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Review: chemokines in transplantation

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Abstract

The alloimmune response in solid organ transplantation is characterized by antigen presentation, activation of the recipient's immune system, and an effector response. Chemokines are chemotactic cytokines and play a role in all 3 components of the alloimmune response. Early studies showed an effectiveness of chemokine receptor blockade in experimental transplant models; chemokine receptor blockers will become more widely available because of their development for other applications. This review intends to summarize the available experimental evidence surrounding chemokines in transplantation. It will first describe the inflammatory chemokine/receptor pairs IP-10/CXCR3, Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES)/CCR5, and MCP-1/CCR2 and then cover studies regarding dendritic cell trafficking, memory cell trafficking, and regulatory cell trafficking, as well as the role of chemokines in the innate immune system. The role of S1P receptors and its antagonist FTY720 will be covered because it exemplifies the importance of trafficking for the immune response. Especially as subsets of lymphocytes and dendritic cells will be better defined as far as their regulatory and memory function is concerned, chemokine targeting strategies will be important in transplantation.

1. Introduction

Chemokines are a family of chemotactic cytokines that were first characterized by their ability to induce migration of leukocytes. Ischemia/reperfusion (I/R) injury and acute and chronic allograft rejection are characterized by leukocyte infiltrates; and therefore, chemokines were thought to play a role in allograft dysfunction. Early descriptive studies showed the presence of chemokines and their receptors in dysfunctional allografts [1]. In 1997, the chemokine field received increased attention because of the finding that both the chemokine receptor CCR5 and the chemokine receptor CXCR4 were coreceptors for the entry of human immunodeficiency virus (HIV) into leukocytes. Several small molecular antagonists against the 7-membrane spanning chemokine receptors have since been developed for wide applications in infectious diseases and rheumatology and after clinical approval will be available for application in the transplantation field [2]. Further investigations have shown that chemokines not only control leukocyte migration into areas of inflammation, but also play an important role in activation and effector functions of leukocytes, modulate hematopoiesis and angiogenesis, and play a central role in homeostatic trafficking of lymphocytes, macrophages, and dendritic cells (DCs) through tissues and lymphatic organs [3]. Chemokines and their receptors modulate the adaptive immune response by positioning cells in lymph nodes [4].

Approximately 50 chemokines have been described to date. They can be divided into 4 families based on their molecular structure and into 2 families based on their function. The classification of chemokines into 4 branches (C, CC, CXC, and CX3C) is based on the position of the first 2 cysteine residues in a 4-cysteine motif in their amino acid sequence: X stands for an intervening amino acid. The second classification is based on the observation that some chemokines are upregulated on leukocytes infiltrating areas of inflammation ("inflammatory chemokines") and others mediate normal trafficking of leukocytes through primary and secondary lymphoid organs ("homeostatic chemokines") [5]. Chemokine receptors and their relevant ligands in transplantation are summarized in Table 1.

Because of the complexity of molecular interplay of hundreds of molecules in transplant rejection, progress in this field has often been gained by empirical study of

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Table 1
Chemokine receptors and their ligands in transplantation

Receptor	Chemokine	Cell types
CXCR1	CXCL8 (IL-8), CXCL6 (GCP2)	Neutrophils, monocytes
CXCR2	CXCL8 (IL-8), CXCL1 (GROα), CXCL2 (GROβ)	Neutrophils, monocytes, endothelial cells
CXCR3	CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC)	T cells, Th1 T cells
CCR2	CCL2 (MCP-1), CLL8 (MCP-2)	T cells, memory T cells, monocytes, DCs,
CCR4	CCL17 (TARC), CCL22 (MDC)	T cells (regulatory?), DCs
CCR5	CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES)	T cells, monocytes
CCR7	CCL19 (ELC), CCL21 (SLC)	T cells, DCs
CCR8	CCL1 (I309)	T cells (regulatory?)

DCs indicates dendritic cells.

deficiency or antagonism of single candidate molecules in either animal models or human transplantation. This review intends to summarize the published data for chemokines and their receptors in solid organ transplantation.

2. Inflammatory chemokines I: IP-10/CXCR3 story

The expression of CXCL10 (IP-10) was first described in human cardiac allograft rejection [6,7]. IP-10 and MIG have been found to be elevated in the urine of rejecting kidneys[8], and diagnostic assays have been established based on these findings [9]. Mechanistic studies about the role of CXCR3 in transplantation models have been summarized in Table 2.

The first mechanistic study examined the Cxcr3 gene–deficient mouse engineered by Craig Gerard at Boston Children's Hospital: cardiac allograft survival in the vascularized heterotopic heart transplant model in the Cxcr3$^{-/-}$ mouse was found to be prolonged from 7 to 60 days, and coadminstration of cyclosporine A led to >100 days survival in the Cxcr3$^{-/-}$ mouse (Table 1). This finding was accompanied by reduced mononuclear cell infiltration and reduced cytokine/chemokine expression by reverse transcriptase polymerase chain reaction [10].

Further studies in the mouse heart model showed that donor hearts from IP-10–deficient mice resulted in a mean graft survival of 45 days compared with 7 days from wild type (WT), whereas IP-10 deficiency in the recipient had no effect [11]. Polyclonal antibodies against MIG resulted in a somewhat increased mean graft survival in the murine vascularized heart model of 19 days [12].

These findings were confirmed in experimental tracheal, lung, and small bowel transplantation in rodents [13-15]. Blocking antibody studies against Cxcr3 yielded prolongation of survival in tracheal and lung transplantation [13]. In the small bowel transplant model in the mouse, chemokine deficiency was more effective in the donor than in the recipient and lack of the receptor in the IP-10/MIG/ITAC→CXCR3 pathway was more effective in delaying rejection than blockade of the chemokine [15].

