The evolving role of chemokines and their receptors in acute allograft rejection

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Introduction

In renal transplantation, the occurrence of one or more episodes of acute allograft rejection (AAR) is a major determinant of graft survival [1]. Most episodes of AAR are caused by cell-mediated processes and require the infiltration of alloactivated T cells into the engrafted organ. These cells are characterized by the expression of surface markers indicating a memory (CD45RO+) and/or activated (CD25+) phenotype [2,3], which develop subsequent to T-cell receptor (TCR) interactions with MHC–alloantigen complexes or donor MHC in secondary lymphoid tissue [4].

A further result of this process is the expression of chemokine receptors that direct the trafficking of alloactivated T cells into the graft in response to local production of chemokines, initially by resident cells. There is now deep interest in this area that reflects the recent identification of restricted chemokine–receptor interactions as key functional events in T-cell recruitment and potential therapeutic targets for the prophylaxis of AAR. We discuss this recent evidence and how it relates to our current knowledge of chemokine–receptor expression in human renal transplantation.

T-cell trafficking

The migration of T cells to extravascular sites requires the successful completion of a series of co-ordinated molecular interactions. These processes are common to all trafficking leukocytes [5,6]. First, leukocytes margined (usually in post-capillary venules) by flow dynamics, roll through transient association and dissociation between glycosylated and sialylated ligands and endothelial cell (EC) surface expressed selectins. This allows leukocytes to sample the EC surface microenvironment where high concentrations of chemokines are sequestered. Chemokines are a superfamily of small proteins that direct leukocyte migration and position [7]. They bind G-protein linked counter-receptors (chemokine receptors) on the leukocyte cell surface to trigger intracellular signalling cascades that rapidly promote the activation of leukocyte integrins such as LFA-1 and VLA-4. Integrins then facilitate firm leukocyte adhesion by binding to super-immunoglobulin counter-receptors (e.g. ICAM-1, VCAM-1) expressed on EC. Adhered cells then transmigrate, across EC junctions and into the extracellular matrix, by directed movement along chemotactic and haptotactic gradients of chemokines and adhesion molecules.

In vivo, intravascular flow may be central for this process, as adhered T cells traffic in response to adequate chemokine signals transmitted at pro-migratory (cell contact) zones within a milieu of continuous application of shear flow [8,9]. This process has been termed chemorheotaxis.

T-cell subsets express different patterns of chemokine receptors that modulate recruitment to different organ sites in physiological and inflammatory states. For example, naive (CD45RA+, CD25−) T cells display chemokine receptors that, on ligation by constitutive chemokines expressed by high endothelial venules, direct their preferential trafficking to secondary lymphoid tissue [10]. At these sites, recruited T cells may encounter antigens, which trigger and sustain T-cell activation. These cells then proliferate into activated (effector) and memory cells and their migratory phenotype changes to one responsive to signals from inducible chemokines produced in inflamed organs. This phenotype may reflect the origin of the antigen encountered and allows T cells to target specific environments in response to local chemotactic signals. For example, the chemokine receptor CCR4 is expressed on a subset of T cells with memory for skin and intestinal antigens and promotes trafficking to skin (in cells co-expressing the E selectin ligand cutaneous lymphocyte antigen) and to intestinal...
Chemokine number of chemokines and chemokine receptors in human renal transplantation that show their increased expression in AAR (see Table 1 and below). Most of these chemokine–receptor combinations are mononuclear cell targeted or expressed. Detailed reviews of the biology of these molecules have been published elsewhere [12]. Until recently, the significance of these in vitro studies was difficult to ascertain as both resident renal cells and infiltrating cells are promiscuous sources of chemokines [12,19–21], and the pro-inflammatory cytokines that promote their production in vitro are present in the kidney in inflammation in vivo [3]. However, studies in organ transplantation models in knockout animals and with blocking antibodies are now clarifying these observations (see below and Tables 2 and 3). These indicate key roles for the receptors CXCR3 and CCR5 and selected targeting chemokines.

