Role of Chemokines for the Localization of Leukocyte Subsets in the Kidney

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Summary: Chemokines comprise a family of structurally related chemotactic proteins. They bind to about 20 corresponding receptors. Chemokines provide a general communication system for cells, and regulate lymphocyte migration under normal (homeostatic) and inflammatory conditions. Chemokines organize microenvironments in lymphoid tissue, lymphoid organogenesis, and participate in vascular and lymphatic angiogenesis. Expressed at the site of injury in the kidney, chemokines are involved in the recruitment of specific leukocyte subsets to particular renal compartments. Here we summarize recent data on chemokine biology with a focus on the role of chemokines in the recruitment of neutrophils (polymorphonuclear leukocytes), monocytes/macrophages, dendritic cells, T cells, including regulatory T cells, and B cells in renal inflammation.

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Inflammation is a prerequisite for renal fibrosis in all chronic human kidney diseases and in animal models.[1] Chemokines are involved in the recruitment of specific subsets of leukocytes to the injured kidney and to renal allografts.[2,3] Various aspects of chemokine biology in renal diseases have been reviewed extensively by us and others.[2-6] In the present review we use a cell-centered approach to describe recent developments in our understanding of renal inflammation, focusing on the involvement of chemokines and in the recruitment of 5 cell types (ie, polymorphonuclear leukocytes [PMNs], B cells, and T cells, including regulatory T cells, monocytes/macrophages [Mo/Mac], and dendritic cells [DCs]).

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CURRENT ASPECTS OF CHEMOKINE BIOLOGY

The Chemokine Family

During recent years the number of chemokines (L for ligand) and chemokine receptors (R) has not increased significantly and, because of the knowledge of the human genome, little additional identifications are expected. However, posttranscriptional modifications of chemokines and heterodimerization of chemokine receptors are increasingly recognized and considerably expand the spectrum of biological effects of the system.[7,8] N terminal changes via naturally occurring proteases significantly changes chemokine functions, with responses ranging from activation (eg, for CXCL7/NAP-2) to the biological active chemokine, increased activity (eg, for CXCL8/interleukin [IL]-8), to decreased activity (eg, for CXCL9/MIG), or even antagonistic function.[8] Agonistic and antagonistic functions of chemokines on different receptors also have been described.[9,10]

A motif of 4 conserved cysteine residues in the primary structure is used to divide the chemokines into 4 subgroups (CCL, CXCL,

CX3CL1, and XCL1), according to the position of the N terminal 2 cysteines and intervening nonconserved amino acids (X).[11,12] The N terminus of chemokines is important for receptor binding and specificity, whereas areas in the core and the C terminus are important for matrix binding and presentation of the chemokine on the glycocalyx of the endothelial surface.[13]

Chemokine Receptors

Chemokine receptors are 7 transmembrane-spanning proteins, coupled to heterotrimeric G proteins, and activate various intracellular pathways such as small guanosine triphosphatases and kinases.[12,14] It has been shown that chemokine receptors can cross-desensitize other chemokine receptors. Chemokine receptors can form homodimers and heterodimers.[15,16] CC chemokine receptor (CCR5), for example, forms heterodimers with CCR2b, and binding of CCR5 ligands prevents CCL2/ MCP-1 signaling.[15] Therefore, paradoxically, chemokine agonists might be suitable anti-inflammatory therapeutics under specific conditions.[17]

By binding to their respective receptors, chemokines fulfill major functions in leukocyte recruitment to the site of tissue injury.[12] Presented by glycosaminoglycans (GAGs) on the endothelial surface, chemokines activate integrins on rolling leukocytes. This transforms integrins to a high-affinity state, and mediates firm adhesion of the leukocyte to the endothelium, and allows their extravasation.[18,19] Therefore, chemokines that activate integrins may be called *arrest chemokines*.[18] To mediate firm adhesion, chemokines need to be transported to the endothelial surface. The involvement of nonsignaling chemokine receptors such as the duffy antigen/receptor for chemokines (DARC) in this process is very likely.[20-22] After having left the vasculature, chemokines mediate the directed migration of leukocytes toward the site of injury, likely following matrix-bound chemokine gradients.[23] A rapid change in the chemokine-receptor pattern enables leukocytes to either remain tissue bound, to leave the tissue, or to interact with other cells.[12]