Table 2
Mechanistic studies: CXCR3 in transplantation

Species	Model	Effect	Conclusion	Reference
Mouse	Heterotopic heart tx Major mismatch Cxcr3$^{-/-}$ Anti-Cxcr3 monoclonal antibodies	Cxcr3$^{-/-}$ MGS 7-58 d Synergism with CsA	Cxcr3 plays a key role in T-cell activation, recruitment, and allograft destruction.	Hancock et al, 2000 [10]
Mouse	Heterotopic heart tx Anti–Ip-10 monoclonal antibodies Ip10$^{-/-}$ recipients Ip10$^{-/-}$ donors	MGS 7-40 d with Ip-10$^{-/-}$ donor heart	Pivotal role of donor-derived Ip-10 in initiating alloresponses.	Hancock et al, 2001 [11]
Mouse	Heterotopic heart tx Polyclonal anti-MIG antibodies	MGS 7-19 d Reduction in cell infiltrates	MIG antagonism delays acute heart allograft rejection. The alloimmune response circumvents MIG antagonism through alternative mechanisms.	Miura et al, 2001 [12]
Mouse	Heterotopic trachea tx Polyclonal anti-Cxcr3 antibodies	Reduced cell infiltrates by FACS Less morphological injury	Ligand/Cxcr3 biology plays an important role in the recruitment of mononuclear cells.	Belperio et al, 2002 [13]
Rat	Orthotopic lung tx Polyclonal anti-Cxcl9 antibodies	Reduced lung rejection score	Cxcl9/Cxcr3 has an important role in the recruitment of mononuclear cells in lung rejection.	Belperio et al, 2003 [14]
Mouse	Small bowel tx to RAG$^{-/-}$ recipients Cxcr3$^{-/-}$ T-cell and WT T-cell transfer	Graft survival delayed in Cxcr3$^{-/-}$ T-cell transfer compared with WT T-cell transfer	Ip-10 is an attractive therapeutic target for humanized monoclonal antibody strategies.	Zhang et al, 2004 [15]
Mouse	Heterotopic heart tx Small molecular antagonist against Cxcr3 and Ccr5 (TAK-779)	MGS 8-13 d Reduction in cell infiltrates by histology	Antagonism of Ccr5 and Cxcr3 has a substantial therapeutic effect on inhibiting both acute and chronic allograft rejection.	Akashi et al, 2005 [16]
Mouse	Tracheal transplantation Cxcr3$^{-/-}$ Cxcl9$^{-/-}$ Cxcl10$^{-/-}$	Cxcr3$^{-/-}$- no airway obliteration Deletion of Cxcl9 of Cxcl10 no effect	Chemokines are differentially regulated after transplantation, and deletion of either chemokine alone does not affect the development of airway obliteration.	Medoff et al, 2006 [17]

MGS indicates mean graft survival; CsA, cyclosporine A; FACS, fluorescence-activated cell sorting; WT, wild type.

Several companies developed small molecular antagonists against both murine and human CXCR3. An antagonist developed by Takeda, Japan, blocking both Ccr5 and Cxcr3 in mice leads to a mild prolongation of survival from 8 to 13 days, by no means as significant as the earlier results seen in gene-deficient mice [16]. Compounds by Merck and Novartis did not lead to significant prolongation of survival in the murine heart model [18-20]. This finding was difficult to reconcile with the previously published results in gene-deficient mice. We tested a rat monoclonal antibody against human CXCR3 in a human CXCR3 knock-in model in the mouse and found only a mildly increased survival from 8 to 17 days [20]. With the intent of moving these findings into the preclinical model, we used a monoclonal rat antihuman CXCR3 antibody as monotherapy in experimental kidney transplantation in class I and class II mismatched cynomolgus monkeys and found rejection within 10 days not different from untreated monkeys (not published). The experiments were discontinued.

We then examined 2 independently derived lines of $Cxcr3^{-/-}$ mice in the major mismatch murine vascularized heart transplant model and found no significant survival advantage and no reduction in mononuclear cell infiltration in the gene-deficient mice [21].

The anticlimax of the CXCR3 story shows that target validation in antitrafficking drugs can be an opaque process and that it cannot be presumed that the expression of a specific chemokine receptor on infiltrating cells is a necessity for chemotaxis to areas of severe inflammation. Chemotactic receptors other than CXCR3 may mediate firm adhesion and transmigration; candidates for this role are abundant such as the leukotriene receptor BLT1 and others [22]. Alternative receptors might therefore direct prominent effector cell markers such as CXCR3 and CCR5 into a bystander role. Furthermore, we have found in the minor mismatched murine vascular heart transplant model (with only 50% rejection) that only about 40% of splenic CD8 cells specific for a minor antigen (and therefore presumably destined to migrate into the graft) express the chemokine receptor Cxcr3 [23].We also found that a small percentage of alloantigen-specific cells is retained in the blood compartment in $Cxc3^{-/-}$ mice, indicating that there is a chemotactic role for Cxcr3 that does not translate into significant differences in allospecific infiltrates inside the graft [23]. The number of allospecific cell infiltrates is the net result of a steady state of immigration into and emigration out of the graft. At 100 days, we observed significantly less neointimal hyperplasia in the minor mismatched murine heart transplant model in $Cxcr3^{-/-}$ mice. We concluded that Cxcr3 deficiency has more effect in a model with overall less T cell infiltrating the organ but in which the T-cell infiltrate is allogen specific [23]. We think CXCR3 remains an interesting target for certain aspects of chronic rejection.

Recent work in the experimental autoimmune encephalitis model emphasizes the immunomodulatory effect on T cells over migratory effects in Cxcr3 deficiency: Cxcr3-deficient T cells have a decreased capacity to produce interferon (IFN) γ that might lead to downstream events affecting disease severity [24,25].

As far as drug development is concerned, several human small molecular antagonists against CXCR3 and IP-10 may receive Food and Drug Administration approval relatively soon and thus become available for applications in transplantation. AMGEN/Tularik (San Francisco, CA) developed T-0906487 that showed disappointing results in phase II psoriasis trials. Its clinical development has therefore not been continued. Medarex (Princeton, NJ) has developed a monoclonal anti–IP-10 antibody (MDX-1100) that is currently in a phase I clinical trial for ulcerative colitis.