The biology of CXCR3 and CCR5

Studies in patients with rheumatoid arthritis first showed that CXCR3 and CCR5 are of particular importance in the recruitment of activated and memory T cells to inflammatory sites in human inflammation [22]. These receptors are also preferentially expressed on Th1 cells, the dominant Th phenotype in AAR and other cell-mediated immune processes [23]. For CXCR3, virtually all T cells at sites of non-renal human inflammation with prominent mononuclear cell infiltrates are receptor positive. CXCR3 is expressed by up to 40% of freshly isolated peripheral blood T cells, but will only mediate adhesion under conditions of flow and cell migration towards targeting chemokines after cell activation.

Chemokines and their receptors in human renal transplantation

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CX3CR1 is ligated by the inflammatory non-ELR CXC chemokines CXCL10/IP-10, CXCL9/Mig and CXCL11/I-Tac [25–27], which are differentially produced by multiple cell types, including EC, on activation by IFN-γ and TNF-α in combination. The expression of CXCR5 is closely linked to CXCR3; all T cells that express CXCR5 are also CXCR3+, indicating that these cells may be chemotrafficked through either receptor [22]. CXR5 is ligated by RANTES/CCL5, MIP-1α/CCL3 and MIP-1β/CCL4 to promote the chemotrafficking of activated T cells in chemotactic assays [28,29]. Also, analogous with CXCR3, there is little chemotrafficking of unstimulated lymphocytes by chemokines active against CXCR5. Studies on the chemotactic potential of the supernatant from activated (IFN-γ and TNF-α treated) PTEC, which is chemokine rich, towards CD3+ T cells confirm this. There is little chemotactic activity towards freshly isolated T cells; however, after activation by TCR ligation and IL-2 treatment there is heavy cell migration. Most of this chemotaxis is mediated through CXCR3 and CCR5, with an additional role for CX3CR1, the CX3CL1/fractalkine receptor [30].

Animal models for CXCR3 and CCR5 and their ligands

In a rat cardiac transplant model, where pre-transplant MHC priming resulted in allograft rejection, there was increased intragraft expression of CXCL10/IP-10 and CXCL9/Mig and infiltration with mononuclear cells expressing CXCR3 [31]. In a murine cardiac model, Kapoor and colleagues [32] showed that both CXCL10/IP-10 and CXCL9/Mig expression was increased from day 2 until the day of rejection (day 8–10) although the infiltrating cellular phenotype was not assessed. In MHC disparate skin allografts, blocking of CXCL9/Mig inhibited T-cell and macrophage migration into the graft [33]. Injection of CXCL9/Mig then restored T-cell infiltration and subsequent rejection.

Hancock and colleagues [34] showed early CXCL10/IP-10 production (at day 1) by EC in cardiac allografts and isografts, presumably as a result of surgical manipulation and reperfusion injury. There was subsequent amplification expression of CXCL10/IP-10 and sequential expression of CXCL9/Mig and CXCL11/I-Tac in allografts but not isografts [34]. Transplanting cardiac allografts from CXCL10/IP-10 knockout mice into wild-type animals had a dramatic effect, with no subsequent development of AAR. This indicates a key role for early induction of CXCL10/IP-10 promoting subsequent production of CXCL9/Mig and CXCL11/I-TAC and the recruitment of alloactivated T cells. The development of this ‘chemokine cascade’ may be dependent on early natural killer (NK) cell recruitment; EC-expressed CXCL10/IP-10 may ligate NK cell CXCR3 to localize these cells within the graft, where they recognize disparate MHC on the surface of EC and other resident cells. Production of IFN-γ and other cytokines by NK cells may then amplify CXCL10/IP-10 production and promote sequential expression of CXCL9/Mig and CXCL11/I-TAC with subsequent recruitment of alloactivated T cells. This is consistent with recent data showing early infiltration of NK cells (by day 1) in a separate animal model of cardiac transplantation [35] and induction of chemokines by resident cells before infiltration of mononuclear cells in an animal model of lupus nephritis [36]. Studies using knockout animals complement these findings, with a mean cardiac allograft survival of