Chemokine Function

Functionally, chemokines were labeled as *inflammatory* or *homeostatic*, but there is some overlap between the groups.[12] The focus of the renal literature has been mostly on inflammatory chemokines such as CCL2/MCP-1, CCL5/RANTES, or CXCL10/IP-10.[2,3,6,24-26] These chemokines are rapidly up-regulated and released by proinflammatory cytokines and cell stress at the sites of injury.[3] Homeostatic chemokines are thought to be involved in cellular localization, recirculation, and cell-cell interactions under noninflammatory conditions. Finally, some chemokines (eg, CCL22/MDC) fulfill both functions. Furthermore, there may be heterophilic interactions between chemokines modifying their function (eg, CXCL4/PF4-CXCL8/IL-8 and CXCL4/PF4-CCL5/RANTES).[27] For example, CXCL4/PF4 together with CXCL8/IL-8 inhibits rather than induces monocyte arrest under flow conditions. Besides the recruitment and positioning of inflammatory cells to sites of injury, chemokines are involved in the recruitment of leukocytes and stem cells from the bone marrow in vascular and lymphatic angiogenesis and in tumor metastasis formation (via recruitment of tumor cells to certain organs). The multiple functions of chemokines and chemokine receptors are achieved by a versatile and dynamic system. For example, ligand binding to chemokine receptors can result in rapid internalization of the chemokine receptor, which stops cell movement. Concomitantly the cells may express different chemokine receptors that enable response to other chemotactic factors.[28] Expression of the same chemokine receptor on 2 different cell types (eg, CCR7 on naive T cells and mature DCs) provides for a rendezvous of these cells in specific mircroenvironments.[29] It has become apparent that the change of chemokine-receptor expression during maturation or activation plays an important role in immune surveillance and that the picture is by far less static than previously expected. Monocytes and immature DCs express receptors for inflammatory chemokines (eg, CCR5, CCR2, and CCR1), which allows them to be recruited to the site of acute tissue injury.[29] T-cell activation results in down-regulation of CCR7 and up-regulation of inflammatory receptors such as CXCR3 and CCR5, which allows them to leave the lymph node and guide them toward the site of injury.[29] On acti-

vation and maturation of DCs the receptors for inflammatory chemokines are rapidly down-regulated, and others, particularly CCR7, are up-regulated. CCL21, the ligand for CCR7, is presented by lymphatic endothelium, thereby MO/DCs can travel from the injured organs to the draining lymph nodes. Immature T cells also express CCR7, therefore the cells meet with DCs in the lymph node–T-cell area.[29] Similar concepts currently are being developed for the various cell partners of the immune system. Another example are B cells, which need to meet with T cells in lymphoid tissue. Here the expression of CXCR5 on B cells and specialized T cells enables this copositioning.[30] Recent data show that the chemokine system not only may regulate leukocyte movement between lymphatic organs and sites of tissue injury, but also may govern leukocyte mobilization from the bone marrow to the periphery.[31]

ROLE OF CHEMOKINES IN THE RECRUITMENT OF PMNS

The CXC chemokine CXCL8/IL-8, binding to its corresponding receptors (ie, CXCR1/2), was the first neutrophil-selective chemoattractant identified.[12,32-34] Initially CXCL8/IL-8 was described as being released by monocytes/macrophages, an observation that may be of significance for the early as well as late PMN recruitment noted in many diseases.[35] Subsequently, many cell types have been reported to be able to generate CXCL8/IL-8 in culture when stimulated with proinflammatory factors (reviewed in Segerer et al[3]). Besides the induction by typical proinflammatory cytokines, CXCL8/IL-8 also has been reported to be induced in cultured tubular epithelial cells by transforming growth factor-β 1[36] and by exposure to high concentrations of albumin.[37] The significance of these in vitro observations for the in vivo situation remains to be determined.

The functional role of CXCL8/IL-8 has been studied in animal models of renal diseases as well as in patients. Early studies by Wada et al[38] showed decreased proteinuria and neutrophil recruitment in rabbits with an immune complex nephritis treated with an anti–CXCL8/IL-8 antibody. In patients with glomerular diseases an increased urinary CXCL8/IL-8 excretion has been shown in various forms of glomerulonephritis (GN), including immunoglobulin A (IgA) nephropathy, membrano-proliferative glomerulonephritis (MPGN), and lupus nephritis.[39] We studied the expression of the corresponding receptor CXCR1 in glomerular diseases.[40] In normal renal tissue CXCR1 protein was expressed by a low number of circulating leukocytes and intrinsic renal cells, including smooth muscle cells of small arteries and arterioles, endothelial cells, and, occasionally, podocytes. A prominent number of CXCR1-positive infiltrating cells were found both in glomeruli and the tubulointerstitium with the highest numbers in MPGN, lupus nephritis, and crescentic GN.[40] The CXCR1-positive infiltrating cells were predominantly PMNs according to the nuclear morphology and consecutive staining for CD15 (a neutrophil marker). Furthermore, the distribution and cell number of CXCR1-positive cells was distinct from that of CD68-positive monocytes/macrophages. Thus, a prominent CXCR1-positive PMN infiltrate was found in chronic glomerular diseases, with a strong co-infiltrate of macrophages. These data point toward an ongoing recruitment of CXCR1-positive PMNs in the course of chronic renal diseases. This is contrary to the widely held view that PMNs only contribute to the induction of the inflammatory processes. In a model of lung inflammation, it was shown that early recruitment and activation of Mo/Mac was a prerequisite for PMN recruitment.[41] This would be consistent with CXCL8/IL-8 generation predominantly by Mo/Mac. This would indicate a role for CXCL8/IL-8, CXCR1, and PMNs also in later phases of the inflammatory process.