3. Inflammatory chemokines II: the RANTES/CCR5 story in transplantation

Pattison et al were the first to describe chemokine expression in acute renal allograft rejection showing RANTES in a human biopsy specimen in 1994 [1]. Histologically, the protein and RNA expression of the chemokines Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES), MIP-1α, and MIP-1β and of their receptor CCR5 has been repeatedly described in human acute and chronic allograft rejection in multiple organs (reviewed by Segerer et al [26]). These descriptive data demonstrate that rejection as an inflammatory process includes chemokine production and that graft leukocytes in rejection express CCR5 and CXCR3 as phenotypic markers for effector cells infiltrating the allograft. Importantly, one epidemiological study has demonstrated improved transplant survival, albeit no decrease in the episodes of acute rejection in the naturally occurring human CCR5 receptor mutation delta 32. This mutation results in defective CCR5 receptor expression [27]. The mechanistic relevance of this observation however requires support with functional data, summarized in Table 3.

Groene et al showed in 1999 that Met-RANTES, a RANTES-like protein with a modified amino terminus with potent CCR5 antagonism in the nanomolar range, reduced cell infiltration and renal injury in the major mismatch rat renal transplant model by blocking firm adhesion of leukocytes on inflamed endothelium [36]. This and other studies spurred a multitude of studies evaluating graft survival and mononuclear cell infiltrates in the Ccr5 gene–deficient mouse that was developed in 2001. The first study examined the heterotopic heart transplant model in the $Ccr5^{-/-}$ line 5427 engineered by Kuziel in a B6/129 background, in both the major mismatch and MHC (major histocompatibility complex) class II mismatch combination; the study also used monoclonal antibodies against Ccr5 and tested synergisms with cyclosporine. Surprisingly, both gene deficiency and blocking antibodies delayed rejection from 7 to 23 and 18 days, respectively. In the MHC II mismatched mouse heterotopic heart transplant model, survival increased from 35 days in the WT to indefinite (>100 days) in the

Table 3
Mechanistic studies: CCR5 in transplantation

Species	Model	Effect	Conclusion	Reference
Mouse	Heterotopic heart tx Major mismatch $Ccr5^{-/-}$ Monoclonal antibody against Ccr5	Major mismatch: $Ccr5^{-/-}$ MGS 7-23 d Major mismatch: Antibody: 7-18 d Class II mismatch permanent survival Synergism with CsA	Ccr5 plays a key role in the mechanisms of host T-cell and macrophage recruitment.	Gao et al, 2001 [28]
Mouse	Islet cell transplantation Major mismatch $Ccr5^{-/-}$	MGS 10-38 d	Ccr5 plays an important role in orchestrating the Th1 immune response.	Abdi et al, 2002 [29]
Pig to mouse xeno	Islet xenograft $Ccr5^{-/-}$	No effect on leukocyte infiltration	There was little effect on leukocyte infiltration in xenografts harvested from $CCR5^{-/-}$ mice.	Solomon et al, 2003 [30]
Mouse	Heterotopic heart tx Major mismatch $CCR5^{-/-}$ Aortic transplant	MGS 7-11 d Less tissue remodeling in aortic transplant	Ccr5 seems to play an important role in transplant-associated arteriosclerosis.	Luckow et al, 2004 [31]
Mouse	Islet cell Major mismatch $CCR5^{-/-}$ + Rapamycin	MGS 11-16 d + Rapamycin, up to 38 d	Disruption of CCR5 signaling, alone or in combination, moderately prolongs islet allograft survival.	Schroeppel et al, 2004 [32]
Mouse	Heterotopic heart B6.CH-2(bm12) to C57BL/6 mice (myosin heavy chain II mismatch) Met-RANTES.	Reduced intimal proliferation Reduced cell infiltration	Ccr5 plays significant roles in the development of chronic rejection.	Yun et al, 2004 [33]
Pig to mouse xeno	Islet xenograft $Ccr5^{-/-}$ Adoptive transfer of macrophages $Ccr5^{-/-}$	$Ccr5^{-/-}$ cannot mediate rejection of islets in adoptive transfer.	Ccr5 is involved in both the activation and recruitment of macrophages to rejecting islet xenografts.	Yi et al, 2005 [34]
Mouse	Heterotopic heart tx Major mismatch $CCR5^{-/-}$	No prolongation of survival Reduced leukocyte infiltration Antibody-mediated rejection	Dysregulation of alloreactive antibody responses in the absence of recipient Ccr5.	Amano et al, 2005 [35]
Mouse	Heterotopic heart tx Major mismatch Class II mismatch SMA Ccr5 and Cxcr3 (TAK 779)	MGS 8-13 d Reduced leukocyte infiltration	Antagonism of Ccr5 and Cxcr3 inhibits both acute and chronic allograft rejection.	Akashi et al, 2005 [16]

$Ccr5^{-/-}$ mouse; similar effects could be achieved with addition of cyclosporine. Again, antagonism of the Ccr5 ligands MIP-1α or RANTES was not as effective as targeting the receptor, presumably because of the redundancy of the chemokine system.

Tripling of survival time with CCR5 deficiency could not be reproduced in subsequent studies. Luckow et al demonstrated in a $Ccr5^{-/-}$ strain, derived independently from Kuziel's, that survival was significantly but only minimally prolonged from a mean survival time (MST) of 8 to an MST of 12 days. A small degree of synergism was achieved with cyclosporine, similar to that achieved in the WT [29]. Amano et al repeated the experiment in a $Ccr5^{-/-}$ strain engineered by Kuziel (strain 5427), which had been backcrossed in a C57BL/6 background. No significant prolongation of survival was observed, but less mononuclear cell infiltration was present [35]. The lack of survival advantage in the absence of cellular infiltration was explained by a profound component of antibody-mediated rejection. Follow-up studies showed that this effect could be abrogated by CD4 T-cell depletion or CD154 blockade [37]. Bickerstaff et al also showed that antibody-mediated rejection occurs in $CCR5^{-/-}$ mice in a mouse renal transplant strain combination that usually shows spontaneous acceptance (A/J to C57Bl/6). All C57Bl/6 $Ccr5^{-/-}$ KO recipients of A/J mice generated high titers of circulating donor reactive antibodies [38]. This effect of CCR5 deficiency seems to again depend on CD4 cells.

