effect of $CCR2^{-/-}$ $CD8^{+}$ T cells is intact was not too unexpected, as CCR2-deficient alloreactive T cells were capable of inducing cutaneous and thymic GVHD comparable to WT alloreactive T cells and showed similar expression of activation markers and inflammatory cytokines. In addition, cytotoxicity was not reduced, showing that the alloreactivity of $CCR2^{-/-}$ $CD8^{+}$ T cells is intact. However, it could have also been possible that a decrease in intestinal and hepatic GVHD is accompanied with a loss of GVT activity, because minimal residual disease after an allogeneic BMT could be localized in these organs, and

because the inflammatory stimuli that accompany intestinal and hepatic GVHD could be necessary for the stimulation of GVT activity.

5.7 CC chemokine receptor 2 as potential therapeutic target

An essential feature of the immune response to inflammatory stimuli is the correct, controlled trafficking of immune cells into target tissues, but excessive, uncontrolled recruitment of immune cells can cause deleterious inflammatory reactions. Current anti-inflammatory therapies mostly act on intracellular targets in leukocytes, meaning they act on the cells that have already emigrated into the tissues. More efficient might be the prevention of excessive recruitment of specific leukocyte subpopulations to sites of inflammation e.g. by antagonizing chemokine receptors, which act upstream of the current anti-inflammatory agents. This approach has been recognized by many investigators, and pharmaceutical companies have launched large programs to develop chemokine receptor antagonists. The discovery that chemokine receptors are required for the human immunodeficiency virus (HIV) to enter target cells (mainly CCR5 and CXCR4 (174) but also CCR2 (175)) has even boosted research on this system. Antibodies, small-molecule compounds or amino-terminus modified chemokines were all shown to be effective chemokine receptor blockers in a variety of animal models of inflammatory diseases (86).

Antagonists, which are currently undergoing clinical trials, include the CCR1-antagonist BX-471 (Berlex Biosciences) for the treatment of multiple sclerosis, psoriasis and eczema, the CCR3-antagonist CAT-213 (Cambridge AT) for the treatment of rhinitis and conjunctivitis, the CCR5-antagonist UK-427858 (Pfizer) for the treatment of HIV and most important for this study, the CCR2-antagonist MLN-1202 (Millenium) for the treatment of rheumatoid arthritis (Table 6).

An important aspect of chemokine receptor physiology that makes these proteins especially attractive therapeutic targets is their specificity. Although ligand-receptor binding contains extensive redundancy *in vitro*, different ligands act in a topographically, temporally distinct and hierarchical fashion *in vivo* so that antagonism of a given chemokine receptor can have a specific action with a limited side effect profile (86).

Target	Compound	Company	Phase	Disease
CCR1	BX-471	Berlex	I/II	MS, psoriasis, eczema
	MLN-3897	Millenium	I	RA, MS, psoriasis
	CP-481715	Pfizer	II	RA
CCR2	MLN-1202	Millenium	II	RA

CCR3	CAT-213 GW-766994 DPC-168	Cambridge AT GlaxoSmithKline Bristol Myers Squibb	II II I	Rhinitis, conjunctivitis Asthma, rhinitis Asthma
CCR5	UK-427857 ONO-4128 Sch-351125	Pfizer Ono Pharmaceuticals Schering Plough	II II I	HIV infection HIV infection HIV infection
CXCR1/2	SB-332235	GlaxoSmithKline	I	COPD, RA, psoriasis
CXCR3	T-487	Tularik	II	Psoriasis
CXCR4	AMD-3100 AMD-070	AnorMED AnorMED	II I	Stem cell transplantation

Table 6: Clinical trials for chemokine receptor antagonists.

MS: Multiple sclerosis, RA: Rheumatoid arthritis. HIV: Human immunodeficiency virus. COPD: Chronic obstructive pulmonary disease (Adapted from (176)).

In the case of CCR2, animal studies had shown that the truncated CCR2 antagonist (9-68)-CCL2 significantly reduced the inflammatory symptoms in a spontaneous model of arthritis in MRL-lpr mice (177). Another CCR2-antagonist, the monoclonal antibody MC-21, almost completely prevented the influx of leukocytes into the peritoneum in a model of thioglycollate-induced peritonitis (112) and the influx of leukocytes into the lung after LPS stimulation (178). Two other compounds which block CCR2, namely propagermanium and RS-504393, proved to be beneficial in progressive renal fibrosis via the decrease in infiltration and activation of macrophages in diseased kidneys (179).

The data presented here demonstrated in two different murine bone marrow transplantation models that lethally irradiated recipients of $CCR2^{-/-}$ $CD8^{+}$ T cells develop less GVHD than recipients of WT $CD8^{+}$ T cells, while the GVT effect and alloreactivity of $CCR2^{-/-}$ $CD8^{+}$ T cells were preserved. This provides the first proof of principle that chemokine receptor antagonism can separate GVHD from GVT and this suggests that CCR2 might be a new promising target for GVHD therapy. However, to reinforce CCR2 as a therapeutic target, the observed effects with donor T cells from knockout mice have to be validated against small molecule antagonists or blocking antibodies.

Caveats against the use of CCR2 antagonists in GVHD therapy are that the lack of CCR2 signaling also impairs the control of infectious diseases such as tuberculosis (120) and Leishmania major (129). In addition, the absence of CCR2 can worsen organ function in

noninfectious inflammatory diseases, as shown in the models of nephrotoxic serum nephritis (180), aspergillus-induced asthma (181), and acetaminophen-induced hepatitis (182). Also, CCR2 might be required for the recruitment of $CD4^+CD25^+$ regulatory T cells (159) and the maintenance of peripheral tolerance might be impaired after CCR2 blockade (183).

The development of GVHD is a complex process that is unlikely to be controllable with a single drug. Very encouraging studies of single-cytokine inhibition in mouse models have not translated well to human trials. Most studies show partial benefits for a variety of approaches, including T cell depletion, cytokine inhibition, manipulation of costimulation, and the use of antiproliferative agents, but these therapeutic interventions are rarely used synergistically. A useful strategy will attempt to control GVHD by interfering with distinct events along the pathway of GVHD pathophysiology. For instance, maintaining gut integrity, preventing cytokine upregulation, interfering with T cell activation and finally blocking T cell infiltration into GVHD target tissues. If interventions are chosen wisely, it may be possible to control the inflammatory aspect of GVHD while sparing the critically important anti-tumor effect.