In a study on human renal allografts, CXCL8/IL-8 expression was increased significantly 30 minutes after declamping of the arterial graft anastomosis.[42] CXCL8/IL-8 expression increased by only 50% in living donor grafts, but by 13-fold in cadaver donor grafts, indicating an important role for ischemia in attracting PMNs.[42]

Furthermore, recent studies have indicated interactions of PMNs with DCs, potentially as danger sensors and for DC maturation. Therefore, PMNs might potentially act as links between innate and adaptive immune responses in chronic kidney diseases and in renal allografts.[43]

ROLE OF CHEMOKINES IN THE RECRUITMENT OF MO/MAC IN RENAL INFLAMMATION

The distinction between Mac and DCs in the kidney, both in health and disease, is becoming more fluent than previously thought.[44] Mac and DCs are part of the mononuclear phagocyte and antigen presenting system. As such, they play pivotal roles in innate and adaptive immune responses.[45,46] It is increasingly becoming apparent that the development of Mo/Mac and DCs is a very dynamic process and that no single cell marker can be assigned to these cells. Functionally, Mac can be activated to various phenotypes, ranging from the classic cytotoxic macrophage to an anti-inflammatory cell involved in tissue remodeling.[46] The heterogenicity of Mo/Macs reflects plasticity rather then defined lineages because a common progenitor has been described in mice.[45-48] For a detailed discussion of the various monocyte activation pathways see Mantovani et al.[48] In brief, Mo treated in vitro with proinflammatory cytokines such as tumor necrosis factor-α and interferon-γ adopt a classic activated phenotype and release toxic mediators (eg, nitric oxide and reactive oxygen intermediates). Chemokines such as CCL2/MCP-1 can additionally increase the cytotoxicity of these cells.[46] The other end of the spectrum of macrophage activation results in alternatively activated cells.[48]

The classically activated macrophage showed a stronger expression of messenger RNA for CCR7, as well as the chemokines CCL5, CCL19, CXCL 9-11, as compared with alternatively activated macrophages in vitro, characterized by low production of proinflammatory cytokines.[49]

In the mouse, 2 monocyte populations have been described in the peripheral blood.[50] One is short lived, expresses low amounts of the fractalkine receptor (CX3CR1), but high amounts of CCR2, and is recruited to inflamed tissue.[50] The other cell type does not express CCR2 and localizes to noninflamed tissue via CX3CR1.[50] Both cell types can give rise to DCs. In human beings these 2 monocyte populations were described as CX3CR1lowCD14(+)CD16(−) and CD14lowCD16+, respectively.[50] Furthermore, freshly isolated blood monocytes express a panel of inflammatory chemokine receptors (CCR1, CCR2, and CCR5).[48] Differentiation into tissue macrophages is associated with a loss of CCR2, but induction of CCR1 and CCR5.[48]

In the kidney, Mo/Mac are the most common inflammatory cells in glomeruli with proliferating GN, and together with T cells are the most common infiltrating cell types in the diseased tubulointerstitium.[46] There are 2 sources for macrophages in inflamed kidneys, those recruited from the circulation and those derived from proliferation of resident monocytic cells. At present their relative contributions are not defined, but the proliferation of intrarenal Mo/Mac may have been underestimated to date.[46]

Currently the most commonly described chemokines for Mo/Mac recruitment during renal inflammation are CCL2/MCP-1, CCL5/RANTES, CCL3/MIP-1α, CCL4/MIP-1β, and CXCL8/IL-8. The corresponding receptors mediate firm adhesion (CCR1 and CXCR2), and shape change (CCR2 and CCR5), spreading (CCR2, CCR5), and transmigration.[46,51] Once Mo/Mac have transmigrated into the interstitium they appear to change their surface expression of chemokine receptors (eg, loss of CCR2). Migration of Mac through the interstitial tissue is facilitated through the release of matrix degradative enzymes by the Mac, which is mediated in part by chemokines.[46] Various models (eg, nephrotoxic nephritis, unilateral ureter ligation, adriamycin nephropathy, and models of lupus nephritis) have been studied for the expression of chemokines and the potential benefits of chemokine blockade. In general, chemokines such as CCL2/MCP-1, CCL5/RANTES, and CX3CL1/fractalkine were found to be expressed in the damaged renal compartment and preceded the recruitment of inflammatory cells. Blockade of CCL2/MCP-1 and CX3CL1/fractalkine significantly improved glomerular and interstitial injury and reduced Mo/Mac recruitment.[52-54] Blockade of CCR1 using a small molecule antagonist prevented Mo/Mac recruitment and interstitial fibrosis in UUO and adriamycin nephropathy.[55,56] Detailed studies on the roles of CCL2/MCP-1 and the receptor CCR2 have been performed in lupus models. The blockade of CCL2/MCP-1 function or genetic deficiency of either the ligand CCL2 or the receptor CCR2

resulted in decreased Mo/Mac recruitment and functional improvement in lupus nephritis models.[57-59] On the other hand, exacerbation of inflammatory models in CCR2-deficient mice and in mice treated with N-terminal modifications of CCL5 also have been described.[60,61] The reasons for these apparently opposing effects remain unclear at present but may be related to other immune functions of the specific chemokines during the development of the renal disease models. Detailed reviews of the available animal studies have been published recently.[2,3,5]

CHEMOKINES AND DCS IN RENAL INFLAMMATION

The realization that the development and relation between Mo/Mac and DCs is much more dynamic than previously thought also applies to the kidney. DCs have a high phagocytic activity in an immature state, and accumulate costimulatory molecules to induce strong T-cell responses during maturation. DCs play a central role in the induction and regulation of adaptive immune responses, controlling B and T cells as mediators of immunity.[62] In vitro DCs can be generated from myeloid and plasmacytoid progenitors (showing different migration behavior). Furthermore, the DC subsets differ according to the lymphoid or nonlymphoid organ where they reside.[63] For example, in the mouse spleen 4 DC subsets have been characterized: 2 myeloid DCs (major histocompatibility complex [MHC] II low, CD11c+, CD11b+, CD205−, CD8α−, B220−), 1 lymphoid (MHC II low, CD11c+, CD11b−, CD205+, CD8α+, B220−), and 1 plasmacytoid (MHC II low, CD11c+, CD11b−, CD205−, CD8α+, CD4 +, B220+, Gr1+).