Several companies have developed small molecular antagonists for human CCR5 because of its coreceptor status to the macrophage-tropic HIV virus [39]. Pfizer's (New York, NY) lead compound Maraviroc is in stage III clinical development as monotherapy in HIV disease and has also been shown to be effective in blocking CCR5 of macaque monkeys [40].We have used a CCR5 human small molecular antagonist in development with cross-reactivity in 2 cynomolgus monkeys as monotherapy in a renal transplant model and observed nonattenuated rejection in this outbred large animal model [41].

Table 4
Mechanistic studies: CCR2 in transplantation

Species	Model	Effect	Conclusion	Reference
Mouse	Trachea tx $CcR2^{-/-}$ Anti–MCP-1 monoclonal antibodies	$Ccr2^{-/-}$ reduced cellular infiltrates.	MCP-1/Ccr2 signaling plays an important role in recruitment of mononuclear phagocytes, a pivotal event in the pathogenesis of BOS.	Belperio et al, 2001 [50]
Mouse	Islet cell tx (subcapsular renal) Anti–MCP-1 monoclonal antibody + subtherapeutic sirolimus dose $CcR2^{-/-}$	MCP-1 antibody minimal effect $CcR2^{-/-}$ alone no effect Sirolimus + anti–MCP-1 antibodies prolonged to >120 d Sirolimus + $CCR2^{-/-}$ prolonged to 120 d	Blockade of MCP-1 binding to CcR2 in conjunction with subtherapeutic immunosuppression can have profound effects on islet allograft survival.	Lee et al, 2003 [51]
Mouse	GVHD model mouse B6-bm12 mice WT non–T cells can attenuate GVHD in a dose-dependent manner $CCR2^{-/-}$	Acute GVHD when $CCR2^{-/-}$ non–T cells were used Delayed macrophage and CD4 T-cell recruitment, enhanced eosinophil influx, and a minor delay in rejection	Ccr2 expression in the non–T-cell compartment may be an important molecular determinant of activation-induced cell death and GVHD pathogenesis.	Rao et al, 2003 [52]
Mouse	Pic islet xenograft $CCR2^{-/-}$ recipients	Minor delay in rejection Synergism with anti-IL4 and anti-IL5 monoclonal antibodies	Mcp-1 plays an important role in regulating macrophage and CD4 T-cell infiltration to xenograft sites via the CcR2 signaling pathway.	Solomon et al, 2003 [30]
Mouse	Mouse islet (Balb/C to B6) in $Ccr2^{-/-}$ Heterotopic vascularized heart in $CcR2^{-/-}$	Islet: MGS 12-24 d Mononuclear infiltrates in WT and $CcR2^{-/-}$ No prolongation for heart transplant	Important role for CcR2 in early islet allograft rejection, not in heart tx model There might be some tissue specificity (heart/islet).	Abdi et al, 2004 [53]
Mouse	Islet cell tx $Ccr2^{-/-}$ and in $Ccr5^{-/-}$ + Sirolimus	MGS $Ccr2^{-/-}$: 12-24 d $Ccr5^{-/-}$: 12-25 d No synergism between Ccr2 and Ccr5 + Sirolimus MST 11-38 d	Disruption of the Ccr2 and Ccr5 signaling, alone or in combination, moderately prolongs islet allograft survival.	Schroeppel et al, 2004 [32]
Mouse	Fetal proislet allografts mouse $Ccr2^{-/-}$ and $Ccr5^{-/-}$	No increase in survival in $Ccr2^{-/-}$ Ccr5 allograft destruction delayed	Mcp-1/Ccr2 signaling controls initial macrophage entry; and the MiP-1α, MIP-1β, and RANTES/Ccr5 pathway contributes to the rejection response.	Solomon et al, 2004 [54]
Mouse	Islet cell tx $Ccr2^{-/-}$	Level of pretransplantation Ccl2 inversely correlated with isograft function In $Ccr2^{-/-}$ recipients, this correlation was not present.	Ccl2 secreted by islets plays an important role in the immediate islet graft function.	Schroeppel et al, 2005 [55]
Mouse	GVHD	$Ccr2^{-/-}$ $CD8^+$ T cells have an intrinsic migratory defect to the gut and liver. GVT effect mediated by $Ccr2^{-/-}$ $CD8^+$ T cells was preserved.	Interference with T-cell migration by blockade of Ccr2 signaling can separate GVHD from GVT activity.	Terwey et al, 2005 [56]

GVT indicates graft vs tumor; GVHD, graft vs host disease.

Despite the initial promise in epidemiological studies, the pursuit of CCR5 as a potential target for inflammatory leukocyte trafficking has overall been disappointing: in the acute rejection model in the mouse only a moderate survival benefit could be achieved with anti-CCR5 monotherapy. There is evidence for dysregulation of T-helper responses and accumulating evidence of increased susceptibility to viral and fungal infections in the $Ccr5^{-/-}$ mice [42,43].

4. Inflammatory chemokines III: the MCP-1/CCR2 story

Besides RANTES, MCP-1 protein and RNA were found to be expressed in acute rejection in human transplant biopsies and transplant nephrectomy specimens (reviewed in [27]). Its receptor, CCR2, is expressed on monocytes and on a small percentage of T cells.

