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Questions about Chemokine and Chemokine Receptor Antagonism in Renal Inflammation

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Abstract
Chemokines remain attractive therapeutic targets for modulating inflammatory diseases in all areas of medicine including acute and chronic kidney disease. Industry has launched huge programs for the development of chemokine antagonists, and clinical trials with chemokine and chemokine receptor antagonists are ongoing. However, chemokine biology remains an area of unexpected discoveries. Here we discuss a number of questions which need to be addressed to further explore the potential of chemokine antagonism in renal inflammation: Why does renal expression of chemokines and chemokine receptors not always correlate with their functional significance? Why does chemokine antagonism only partially reduce renal leukocyte counts? Will antagonist combinations be more effective in reducing renal inflammation? What are the functional roles of homeostatic chemokines and atypical, nonsignaling chemokine receptors in renal inflammation? And finally, what classes of chemokine antagonists are available to address these questions experimentally?

Introduction

Almost two decades ago, it was found that a group of chemotactic cytokines specifically triggers the migration of leukocytes [1]. The expanding numbers of chemokines and their complex interactions with chemokine receptors (both referred to as chemokine mediators in this review) set off broad research activities in all areas of medicine. Meanwhile, industry has taken over to develop chemokine antagonism as a novel therapeutic concept for inflammatory diseases. While clinical trials with chemokine and chemokine receptor antagonists are ongoing [2], research in the field of chemokine biology remains an area of unexpected discoveries. This review raises and discusses a number of open questions that may help to plan and interpret chemokine research in the context of renal inflammation.

Does Renal Expression of Chemokine Mediators Predict Functional Significance?

The rationale to block chemokines or chemokine receptors in renal inflammation is usually based on data demonstrating their expression in human renal biopsies

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or along the progression of experimental kidney disease in rodents. For example, the monocyte chemoattractant protein (MCP)-1/CCL2 has consistently been shown to be secreted by proximal tubular epithelial cells of proteinuric mice, rats and humans [3]. These observations correlated with chemokine receptor CCR2+ macrophage and T cell infiltrates in adjacent interstitial areas. As such, it was predicted that the known function of MCP-1/CCL2 to trigger the recruitment of CCR2+ macrophages and T cells in in vitro assays would translate to experimental renal inflammation, a prediction confirmed by numerous interventional studies in rodents [3, 4]. CCR5 is also present on monocytes and T cells and was shown to specifically mediate leukocyte spreading in flow chamber experiments. Thus, it was predicted that CCR5 would also contribute to the renal recruitment of CCR5+ macrophages and T cells. However, interstitial macrophage and T cell recruitment turned out to be independent of CCR5, at least in mice with renal interstitial fibrosis [5]. In addition, two CCR5 antagonists, AOP-RANTES and Met-RANTES, did not affect interstitial leukocyte recruitment in the same model (own unpubl. data). Moreover, lack of CCR5 rather increased interstitial leukocyte numbers and renal injury in murine crescentic glomerulonephritis via increased expression of CCL3 and CCL5 [6]. These two CC chemokines mediate the recruitment of T cells via CCR1, as such CCR1-deficient mice show altered macrophage and T cell recruitment into the tubulointerstitial compartment after unilateral ureteral obstruction [5] or in crescentic glomerulonephritis [7]. Hence, expression studies may suggest, but not always predict the functional role of chemokine mediators in experimental renal inflammation. A possible explanation for this phenomenon is that multiple chemokine receptors are present on the surface of infiltrating leukocytes and some of them may shuttle passively into the kidney without being functionally involved in the recruitment process. Still, they will be detectable by descriptional expression studies, e.g. transcriptome analysis or immunostaining. Whether a single molecule has a functional role for recruitment or not can only be answered by blocking the function of the factor of interest, e.g. by appropriate antagonists. In order to obtain reliable results, it is necessary that such compounds have sufficient species specificity, selectively inhibit the target molecule, and are administered at an appropriate dose based on compound-specific pharmacokinetics and pharmacodynamics in the species studied [8].

Why Does Chemokine Antagonism Only Partially Reduce Renal Leukocyte Counts?