For the kidney we will refer to Mac/DC cells rather than DC only because there are fluid functions and definitions between Mac and DCs. The kidney contains an interstitial network of cells that previously were labeled as *interstitial cells*, or resident monocytes.[64] In the mouse kidney these F4/80+ cells may in part represent a DC network, performing an intrarenal surveillance role. Such DCs reside in most tissues under normal conditions and sense danger signals.[65] After activation some of these DCs travel from the tissue to the draining lymph node, whereas others remain and potentially proliferate locally. In these processes the different Mac/DCs change their phenotype, so that by surface markers different types can be identified. At present it remains unclear if this truly represents differentiation of Mac/DCs or different stages of a dynamic cell function. In terms of chemokine-receptor expression, immature DCs express receptors for inflammatory chemokines (CCR1, CCR2, CCR4, CCR5, and CXCR3), which allow them to migrate toward the site of injury.[66] During maturation mediated through a variety of factors (eg, IL-1, tumor necrosis factor, and lipopolysaccharide), DCs acquire costimulatory potential and MHC molecules. This is associated with a functional change in the chemokine-receptor pattern. Down-regulation of inflammatory chemokine receptors and induction of CCR7 allows them to leave the site of injury and migrate toward lymphatic vessels. The corresponding ligand CCL21/SLC is expressed and presented by lymphatic endothelial cells.[66] Mature DCs, which are characterized by less phagocytic activity and a strong expression of costimulatory molecules, find their way into the draining lymph nodes where they interact with T cells in antigen presentation and T-cell activation. On the other hand, DCs also can have tolerizing effects when they express autoantigens in the absence of costimulatory molecules.

It should be apparent from this brief description that, similar to the development of monocytes to macrophages, the macrophage to DC changes are highly dynamic, so that it is difficult to put the cells into specific categories. Furthermore, the transition from Mo/Mac to DCs is a fluent one. Because unique markers specific for DCs do not exist, DCs may have been described under various labels in the kidney. In the normal mouse kidney a network of F4/80-positive cells in the tubulointerstitium was described more than 20 years ago.[64] Kruger et al[67] described a population of CD11c and MHC II–positive cells with DC functionality in mouse kidneys. Most of these cells also expressed F4/80. Others have taken advantage of the fact that Mac/DCs express the fractalkine receptor CX3CR1 constitutively, which mediates their

localization to nonlymphoid tissue under noninflammatory conditions.[68,69] Soos et al[70] recently described an interstitial network of cells with dendritic morphology and CX3RCR1 positivity in kidneys of mice transgenic for green fluorescence protein under control of the CX3CR1 promoter. By fluorescence-activated flow cytometry the green fluorescence protein–positive cell population showed co-expression of CD11c, and was F4/80low, CD4−, CD8α−, B220−, and Gr-1negative, with high expression MHC II, consistent with myeloid DCs.[70] These cells showed immature costimulatory competence (low expression of CD80, CD86, and CD40), but strong phagocytic ability.[70] This would be consistent with immature DCs and closely resembles interstitial DCs in other organs (eg, the lung). Clearly, evidence for a cell population with DC morphology and function is evolving in the mouse kidney. Because a significant part of these cells also expressed the marker F4/80, which is a common marker used to describe macrophages, the distinction between monocytes/macrophages and DCs has become blurry. Although these Mac/DC cell markers have been described for the mouse system, the expression of cell antigens by Mac/DCs in the human kidney are not well established. Kerjaschki[71] used the marker S100 to describe a DC population in human allografts. We recently compared CD68 (a marker commonly used for human Mo/Mac), dendritic cell-specific intercellular-adhesion-molecule-grabbing non-integrin (DC-SIGN) (for immature DCs), S100, and langerin (a marker of Langerhans cells) in renal biopsy specimens with various forms of glomerulonephritis. We found a strong recruitment of CD68-positive cells to glomeruli and the tubulointerstitium in proliferative GN. A high number of DC-SIGN–positive cells were present in the tubulointerstitium (some cells being CD68 double positive), but no DC-SIGN expression was present in glomeruli. This indicates that CD68-positive cells differ in the 2 renal compartments (ie, glomerular and tubulointerstitial). Mature DCs identified by S100 or langerin were detectable but rare in both normal and inflamed kidneys (Segerer, unpublished observation).