CCR2-deficient mice showed diminished monocyte recruitment and lesion formation in murine models of atherosclerosis [44], experimental autoimmune encephalitis [45], and pulmonary tuberculosis [46]. CCR2-deficient mice may also play a role in T-cell differentiation with a decreased TH1-type immune response and an increased Th2-type immune response compared with WT controls [47].

After it was demonstrated that human renal transplant recipients with a defective CCR5 receptor have better graft survival, similar studies were performed examining polymorphisms in the MCP-1/CCR2 axis. Contrary to the results with the Delta 32 mutation in CCR5, renal transplant recipients homozygous for the −2518 G mutation of the MCP-1 gene were shown to be at risk for premature kidney

graft failure [48]. Another study showed that heterozygosity of the CCR2-64I allele resulted in a 70% decreased risk of rejection [49].

Mechanistic studies (summarized in Table 4) were first performed in the tracheal transplant model in the mouse and showed a decreased mononuclear cell infiltrate and improved histology in the $Ccr2^{-/-}$ recipients and in animals treated with anti–mouse MCP-1 monoclonal antibody [48]. Most subsequent studies were performed in the islet transplant model. Lee et al demonstrated that both blocking mouse MCP-1 and the use of $Ccr2^{-/-}$ recipients of major mismatched mouse islets (BALB/C to B6) had no effect on graft survival. However, the addition of a short course of rapamycin, ineffective alone, resulted in graft survival for more than 120 days. Mononuclear cell recruitment to renal subcapsular allogeneic islets was unchanged in all of these models, calling into question a role for Ccr2 as a chemokine receptor mediating migration of inflammatory cells. The cause for the synergisms between lack of Ccr2 and rapamycin remains unclear [51]. Solomon et al confirmed that rejection was unchanged in $Ccr2^{-/-}$ mice using a xeno-islet model, although he noticed delayed infiltration of macrophages [30]. A study using $Ccr2^{-/-}$ mice in a graft vs host disease (GVHD) model suggested a role for CCR2 in regulation because lack of Ccr2 in the non–T-cell compartment of bone marrow transplants resulted in accelerated GVHD [52].

Abdi et al shed an interesting light on the MCP-1/CCR2 pathway by demonstrating that although CCR2 deficiency alone results in doubled survival time from 12 to 24 days in a mouse major mismatch renal subcapsular islet transplant model in his hands, heart transplant model survival under major mismatch conditions was not prolonged [53]. Interestingly, some of the islet recipients even showed prolonged graft survival beyond 24 days, with donor-specific peripheral tolerance. This tolerance could be broken by performing a heart transplant procedure from the same donor strain. It was concluded that chemokine pathways might be organ specific. It is possible that CCR2 is less involved in inflammatory trafficking of T cells than in the trafficking of antigen-presenting cells (APCs). The differences between antigen presentation pathways of islet cell transplantation and the heterotopic heart transplant model remain unclear, but could certainly have played a role in the differential survival times that Abdi et al have observed for the 2 models. This role of the MCP-1/CCR2 axis in the generation of alloresponses is also suggested by more recent studies that demonstrate an impairment of memory cell generation in MCP-1–deficient mice [57]. The study suggests that the MCP-1/CCR2 axis (most likely for APCs) is required to generate memory CD8 cells. The islet cell transplant model might be more dependent on this antigen-presenting pathway than the mouse heart transplant model. It has to be added though that the effect of CCR2 deficiency alone on graft survival in the islet model remains somewhat unclear after 2 more recent studies [32,54] with diverging results.

Unfortunately, the MCP-1/CCR2 axis has not been extensively studied in the heterotopic heart model in the mouse or in higher animal models. It seems that more needs to be learned about the role of the MCP-1/CCR2 axis in transplantation to better dissect its role.

5. Memory cell trafficking and chemokines

Alloreactive memory cells pose a considerable problem in organ transplantation because of their high frequency, low costimulatory requirements, low activation thresholds, and relative resistance to apoptosis. Presence of donor reactive memory T cells may represent an independent risk factor of poor posttransplant outcome [58,59]. T cells of a memory phenotype increase from 1% to 5% at birth to constitute 60% of all CD4 T cells by age 30 [60]. The origin of allospecific memory cells in transplant recipients is manifold. Either they arise from pretransplant sensitization events such as blood transfusions or they represent residual effector T cells after their majority undergoes apoptosis after the transplant. Alternatively, in a phenomenon called *heterologous immunity*, T cells that are primed through infections or environmental antigens cross-react with allogeneic MHC molecules.

There is evidence that CD4-memory cells can be divided into 2 or maybe 3 populations based on their trafficking receptors: central memory cells with a $CCR7^{hi}$ and $CD62L^{hi}$ phenotype reside in lymph nodes, where they await stimulation by professional APCs to give rise to a second wave of effector cells. Effector memory T cells with a $CCR7^{lo}$ and $CD62L^{lo}$ phenotype reside in the peripheral blood compartment to act as a first line of defense. The third population has only recently been identified as peripheral immune surveillance T cells (T_{ps}) and is characterized by preferential localization in healthy extralymphoid tissues such as normal skin and the respiratory and gastrointestinal tract [61].

The therapeutic potential of interfering with memory cell trafficking has recently become obvious in studies with the S1P receptor blocker FTY720 that prevents lymphocyte egress from lymphoid tissue and prolongs allograft survival in several animal models of solid organ transplantation. FTY720 causes a reversible sequestration of alloantigen-specific effector memory T cells in regional lymphoid tissue that is associated with a decrease in T cell infiltration within the mouse cardiac allograft [62]. It has also been suggested that FTY720 selectively affects central but not effector memory cells [63].