Although considerable redundancy of single chemokine mediators was initially suspected, many interventional studies revealed that specific antagonists of single chemokine mediators can have significant effects on leukocyte recruitment to diseased tissues. Such data supported the concept that at least some mediators have predominant roles in certain disease entities. On the other hand, data from different groups using various methodological approaches documented that blocking a single chemokine mediator never entirely abrogated leukocyte recruitment. For example, MCP-1/CCL2 or CCR2 blockade both reduced glomerular or interstitial macrophage numbers by approximately 50%, no matter what dose or treatment duration was applied [3, 4]. Three potential explanations have been discussed: (1) redundancy of single chemokine mediators, (2) local leukocyte proliferation which affects tissue leukocyte numbers independent of influx of circulating cells, and (3) variable leukocyte phenotypes with variable expression patterns of chemokine receptors. It is now becoming clear that probably all three mechanisms limit the ability of chemokine antagonism to reduce tissue leukocyte numbers near to zero. Especially the evolving concept of macrophage heterogeneity and phenotypic flexibility in changing cytokine microenvironments offers fascinating perspectives either to explain the aforementioned phenomenon or to learn more about how to specifically modulate renal inflammation [9]. For example, classically activated macrophages of a proinflammatory phenotype express CCR2 on the surface, and alternatively activated macrophages with a wound-healing anti-inflammatory capacity lack CCR2 expression. In this regard, MCP-1/CCL2 and CCR2 blockade may selectively inhibit the influx of monocytes with a predominant proinflammatory phenotype, and may not affect those macrophage phenotypes with predominant immunoregulatory or anti-inflammatory functions. In addition, leukocytes with anti-inflammatory properties also recruit to the kidney, e.g. NKT cells [10] and regulatory T cells [11]. Blocking the recruitment of these cells may also explain unexpected outcomes of renal disease models in chemokine receptor knockout mice or mice treated with chemokine antagonists [6, 7, 10].

Will Antagonist Combinations More Effectively Reduce Renal Inflammation?

The aforementioned concept would suggest that strategies targeting two or more chemokines attracting identical leukocyte subsets may not necessarily elicit additive effects on leukocyte counts or tissue damage. The same would apply for chemokine receptors that are coexpressed by the same cell type and share redundant functions. Some of our own unpublished data seem to support this hypothesis. Simultaneous blockade of CCR2 and CCR5 was not superior to MCP-1/CCL2 inhibition in terms of reducing glomerular macrophage counts and glomerular scaring in a murine model of glomerulosclerosis. In contrast, combined chemokine antagonism may be more likely to have additive effects on tissue damage when different disease pathomechanisms are targeted. In view of the suspected role of regulatory and wound-healing macrophage phenotypes for the progression of chronic glomerulopathies and interstitial fibrosis, it will become very important to identify the chemokines that mediate recruitment of these macrophage subclasses into the kidney. Thus, antagonist combinations that simultaneously prevent the recruitment of proinflammatory as well as profibrotic leukocyte subsets have the potential for additive therapeutic effects. It may also be important to develop different strategies for acute or chronic renal inflammation as wound-healing leukocyte phenotypes may either support the resolution of renal inflammation, e.g. in the healing phase of acute tubular necrosis [12], or facilitate interstitial fibrosis in chronic kidney disease.

What Are the Roles of Homeostatic Chemokines in the Kidney?

Not much is known about the roles of the homeostatic chemokines in the kidney because these are thought to mainly orchestrate the migration to and the spatial distribution within lymphoid organs and bone marrow. For example, B cell-attracting chemokine-1/CXCL13 and its receptor CXCR5, physiologically orchestrating B cell homing in lymph nodes, is also associated with renal B cell infiltrates and clusters in lupus nephritis and renal vasculitis [13]. As another example, stromal cell-derived factor (SDF)-1/CXCL12 mediates stem cell-homing in the bone marrow but also recruits mesenchymal progenitor cells to the postischemic kidney [14]. CXCR4 blockade can also prevent chronic lupus-like glomerulonephritis in MRLlpr mice, but the contribution of intrarenal SDF-1/CXCR12 expression in this model remains unclear [15]. We have recently discovered that glomerular SDF-1/CXCL12 expression derives from podocytes in db/db mice with type 2 diabetes and that SDF-1/CXCL12 antagonism can prevent diabetic glomerulosclerosis without affecting glomerular leukocyte numbers [16]. Such data indicate that as yet unknown functions of chemokines remain to be discovered and that these may reveal novel pathomechanisms and therapeutic targets in kidney disease. To further extend the aforementioned concept of chemokine co-blockade, it might be more effective to simultaneously target proinflammatory and homeostatic chemokines rather than different members of the same group. However, experimental data to support this hypothesis are not yet available in the public domain.

What Are the Roles of Atypical, Nonsignaling Chemokine Receptors?