Table 2. Chemokine receptor knockout models in allograft rejection

<table>
<thead>
<tr>
<th>Chemokine receptor</th>
<th>Organ</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR1</td>
<td>Heart</td>
<td>CXR1−/− model survived 2 × longer than wild-type (WT)</td>
<td>Gao et al., 2000 [49]</td>
</tr>
<tr>
<td>CCR2, CCR5</td>
<td>Heart</td>
<td>CXR2−/− have 2 × survival, and CCR5−/− × 3 × survival compared with WT</td>
<td>Hancock et al., 2001 [37]</td>
</tr>
<tr>
<td>CXCR3</td>
<td>Heart</td>
<td>CXCR3−/− survived &gt; 8 × longer than WT. Indefinite survival with</td>
<td>Hancock et al., 2001 [37]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>low-dose cyclosporin. Minimal cellular infiltration and injury in</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sacrificed CXCR3−/− animals</td>
<td></td>
</tr>
<tr>
<td>CX3CR1</td>
<td>Heart</td>
<td>CX3CR1−/− mice survived 3 × longer if treated with subtherapeutic</td>
<td>Haskell et al., 2001 [46]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cyclosporin</td>
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</table>

Table 3. Studies using antibodies against chemokines and chemokine receptors in AAR

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Species</th>
<th>Organ</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met-RANTES</td>
<td>Rat</td>
<td>Kidney</td>
<td>Reduced vascular and tubular injury</td>
<td>Grone et al., 1999 [63]</td>
</tr>
<tr>
<td>Anti-Mig/CXCL9 (serum)</td>
<td>Mouse</td>
<td>Skin</td>
<td>Treatment improved skin graft survival (×4).</td>
<td>Koga et al., 1999 [33]</td>
</tr>
<tr>
<td>Anti-CXCR1</td>
<td>Mouse</td>
<td>Heart</td>
<td>Prolonged survival (× 7) in anti-CXCR1 group</td>
<td>Robinson et al., 2000 [64]</td>
</tr>
<tr>
<td>Anti-CXCR3</td>
<td>Mouse</td>
<td>Heart</td>
<td>Doubled allograft survival compared with control</td>
<td>Hancock et al., 2000 [37]</td>
</tr>
<tr>
<td>Anti-IP-10/CXCL10</td>
<td>Mouse</td>
<td>Heart</td>
<td>Improved survival (×3 ×)</td>
<td>Hancock et al., 2001 [34]</td>
</tr>
</tbody>
</table>
58 days in CXCR3−/− compared with 7 days in the wild-type (CXCR3+/+) [37]. This increased survival was consistent with the histology of the graft, which showed greatly decreased cellular infiltrate and damage compared with controls. Similar effects on graft survival were obtained using an anti-CXCR3 antibody in CXCR3+/+ recipients.

Transplantation models for CCR5 knockouts show less dramatic results but are still highly significant. MHC disparate cardiac allografts transplanted into CCR5−/− mice show a tripling of graft survival, and addition of low-dose cyclosporin results in permanent allograft survival [38]. This demonstrates an important principle for the prophylaxis of AAR; increasing the threshold for T-cell infiltration may dramatically lower the doses of immunosuppression required. However, whilst these models provide major evidence for a role for CXCR3 and CCR5 in AAR, in cardiac allografts chemokine expression in the heart is primarily by EC or infiltrating mononuclear cells, whereas kidneys have a heterogeneous population of resident cells, which express inflammatory chemokines when stimulated. Therefore, use of these knockout animals in studies on renal transplantation would help prove the applicability of this data to other organ systems. More focused ex vivo analyses on the role of CXCR3 in human renal transplantation are also required.

CXCR3 and CCR5 and their ligands in human renal transplantation

Despite the evidence for a role for CXCR3 and its ligands in AAR, there is relatively little data in human renal transplantation. In rejecting human lung allografts, infiltrating T cells express CXCR3 and these cells were highly responsive to CXCL10 u grafts, infiltrating T cells express CXCR3 and these renal transplantation. In rejecting human lung allografts, there is relatively little data in human renal transplantation.