Studies in vitro and in organs other than kidney have indicated that CCR7 and various inflammatory chemokine receptors (eg, CXCR3, CCR1, CCR5, and so forth) also might be involved in the recruitment and positioning of these cells.[72] It will be a major task for future studies to describe the localization and function of the various Mac/DC types in the human kidney and the expression of chemokine receptors by these cells, as well as the cytokine pattern expressed by these cells. The resident network of Mac/DCs in the kidney may perform a constant surveillance function and may after insult also serve as an early and major source for cytokines (perhaps including chemokines) during tissue injury.[73]

CHEMOKINES, B CELLS, AND RENAL INFLAMMATION

B cells function as effector cells primarily via antigen-specific expansion and plasma-cell differentiation. The renal literature has focused on their role as antibody-producing cells, whereas B cells infiltrating the kidney have received very little attention. B cells can release a variety of cytokines (including chemokines), present antigens, and are involved in lymphangioneogenesis and lymphoneogenesis.[74-76] A role for B cells in tissue fibrosis has been shown in a model of CCl_4-induced liver injury. In this model about half of the infiltrating cells in the liver were found to be B cells, and B-cell deficiency significantly improved liver injury.[77] This improvement was not T-cell or immunoglobulin dependent.[77]

Most B-cell responses to antigens need the involvement of DCs and antigen-specific T-helper cells. T-cell–dependent responses result in short-lived plasma cell differentiation and germinal center development of long-lived plasma cells and B memory cells.[78] Chemokines are involved in the positioning of the interacting cell types in lymphatic microenvironments.[79] Recirculating naive B cells localize to B-cell follicles in secondary lymphoid organs owing to the expression of CXCR5 on B cells and CXCL13/BLC by follicular stromal cells.[80,81] Furthermore, naive B cells express moderate amounts of CCR7. Having encountered their specific antigen, B cells rapidly relocalize to the

B-/T-cell boundary area. This is associated with a rapid induction of CCR7, whereas CXCR5 remains expressed.[82] The expression of CCR7 by B cells and T cells enables them to meet. The CXCL13/BLC and CXCR5 knockout mice were severely deficient in lymph nodes and Peyer's patches, indicating a role during lymph-organogenesis.[30,83] Furthermore, CXCL13/BLC has been shown to act as an arrest chemokine on high endothelial venules.[84,85]

B cells accumulating in renal allografts have been shown to be associated with a poor outcome.[86] Steinmetz et al[87] studied serial sections of 23 allograft biopsy specimens for the expression of CD20, CXCR5, and CXCL13/BLC. Acute rejection was found in 13 biopsy specimens, 4 of which contained clusters of B cells. Biopsy specimens with acute rejection showed a significantly increased expression of CXCL13/BLC as compared with nonrejecting allografts. By immunohistochemistry, sites of CXCR5-positive cells correlated with CXCL13/BLC, and both were restricted to sites of nodular B-cell accumulation.[87]

We studied renal biopsy specimens from patients with acute and chronic primary interstitial nephritis, and from patients with chronic IgA nephropathy and interstitial involvement.[88] CD20-positive B cells formed a prominent part of the interstitial infiltrates and formed lymphoid-like nodular structures in about a third of the biopsy specimens. These infiltrates were associated with expression of CXCL13/BLC and CXCR5 (consistent with the data shown for allografts). These data indicate that B-cell infiltrates appear to play a role during renal inflammation that was not previously appreciated. CXCL13/BLC and the corresponding receptor CXCR5 likely are involved in the recruitment of B cells to the kidney, particularly to lymphoid follicle-like structures. These tertiary lymphoid structures could be a site of an intrarenal immune response.

CHEMOKINES AND T CELLS

Chemokines play crucial roles during early thymocyte development, positioning, T-cell selection, and central memory.[89] Self-tolerance is mediated by deletion of self-reactive cells in the thymus. T cells need to meet with antigen-presenting cells in T-cell areas to be activated (see previously). During maturation, T cells change the pattern of chemokine-receptor expression from constitutive receptors such as CCR7, to a receptor pattern for inflammatory chemokines such as CXCR3 and CCR5.[29] The chemokine CXCL10/IP-10 has been shown to mediate a T-helper type I (TH1) response, whereas CCL2/MCP1 and CXCL4/PF-4 are involved in TH2 responses.[90] The different T-helper cell types also are reflected by different chemokine-receptor expression. CCR4 and CCR8 are expressed mainly by TH2 cells, whereas TH1 cells express predominantly CCR5 and CXCR3. A third subset of T effector cells (ie, TH17 cells) has been described recently, which is involved in protection against extracellular bacteria as well as in various autoimmune diseases.[91,92] These cells express IL-17, but the chemokine-receptor pattern of these cells has not been described so far.