The issue of memory cell trafficking is particularly pressing because commonly applied and investigated approaches to tolerance induction including lymphocyte depletion, costimulatory blockade, and induction of regulatory cells have been shown to be ineffective in the face of memory cell–mediated allograft rejection. Pearl et al showed that the human T-cell repertoire after depletion with

monoclonal anti-CD52 antibody (Campath1-H) was of a single phenotype ($CD3^+CD4^+CD45RA^-CD62L^-CCR7^-$) consistent with depletion-resistant effector memory T cells that expanded in the first month and were uniquely prevalent at the time of rejection [64]. Experiments using CD40-deficient and CD80/86-deficient APCs demonstrated that memory T cells do not require signaling through these 2 main costimulatory pathways [65]. Moreover, a recent study demonstrates in a mouse model with polyclonal alloantigen-specific memory CD4 cells that memory cell–mediated rejection of skin grafts could not be delayed or prevented by regulatory cells [66].

Given the expression of CCR2, CXCR3, and CCR5 by effector memory T cells, interference with trafficking could perhaps be achieved with inflammatory chemokine receptor blockade. Future research will have to determine how central memory T-cell trafficking can be blocked. It seems that CD4 memory cells in the allografts are required to enable CD8 effector cell function in memory cell–driven models of rejection in which FTY 720 sequesters memory cells in the lymph node and prolongs graft survival [63]. There is also evidence that CD8 central memory T cells preferentially home to the bone marrow, where they await DCs for restimulation [67]. Blocking CD8 T-cell and DC trafficking into the bone marrow could prevent this reactivation of central memory CD8 cells. This is particularly interesting in view of the fact that the bone marrow is a preferred site for homeostatic proliferation of memory CD8 cells [68].

6. Regulatory cell trafficking and CCR4

Regulatory cells require specific homing signals to encounter foreign MHC antigen in transplantation to be activated in graft or secondary lymphoid organs and to finally execute their regulatory effector response. Early studies examining the chemokine receptor phenotype of regulatory cell populations have recently been reviewed [69].

Iellem et al described the expression of 2 distinct chemokine receptors, CCR8 and CCR4, on $CD4^+CD25^{+-}CD45RB^{lo}$ regulatory cells in both mice and human beings. They demonstrated that regulatory cells migrated preferentially to the corresponding chemokines CCL22 (CCR4) and CCL17 and CCL1 (CCR8) in vitro. They also demonstrated that bloodborne CD4 cells that migrate in response to CCL1 and CCL22 exhibit a reduced alloproliferative response dependent on the increased frequency of T regulatory (T_{reg}) cells in the migrated population. This was felt to be consistent with the observation that mature DCs preferentially attract T_{reg} cells among circulating T cells by virtue of their secretion of CCR4 agonistic chemokines [70]. CCR4 and CCR8 have an overlapping pattern of expression and are both expressed not only by T_{reg} cells but also by thymocytes, cutaneous memory T cells, and Th2 cells [71].

The functionality of CCR4 as a trafficking receptor for regulatory T cells was established in a study that used $Ccr4^{-/-}$ mice as recipients of mouse heterotopic vascularized heart transplants. Tolerance induction was induced using CD154 monoclonal antibody plus donor-specific transfusions (DSTs). Tolerance induction with this protocol could not be achieved in $Ccr4^{-/-}$ recipients presumably because of lack of infiltration of the allograft with $CCR4^+$ $Foxp3^+$ regulatory cells. According to this study, there is evidence that $CCR4^+$ cells leave the spleen and migrate to the graft. The graft expressed more CCL22 (CCR4 ligand) than CCL17 (CCR8 ligand) [70]. This confirmed observations of a previously published study showing that host responses to ovarian carcinoma tumor cells were impaired by local $Foxp3^+$ $CCR4^+$ T_{reg} cells, recruited as a result of tumor production of CCL22 [73].

The anatomy of regulatory cell trafficking is unclear. T cells may loose their transendothelial migration capacity in vitro and in vivo after tolerance induction to exert their regulatory function in the compartment where they were tolerized [74]. Ochando et al have demonstrated that lymph node–homing plasmocytoid DCs express CCL17 and interact with CCR4-expressing T cells. In a mouse tolerance model with DST and monoclonal antibody against CD40L, T_{reg} cells did not develop and rejection occurred [75]. It remains to be seen whether lymph node–based tolerant T_{reg} cells infiltrate into the allograft in tolerance under the influence of CCL22, as other studies suggest [72]. Whether regulatory cells persist in the allograft or in secondary lymphatic organs is unclear. Exploring a model of autoimmune diabetes, Szanya et al found that there are 2 regulatory cell populations in autoimmunity that express distinct chemokine receptors: $CD4^+CD25^+CD62L^+$ T_{reg} cells express high levels of CCR7 and home to lymph nodes, whereas $CD4^+CD25^+CD62L^-$ T_{reg} cells express inflammatory chemokine receptors CCR2, CCR4, and CXCR3 and migrate into areas of inflammation [76]. Kleinewietfeld et al introduced $CCR6^+$ T_{reg} cells as a population that seems to represent regulatory effector memory cells. It is unclear at this point whether there also exists a population of regulatory central memory T cells that follows different trafficking pathways [77]. This is interesting because it might raise new mechanistic insights to the field of therapeutic vaccination for the induction of tolerance [78,79].

Wysocki et al described the expression of CCR5 on regulatory T cells that inhibit GVHD and showed that $CCR5^{-/-}$ T_{reg} cells lacked the ability to control GVHD. Contrary to the predominating theory that CCR5 plays a major role in inflammatory leukocyte trafficking, this work established a role for Ccr5 in regulation [80]. It would be interesting to determine whether tolerance induction is impaired in $Ccr5^{-/-}$ with the commonly used protocols of costimulatory blockade and DST.

In summary, preliminary data about the mechanisms of regulatory cell trafficking suggest that deficiency of CCR4 abrogates tolerance induction by regulatory cell–dependent tolerance strategies in the mouse model. Given more recent

data, it seems that CCR5, CCR6, and CCR8 might also be involved in regulatory cell activation and/or trafficking. There are no data on the importance of these trafficking molecules on human regulatory cells at this point.