Some 'atypical' chemokine receptors do not mediate intracellular calcium ion mobilization upon binding of their chemokine ligands, i.e. the Duffy antigen/receptor of chemokines (DARC), D6, the chemocentryx chemokine receptor (CCX-CKR), and CXCR7 [17]. The atypical receptors share alterations in the canonical DRYLAIF motif which mediates G-protein coupling in all other chemokine receptors. DARC binds several inflammatory CC and CXC chemokines, D6 binds to almost all proinflammatory and homeostatic CC chemokines, CCX-CKR binds the homeostatic chemokines ELC/CCL19, SLC/CCL21, and TECK/CCL25, and CXCR7 binds I-TAC/CXCL11 and SDF-1/CXCL12 [17, 18]. There is emerging evidence that atypical chemokine receptors regulate chemokine functions, e.g. by binding, internalization and intracellular degradation like other functional decoys and scavenger receptors. Dependent on their cell-, organ- and context-specific expression, the atypical receptors can negatively regulate chemokine signals, and hence, control bioavailable chemokine levels in a particular microenvironment to influence the recruitment of inflammatory cells to that site. Current experimental data suggest that D6, CCX-CKR, and CXCR7 act as scavengers for their respective ligands, with D6 mainly reducing local levels of inflammatory chemokines, and CCX-CKR regulating homeostatic chemokine concentrations [18]. DARC internalizes chemokines from the basolateral cell surface of vascular endothelial cells. Interestingly, this

Table 1. Chemokine receptor antagonists in clinical trials

Target	Company	Compound name	Trial phase	Indication	Comments
CCR1	Berlex Biosciences	BX-471 (ZK-811752)	II	MS, psoriasis, endometriosis	no longer reported
	Millennium/Aventis	MLN-3897	II	RA, MS	no efficacy in RA
	ChemoCentryx	CCX354	I	RA	ongoing
	Pharmacopeia	PS-031291	IIa	myeloma, RA	ongoing
	Pfizer	CP-481,715	II	RA	no efficacy
	Millennium	MLN-3701	I	RA, MS	ongoing
CCR2	Millennium Pharmaceuticals	MLN-1202 (antibody)	II	atherosclerosis, MS, RA	no efficacy for RA, others ongoing
	Incyte Pharmaceuticals	INCB8696	I	MS, SLE	ongoing
	ChemoCentryx	CCX140	I	MS, restenosis	ongoing
	Merck	MK-0812	II	RA, MS	no efficacy
	Pfizer	PF-4136309	II	pain	ongoing
CCR3	Cambridge AT/Vancouver Biotech	CAT-213 (antibody, bertilimumab)	II	ocular allergies, allergic conjunctivitis	ongoing
	Topigen	TPIASM8	II	asthma	ongoing
	GlaxoSmithKline	GW-766994	II	asthma, allergic rhinitis	no longer reported
	Bristol-Myers Squibb	DPC-168	I	asthma	no longer reported
CCR4	Amgen	KW-0761	I	oncology	ongoing
CCR5	Pfizer	Maraviroc (UK-427857)	approved	HIV	approved
			II	RA	ongoing
	Schering-Plough	Vicriviroc (Sch-417690)	II	HIV	ongoing
	GlaxoSmithKline	Aplaviroc	III	HIV	toxicity reported
	Incyte	INCB9471	II	HIV	ongoing
	Progenics	Pro 140	I	HIV	ongoing
	Schering-Plough	Sch-351125	I	COPD	ongoing
	Ono Pharm. GlaxoSmithKline	ONO-4128	II	HIV	terminated because of liver toxicity
CXCR1/2	Schering-Plough	SCH 527123	II	COPD	ongoing
	Dompe	Reparixin	II	reperfusion injury	ongoing
	GlaxoSmithKline	SB-656933	I	COPD, cystic fibrosis	ongoing
CXCR3	Amgen/Tularik	T-487/AMG-487	II	psoriasis	no efficacy, trials halted
CXCR4	Genzyme/AnorMED	Plerixafor (AMD-3100)	approved	stem cell transplantation	approved
			III	multiple myeloma	ongoing
	AnorMED	AMD-070	II	HIV	ongoing
	Chemokine Therapeutics	CTCE-0214	II	stem cell transplantation	ongoing

Modified from Horuk [2]. MS = Multiple sclerosis; RA = rheumatoid arthritis; COPD = chronic obstructive pulmonary disease; SLE = systemic lupus erythematosus.