In the periphery, self-tolerance is mediated in part by a group of special T cells (ie, regulatory T cells [Tregs]). These cells actively suppress autoreactive T cells.[93] A role of Tregs in various pathologies such as autoimmune disease, allograft injury, and so forth, is evolving rapidly. Tregs express CD4, CD25, and the transcription factor FOXP3.[93] Human CD4-CD25 double-positive Tregs have been shown to express the chemokine receptors CCR4 and CCR8, and migrate toward the corresponding ligands CCL17/TARC and CCL1/I-309.[94,95] Migration toward CCL22/MDC, another ligand of CCR4, also has been described. In human bone marrow a high number of Tregs has been described, which traffic via CXCL12/SDF-1 as a ligand for CXCR4.[96] A recent study showed a change in the chemokine-receptor pattern during activation of Tregs from a lymphoid-homing pattern (CCR7) to a tissue-homing pattern.[97]

In the mouse, CD25-positive Tregs respond to CCL4 via the receptor CCR5.[98] In a mouse heart allograft model, the recruitment of FOXP3-positive T cells also was related to CCL17/TARC and the corresponding receptor CCR4.[99]

Inflammatory cells, such as activated B cells and mature DCs, might be the source of the chemokine CCL17/TARC attracting Tregs.[100]

Tregs interact directly with effector T cells and antigen presenting cells (APC), and therefore CCR4, CCR5, and CCR8 might be involved in this rendezvous.

Mahajan et al[101] recently showed that Tregs ameliorated responses of macrophages in vitro, resulting in down-regulation of CCL3/MIP-1α release by macrophages. These investigators[101] induced adriamycin nephropathy in scid mice and reconstituted them with FOXP3-positive CD4/CD25-positive cells. Tregs reduced glomerular and tubulointerstitial injury, with reduced numbers of infiltrating macrophages.[101] Similarly, FOXP3-transduced T cells showed a protective effect in this model.[102]

Muthukumar et al[103] measured FOXP3 messenger RNA in the urine of allograft recipients. Thirty-six subjects with acute rejection and 18 with chronic allograft nephropathy were compared with 29 subjects with normal transplant biopsy specimens. A significantly higher level of FOXP3 messenger RNA expression was found during acute rejection, but was correlated inversely with serum-creatinine level and with graft loss at 6 month.[103]

These results indicate that part of the interstitial infiltrate, especially Tregs, and potentially also DCs, may have regulatory properties and might be of positive prognostic impact. It is likely that ongoing studies will shed further light on the role of regulatory cells in mouse renal models and in human kidney diseases. Furthermore, some of the negative effects that have been described for chemokine blocking agents might relate to the blockade of regulatory cells. Obviously this adds another layer of complexity to the interaction of chemokines with various types of leukocytes.

CHEMOKINES AND FIBROCYTES

Fibrocytes are bone marrow–derived circulating cells with fibroblast-like properties.[104] These cells initially were described in wound repair, but also play important roles in granuloma formation and in fibrosing diseases of several organs.[105] Fibrocytes express vimentin, collagens I and III, CD34, and produce matrix metalloproteinases.[105] By bone marrow transplantation experiments it has been shown that bone marrow–derived donor cells accumulated at the site of granulation tissue. These cells substantially contributed to collagen expression at the site of injury and expressed fibroblast specific protein-1.[106] In pulmonary fibrosis, recruitment of fibrocytes was mediated by CXCR4 and CCR2.[107,108] Furthermore, in vitro fibrocytes migrate to CCL21/SLC, a ligand for the receptor CCR7.[109] In a very recent publication on a mouse model of unilateral ureter ligation, cells double positive for CD34 and collagen I were recruited to the kidney at least in part via CCR7.[110] These cells were mostly CCR7 positive and blocking CCL21/SLC by an antibody or deficiency of CCR7 reduced fibrosis and the infiltration of fibrocytes. In addition, the number of macrophages and the expression of CCL2/MCP-1 was reduced.[110] Therefore, chemokine-mediated recruitment of bone marrow–derived fibrocytes into injured tissue may represent a hitherto underestimated mechanism of organ fibrosis, including in the kidney. This rapidly developing field most likely will have considerable impact on our understanding of fibrosing diseases and their therapy. In this context the observation may be of interest that deficiency of the von Hippel-Lindau (VHL) gene in podocytes of mice leads to overexpression of hypoxia-inducible factor (HIF)-regulated vascular endothelial growth factor (VEGF) and the development of a crescentic GN. The proliferating cells in crescents were CXCR4 positive and a blocking antibody for CXCR4 prevented the crescentic GN.[111] If this relates to the proliferation of CXCR4-positive podocytes or potentially CXCR4-positive stem cells or even fibrocytes (see later) remains to be evaluated. In any case, fibrocytes constitute an interesting and hitherto probably underestimated cell population that potentially may become therapeutic targets during progressive renal fibrosis.

ROLE OF CHEMOKINES IN STEM CELL RECRUITMENT

Because in-depth discussion of stem cell recruitment is beyond the scope of this article, CXCL12/SDF-1 is considered to mobilize stem cells via CXCR4. CXCL8 has been reported to be expressed in the normal mouse kidney.[112] CXCL12/SDF-1 expression increased after ischemia-reperfusion in the kidney, but decreased

in the bone marrow. This caused mobilization of CD34-CXCR4 double-positive cells into the circulation and their homing to the kidney. In vitro and in vivo chemotaxis of bone marrow cells toward damaged kidney epithelium was inhibited reversibly by anti-CXCR4 antibodies.[112]