7. Dendritic cell trafficking and CCR7

The first experiments with the CCR7 gene–deficient mouse showed severely delayed kinetics regarding antibody response, lack of contact sensitivity, and lack of delayed-type hypersensitivity. CCR7 proved to be not only an essential homing receptor for naive T cells as well as mature DCs through high endothelial venules into the T-cell zone of lymph nodes but also a guide for them in case they arrive in the lymph nodes through the afferent lymphatics [81]. The CCR7 ligand CCL21 (SLC) is expressed on high endothelial venules in Peyer patches and in lymph nodes and on stroma cells within the T-cell zone of the spleen. CCL19 (ERC), the second CCR7 ligand, was found to be expressed on interdigitating DCs in the T-cell zone of the spleen [81]. Because of the importance of allogen presentation in transplantation, several groups set out to answer the question of how deficiency of the CCL21/CCL19/CCR7 axis influences transplant survival in the mouse model (summarized in Table 5).

The question was first approached in the plt/plt mouse. 'Paucity of lymph node T-cell mice' (plt/plt mice) lack CCL21 and CCL19 expression. Donor DC trafficking to secondary lymphoid tissue was markedly reduced in the plt/plt heart but not skin allograft recipients; heart, but not skin, allografts survived significantly longer [82]. Another study demonstrated permanent engraftment of islets engrafted under the kidney capsule but no prolongation of survival of intrahepatically injected islets or primarily revascularized cardiac allografts in plt/plt mice [83]. When the role of CCR7 was examined, only a modest prolongation of survival from 7 to 11 days (and in another study, 13 days [85]) was noticed in $Ccr7^{-/-}$ recipient mice. T-cell accumulation and expansion in the draining lymph nodes were severely impaired [84]. This suggested that homing of T lymphocytes and APCs to lymph nodes by means of CCR7 is not an absolute requirement for allograft rejection. Whether this should be interpreted in the sense that homing to secondary lymphoid organs is not required for rejection or that additional chemotactic molecules compensate for the deficiency of CCR7 is unclear. When $CCR7^{-/-}$ donor organs were used in $CCR7^{-/-}$ recipients in a multiple minor mismatch model, no significant difference of survival was observed, suggesting that both the direct and indirect pathways do not depend on CCR7-dependent trafficking. Interestingly, cyclosporine alone led to a significant prolongation of survival of the cardiac allograft in WT mice but had no effect in $CCR7^{-/-}$ mice, suggesting that activation of regulatory T cells might depend on CCR7-mediated APCs or lymphocyte trafficking and might be inhibited by the presence of cyclosporine [85].

Recently, the importance of CCR7-mediated homing for the induction of tolerance was demonstrated when it was shown that the engineered expression of CCR7 on immature DCs markedly increased DC homing to lymphoid organs and led to a graft survival >100 days when combined with the expression of the immunomodulatory molecule viral interleukin 10 (IL-10) [86]. By using a monoclonal antibody against a specific donor-derived peptide in the context of MHC class II, it was found in the vascularized heterotopic heart transplant model in the mouse that recipient plasmocytoid DCs are the DC type that induces tolerance through activation of regulatory cells and that this activation occurs in secondary lymphoid tissue [75]. This study found that tolerogenic plasmocytoid DCs expressed the chemokine receptors Ccr7 and Ccr2. The study showed that their ligands

Table 5
Mechanistic studies: CCR7 in transplantation

Species	Model	Main effect	Main conclusion	Reference
Mouse	Het. heart tx Skin tx Major mismatch plt/plt mouse	Heart: MGS 9-14 d Skin: MGS 12-13 d	Heart, but not skin, grafts survived significantly longer in plt/plt recipients.	Colvin et al, 2005 [82]
Mouse	Islet cell tx Major mismatch	MGS 12-120 d	Chemokine-directed homing of donor DC to secondary lymphoid tissues is essential for host sensitization and allograft rejection.	Wang et al, 2005 [83]
Mouse	Het. heart tx Skin tx Major mismatch $Ccr7^{-/-}$ mouse	Heart: MGS 7-10 d Skin: MGS 11-17 d	CCR7-dependent processes support allograft rejection yet are dispensable for the rejection response.	Beckmann et al, 2004 [84]
Mouse	Het. heart tx Major mismatch $Ccr7^{-/-}$	Heart: MGS 7-13 d	CCR7 deficiency resulted in significantly prolonged but not indefinite allograft survival.	Hopken et al, 2004 [85]
Mouse	Het. heart tx Skin tx Major mismatch Transfer of immature DC transduced with Ccr7 and viral IL-10	Heart: MGS 10-100 d	Showed that a single infusion of DC coexpressing CCR7 and the immunomodulatory molecule viral IL-10 markedly prolongs cardiac allograft survival	Garrod et al, 2006 [86]

Ccl2 (JE/MCP-1), Ccl7 (MCP-3), and Ccl 21 (SLC) were upregulated in high endothelial venules during tolerization, but provided no functional evidence that chemokine-mediated homing is necessary during the tolerization process. This seems to be an important question because of the observation that 95% of the allo-APCs in the allograft were plasmocytoid DCs and because it is currently unclear whether myeloid DCs are absent from tolerized grafts because of different homing receptors compared with plasmocytoid DCs.

In summary, CCR7 is a lymph node homing receptor for both APCs and T cells. Initially, it was felt that blockade of this pathway would interfere with sensitization of the recipients of solid organ transplants. Experimental support in the mouse model is not convincing. This could be explained by the fact that antigen presentation does not depend on the lymph nodes. However, it seems as though CCR7-mediated homing to lymph nodes might play a role in tolerogenic DC homing.

8. Leukocyte retention and S1P agonists

FTY 720 is a derivative of the sphingolipid myriocin, which is a metabolite produced by the fungus *Isaria sinclairii*. It was found to induce profound leukopenia in animals and prolonged graft survival in animal models [87-89]. Initially, it was suspected that the FTY720-induced leukopenia was caused by apoptosis until Chiba et al observed in a skin allograft model that peripheral lymphopenia was associated with an increased number of lymphocytes in secondary lymphoid tissue [90].