CX3CL1/fractalkine

A further chemokine of potential importance in AAR is CX3CL1/fractalkine. Unlike virtually all other chemokines it has a membrane-anchoring domain [44]. In addition, CX3CL1/fractalkine is the only ligand for its targeting receptor (CX3CR1) shown to date. CX3CL1/fractalkine can promote chemotaxis via a shed soluble domain as well as adhesion when transmembrane bound. CX3CR1 is expressed by NK cells, monocytes and IL-2 activated CD8+ T cells [45]. Recently, Haskell and colleagues [46] have shown that in CX3CR1−/− heterotopic MHC mismatched cardiac transplants there was no difference in graft survival between knockout and wild-type animals; however, on addition of low-dose cyclosporin there was a threefold increase in graft survival. In renal biopsies from patients with AAR we have shown expression of CX3CL1/fractalkine mRNA and protein (by EC and tubular epithelial cells) that correlates with infiltration of mononuclear cells [47]. Supernatant from activated PTEC has some soluble CX3CL1/fractalkine attributable chemotactic activity towards activated T cells [30] and in its transmembrane-bound form may have a role in the retention of mononuclear cells at tubular sites [48].

Other chemokines and their receptors in AAR

Both CCR1 and CCR2 have been implicated in acute AAR, although to a significantly less degree than CXCR3 and CCR5. Mismatched heterotopic cardiac transplants survive twice as long in CCR1−/− mice compared with wild-type mice [49]. In recipients treated with low doses of cyclosporin or anti-CD4 monoclonal antibodies there was prolonged allograft survival and no development of chronic rejection. Two chemokines that ligate CCR1, RANTES/CCL5 and MIP1-β/CCL4, have been demonstrated in AAR in human renal transplants [42,43]. CCR2−/− recipients of cardiac allografts have a similar phenotype. The ligating chemokines for CCR2 are MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7 and MCP-4/CCL13. In renal transplantation, the main role of these molecules may be in directing macrophage infiltration as predominant CCR2 expression is by this cell type; MCP-1/CCL2 and MCP-4/CCL13 expression in AAR in human renal transplants correlate with, and colocalize to, infiltrating macrophages [50,51].
analyses also indicate that the major role of PTEC-derived MCP-1/CCL2 may be for monocytes/macrophage rather than T-cell recruitment [52].

Schmouder and colleagues [53] used RT-PCR to study the expression of the CXC chemokine ENA-78/CXCL5 in acute renal transplant rejection. This is predominately a neutrophil- and monocyte-directed chemokine that targets the receptor CXCR2. They demonstrated increased transcripts of ENA-78/CXCL5 localized to epithelial cells in vivo when stimulated by IL-1β. There is also heavy local expression of IL-8/CXCL8 (which targets CXCR1 and CXCR2) in other models of human renal inflammation [54], and elevated levels of this chemokine have been identified in the urine of patients with AAR [55,56]. However, there is little expression of the IL-8/CXCL8 receptors CXCR1 and CXCR2 on freshly isolated or activated T cells [57], so these chemokines are unlikely to have a central role in directing T-cell infiltration. A potential role has also been identified for CXCR4, which is also expressed by infiltrating T cells; however, the biological relevance of this is uncertain as it is expressed by all circulating leukocytes and targeted by SDF-1/CXCL12, a constitutively expressed chemokine important for homeostatic trafficking [58].

Conclusions

Although a number of chemokines and their receptors are up-regulated in AAR, recent studies indicate a key role for CXCR3 and CCR5. Assessment of the applicability of this data to renal transplantation requires further expression studies in humans and analyses of animal models of renal transplantation. If confirmatory, then targeting CXCR3 and/or CCR5-mediated processes in the first few days after transplantation may prevent the early recruitment of NK cells and alloactivated T cells whilst the graft is activated from donor factors and ischaemia re-perfusion injury. This directed approach might allow lower induction and maintenance immunosuppressive therapy and sustain long-term graft survival. Finally, whilst recent studies provide essential insights into the mechanisms that promote AAR, there is little known of the role of chemokines and their receptors in the development of chronic allograft nephropathy.

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