ROLE OF CHEMOKINES FOR THE RECRUITMENT OF LEUKOCYTES TO DIFFERENT RENAL COMPARTMENTS

Evidence is increasing that renal compartments (ie, glomerular and tubulointerstitial capillaries) differ in the recruitment of inflammatory cells. For example, PMNs and Mo/Mac can be recruited to both the glomerular tufts and the tubulointerstitial compartment. By contrast, T and B cells almost exclusively are recruited to the tubulointerstitium even in glomerulonephritis. Especially T cells frequently conglomerate around Bowman's capsules. This differential distribution of leukocyte subsets is reflected by the respective expression of chemokine receptors. For example, CCR5 and CXCR3 are expressed mainly by T cells in the tubulointerstitium.[113,114] In contrast, CXCR1-positive PMNs and CCR2-positive Mo/Mac are found in glomerular tufts and in the tubulointerstitium,[40,115] However, macrophages in glomeruli and the tubulointerstitium may be in a different state of activation or maturation because they differ in the expression of markers. For example, in mice interstitial but not glomerular macrophages express the F4/80 antigen (see also the discussion on DCs previously).[116] The differential recruitment of the various leukocyte populations to the glomerular and tubulointerstitial compartment could involve differences in shear stress, chemokine expression, chemokine presentation on the endothelium, and expression of adhesion molecules. We briefly discuss the role of chemokines here.

Although all intrinsic renal cells can be stimulated to release chemokines in vitro, a compartment-specific expression of chemokines in vivo has been described in 2 studies.[117,118] In a rat model of renal microvascular endothelial injury, the expression of the chemokine CXCL10/IP-10 was restricted to the tubulointerstitium, although the glomerular capillaries also showed severe injury. The tubulointerstitial expression of CXCL10/IP-10 correlated with T-cell accumulation in this compartment. CCL2/MCP-1 was expressed both in glomeruli and in the tubulointerstitium, consistent with macrophage recruitment to both compartments.[118] In Goldblatt hypertensive rats the CXCR3 ligands also were expressed predominantly in the tubulointerstitium.[117]

Chemokines are thought to function in vivo when presented by GAG structures on cell surfaces or extracellular matrix.[119,120] The presentation of chemokines on GAGs facilitates adhesion and migration under flow and potentially also by haptotaxis (ie, migration along extracellular matrix–bound chemokines) in tissues. Heparin sulphated GAGs may be different in glomeruli and on endothelial cells of peritubular capillaries. By using an in situ binding assay we recently showed that binding of CCL5/RANTES protein to normal renal tissue is restricted mainly to the tubulointerstitium, at the exclusion of glomerular structures. However, CCL5/RANTES binding to the glomerular tuft was found in renal allograft biopsy specimens with acute transplant glomerulitis. Acute glomerulitis in transplants, in contrast to other renal diseases, is characterized by a high number of T cells in glomeruli. Therefore, these data indicate that differences in chemokine binding structures between renal compartments might be important for compartmentalization of different leukocyte subsets infiltrating diseased kidneys.[121]

Nonsignaling chemokine receptors such as DARC and D6 also might be involved in the compartment-specific presentation of chemokines. The chemokine binding protein DARC binds various chemokines including CXCL8/IL-8 and RANTES/CCL5.[122] DARC is expressed on peritubular capillaries, the site of leukocyte recruitment to the tubulointerstitium, but not on glomerular capillaries.[123,124] During renal inflammation including crescentic glomerulonephritis, and allograft rejection (interstitial, vascular, and humoral), the number of interstitial DARC-positive vessels increases in association with recruitment of CCR5-positive T cells.[123,124] The role of an increased number of DARC-positive peritubular capillaries currently is un-