The importance of S1P receptors in FTY720-induced leukopenia was first discovered when it was found that FTY720, a sphingosine analogue with immunosuppressive properties, binds and activates 4 of the 5 known S1P receptors [91]. Matloubian et al showed that FTY720's blockade of the egress of T cells from thymus and B cells from secondary lymphoid organs and thymus was dependent on the activation of those S1P receptors [92]. It was shown that FTY720 stimulates S1PR1 to drive T cells into the lymph node in a CCR7-dependent fashion and then causes downregulation of S1PR1 leading to retention of T cells in the lymph nodes [93]. Agonism of S1PR1 was found to have an additional effect by inhibiting vascular endothelial growth factor–induced vascular permeability [94].

Human trials with FTY 720 were initially very promising. Single-dose studies established the safety and tolerability of the agent with no serious adverse effects [95]. Because of its immunosuppressive efficacy associated with circulating lymphocyte depletion, increased speed of recovery of leukocytes when FTY720 is withheld, and recovery of all subsets of lymphocytes and no increase in infections, it was felt to be an appropriate candidate for maintenance immunotherapy. Tedesco-Silva et al reported one of the first clinical studies with FTY720 and suggested that FTY720 was as efficacious as mycophenolate mofetil in combination with cyclosporine [96]. The results of a larger phase III trial have not been published, but Novartis discontinued the development of FTY720 because of a lack of benefit of FTY720 compared with mycophenolate mofetil and several adverse effects including bradycardia and macular edema. The drug remains in development for other applications [97]. Preventing lymphocyte egress with other S1P agonists remains an option for experimental immunosuppression and drug development.

9. Chemotaxis in the innate immune system

The effector cells of the innate immune system are neutrophils, macrophages, and DCs. Their antigen-independent activation is mediated by toll-like receptors, cytokine receptors, and chemokine receptors. Eighty percent of the initially infiltrating cells in acute rejection models are neutrophils [98]. Chemotaxis of neutrophils into areas of ischemic injury is mediated by CXCR1(KC)/CXCL1 and MIP-2/CXCL2; and macrophages and DCs accompany these infiltrates and infiltrate through the interaction of CCL2, 7, 8, and 13 with CCR2 and CCL3, 4, and 5 with CCR5.

The role of neutrophils has been functionally dissected in studies in animal models in which neutrophil depletion significantly attenuates allograft damage and dysfunction [59]. Blockade of the neutrophil chemotactic receptors CXCR1 and CXCR2 by the drug Repertaxin, developed by the Italian company Dompe (Milan, Italy), leads to markedly decreased inflammatory injury in the I/R phase [99-101]. Repertaxin has been granted orphan drug status and passed phase II clinical trials (http://www.clinicaltrials.gov/ct/gui/show/NCT00224406) and could be used to prevent early neutrophil infiltration in I/R injury.

In experimental solid organ transplantation, the inflammation after reperfusion subsides within 24 to 48 hours after transplantation in the isograft group and is sustained and remains elevated in allografts. Two observations have led to the theory that the CD8 cell arm of adaptive immunity is involved very early on in I/R injury: First, it could be observed that mice lacking T cells are protected from renal I/R injury [102]. Second, it was shown that only depletion of $CD8^+$ cells or use of IFN-$\gamma^{-/-}$ recipients attenuated the inflammatory response that follows early I/R injury in allografts. Because this IFN-γ–mediated/$CD8^+$ T-cell–mediated immune response is detectable before T-cell priming by APCs, 2 peculiarities for this connection between innate and adaptive immune responses have to be postulated: activation through stromal or endothelial cells as opposed to professional APCs and presence of established memory $CD8^+$ cells that are possibly primed to environmental antigens and initially amplify the innate immune response [103]. In this context, it is very interesting that chemokine receptor blockade of CXCR3 expressed on T cells has been found to protect against I/R injury in a liver transplant model

in the rat [104]. Another study showed that mycobacterial heat shock protein 70 actually signals through the CCR5 chemokine receptor, promoting DC aggregation, immune synapse formation between DCs and T cells, and generation of effector immune responses [105]. Blockade of inflammatory chemokine receptors might be an effective strategy to attenuate I/R injury.

In addition, attenuation of I/R injury might have favorable consequences for the adoptive immune response. A recent study added CXCR1/2 blockade to costimulatory blockade with anti-CD154 monoclonal antibody or CTLA-4 immunoglobulin and showed an enhanced effectiveness of costimulatory blockade [106].

10. Conclusion and perspectives

Chemokines and their receptors are essential components of the inflammatory response that leads to allograft dysfunction. Initial reports emphasized the role of inflammatory chemokines to direct leukocyte infiltrates into the graft. Further experimental work in rodents and preclinical models has shown that blocking single inflammatory chemokine pathways most likely will not provide sufficient graft protection. However, more and more antagonists against the G protein–coupled chemokine receptors will be developed by the pharmaceutical industry; and combining inflammatory chemokine blockade with other immunosuppressive approaches will provide more information about the usefulness of trafficking blockade in transplantation.

Immunosuppressive drugs such as FTY720 have been found to be based on the blockade of homeostatic leukocyte trafficking. S1P receptors do not belong to the chemokine receptor family, but FTY720 exemplifies the profound immunosuppressive effects that can be achieved with homeostatic trafficking blockade.

The microanatomy of alloimmune recognition and responses is more redundant and complex than expected. Surprisingly, well-known inflammatory chemokine/receptors pairs such as CCL3, 4, and 5/CCR5 might play a bigger role in lymphocyte activation [107] and immune regulation [80] than in leukocyte transmigration into inflamed tissue. Especially as subsets of lymphocytes and DCs become better defined with respect to their regulatory and memory function, chemokines and their receptors will remain subjects of growing interest.

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