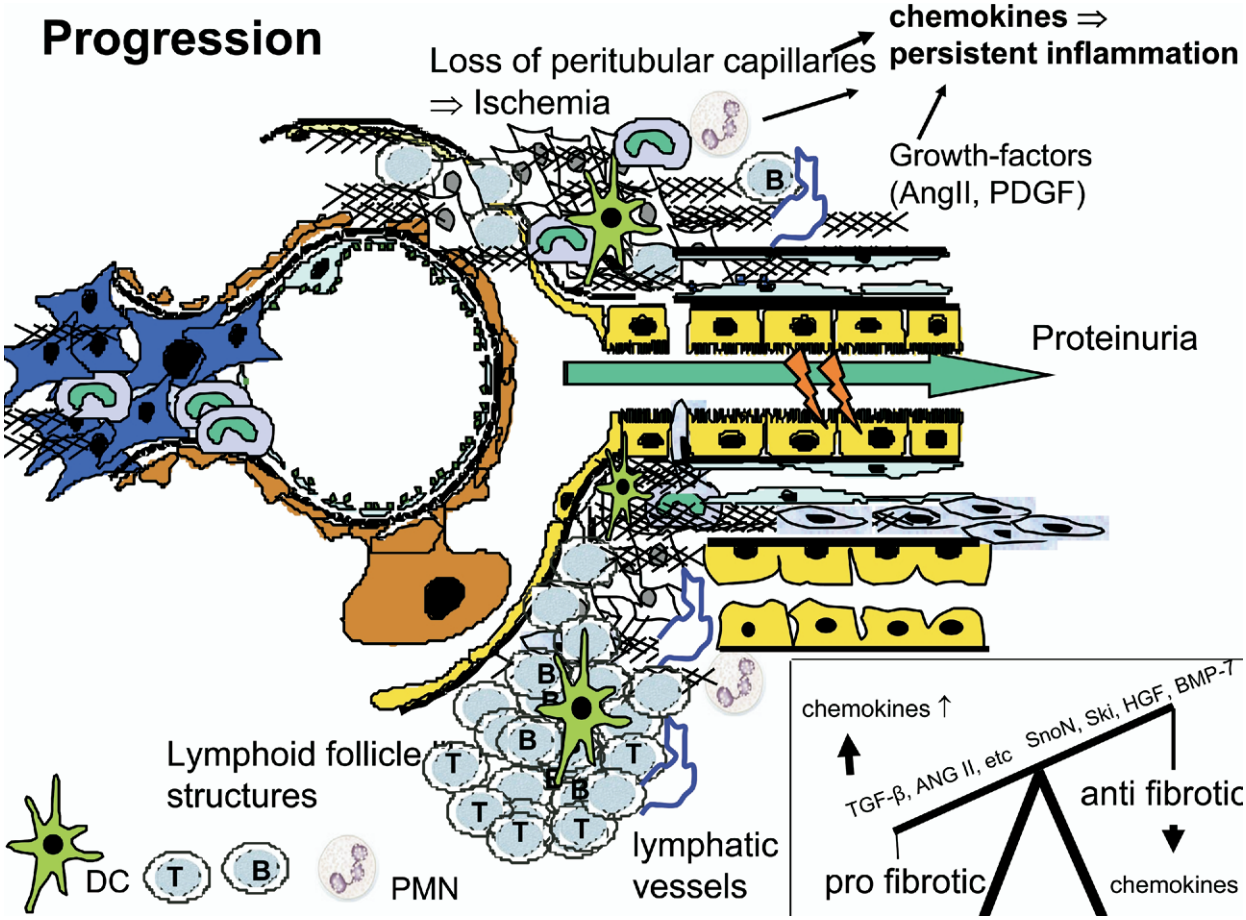

Figure 1. Progression phase in chronic kidney diseases. A proinflammatory milieu results in an ongoing recruitment of leukocytes. The interstitial infiltrate contains CXCR1-positive PMN, CCR2-positive macrophages, and CXCR3- and CCR5-positive T cells. B cells form lymphoid-like structures, including Mac/DCs, T cells, and lymphatic vessels. Activation of tubular epithelial cells by proteinuria or ischemia, release of growth factors inducing further chemokine release, and secretion of chemokines by infiltrating cells all propagate ongoing interstitial inflammation.

known, but the restricted expression in the tubulointerstitium and the positive association with CCR5-positive T cells in the corresponding compartment suggests a role for transcytosis/presentation by DARC on specific endothelial cells.[22]

OUTLOOK

Chronic kidney diseases can result in a smoldering interstitial inflammatory response, contributing to progression of diseases (Fig. 1). Here we have summarized data on the chemokine system pertaining to the infiltration and activity of several leukocyte types (ie, PMNs, Mo/Mac, DCs, B cells and T cells including Tregs, and bone marrow–derived fibrocytes and stem cells).

In the human kidney recruitment of CXCR1-positive PMNs to glomeruli and the tubulointerstitium has been shown in chronic diseases, particularly MPGN, lupus, and crescentic GN. These cells might be recruited via signals of macrophages and might interact with DCs as danger sensors. At corresponding sites Mo/Mac have been shown to express CCR2 and CX3CR1. The expression of CX3CR1 on DCs in other organs and in the mouse kidney has made this receptor a very interesting target for future studies. Little is known about B cells infiltrating the kidney, but B cells account for a significant part of the renal interstitial infiltrates. B cells together with T cells and Mac/DCs can form lymphoid-like follicles in chronic interstitial disease, including renal allografts. CXCR5 and the ligand CXCL13/BLC may recruit B cells to these nodular infiltrates. Interstitial T cells express

the chemokine receptors CXCR3 and CCR5, and correlate with renal function at the time of biopsy. It is currently unclear which percentage of T cells bare Treg markers, and might actually be of positive impact for the resolution of the disease.

It is likely that the chronic inflammatory response is mediated through amplification loops of the system. Involved in the permanent inflammatory process might be proteinuria, causing chemokine induction in tubular epithelial cells or activation of DCs, and ischemia secondary to rarefaction of the microvasculature with generation of proinflammatory cytokines resulting in further chemokine expression. In favor of a contributing role of the chemokine system in these processes are the observations that most of the factors that reduce or even reverse interstitial fibrosis (eg, bone morphogenetic protein [BMP]-7, hepatocyte growth factor [HGF]) also reduce the expression of chemokines and interstitial inflammatory infiltrates.

Finally, novel roles for chemokines still are emerging. For example, CCL2/MCP-1 and CCR2 can be involved in the recruitment of monocytes into the peripheral circulation from the bone marrow during infection.[31] Furthermore, CCR5-deficient mice show an increased humoral immune response in transplant models,[125] and CXCR3 and CCR7 may be involved in the recruitment of fibrocytes and regulatory T cells.[110,126] Therefore, the picture has become increasingly complex, with both proinflammatory, anti-inflammatory, and antifibrotic roles evolving for different chemokines. These new aspects of chemokine biology will have to be considered when interpreting studies with genetic or pharmacologic manipulation of chemokines.

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