does not lead to degradation, but DARC mediates chemokine transcytosis, leading to increased apical retention of intact chemokines, increased presentation of these chemokines to leukocytes and enhanced leukocyte migration across monolayers [19]. Together with the known expression of DARC on high endothelial venules of lymph nodes and postcapillary venules, i.e. sites of leukocyte extravasation, these data suggest that DARC internalizes and transports tissue-derived inflammatory chemokines onto the luminal endothelial cell surface where they enhance local leukocyte recruitment. In inflamed kidneys, expression of DARC is induced in interstitial endothelial cells. Consistently, DARC deficiency ameliorated disease in two neutrophil-dependent models of acute renal injury [20]. However, in renal disease models with a predominant infiltration of renal macrophages and T cells, DARC deficiency did not improve renal inflammation. In contrast, renal leukocyte infiltrates were increased in DARC-deficient mice at early time points, accelerating renal injury without affecting outcomes in the later disease phase [21]. These results suggest that DARC expressed on interstitial endothelial cells contributes to renal neutrophil recruitment, but its role is redundant for the infiltration of macrophages and T cells. Nevertheless,

specific antagonism of DARC on endothelial cells (e.g. by antisense approaches) emerges as a potential therapeutic strategy especially in acute renal inflammation. In contrast, soluble DARC-IgG fusion proteins may have a potential therapeutic effect similar to the suggested sink function of erythrocyte-expressed DARC. To date, it is unknown whether the other atypical chemokine receptors D6, CCX-CKR, and CXCR7 are directly involved in downregulating inflammatory responses in acute or chronic renal injury.

What Classes of Chemokine Antagonists Are Available?

In the early days of chemokine research, antagonists were generated by different technical approaches like neutralizing antibodies to chemokines and chemokine receptors as well as truncated, mutated or modified chemokine proteins. Some chemokine receptor antibodies or modified chemokines were found to elicit partial agonistic effects which compromised the interpretation of the data. Although most companies favored the development of small molecule receptor antagonists, neutralizing antibodies are among those compounds currently tested in clinical trials (table 1). Other technologies like small peptide-based receptor inhibitors or RNAse-resistant RNA aptamers are still in preclinical development. Meanwhile, chemokine receptor antagonists blocking CCR5 or CXCR4 have been approved for therapy in human HIV infection and stem cell transplantation, respectively (table 1). Their lack of efficacy, e.g. in multiple sclerosis or rheumatoid arthritis studies, has dampened the initial enthusiasm regarding their therapeutic potential in chronic inflammatory disease [2]. This may be a result of oversimplifying concepts on chemokine biology in the early days. Thus, more research is required to identify appropriate antagonistic strategies to control inflammation and tissue remodeling in clearly defined disease contexts. The availability of specific antagonists should help experimental nephrologists, provided that the compounds are suitable for the model species from a pharmacodynamic point of view.

Summary and Perspective

The role of chemokine mediators in experimental kidney disease remains an attractive research field holding the potential for unexpected discoveries in the future. We need to identify how specific leukocyte subpopulations like immunoregulatory macrophages, B cells, Th17 cells, regulatory T cells, and the different types of renal progenitor cells recruit to the different renal compartments during the different phases of acute and chronic nephropathies. In addition, we need to learn more about the roles of the homoeostatic chemokines and the atypical chemokine receptors in inflammatory renal disease to identify new therapeutic strategies based on these molecules.

References

1 Rossi D, Zlotnik A: The biology of chemokines and their receptors. Annu Rev Immunol 2000;18:217–242.
2 Horuk R: Chemokine receptor antagonists: overcoming developmental hurdles. Nat Rev Drug Discov 2009;8:23–33.
3 Tesch GH: Mcp-1/ccl2: A new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. Am J Physiol Renal Physiol 2008;294:F697–F701.
4 Vielhauer V, Eis V, Schlondorff D, Anders HJ: Identifying chemokines as therapeutic targets in renal disease: lessons from antagonist studies and knockout mice. Kidney Blood Press Res 2004;27:226–238.
5 Eis V, Luckow B, Vielhauer V, Siveke JT, Linde Y, Segerer S, Perez De Lema G, Cohen CD, Kretzler M, Mack M, Horuk R, Murphy PM, Gao JL, Hudkins KL, Alpers CE, Grone HJ, Schlondorff D, Anders HJ: Chemokine receptor CCR1 but not CCR5 mediates leukocyte recruitment and subsequent renal fibrosis after unilateral ureteral obstruction. J Am Soc Nephrol 2004;15:337–347.
6 Turner JE, Paust HJ, Steinmetz OM, Peters A, Meyer-Schwesinger C, Heymann F, Helmchen U, Fehr S, Horuk R, Wenzel U, Kurts C, Mittrucker HW, Stahl RA, Panzer U: CCR5 deficiency aggravates crescentic glomerulonephritis in mice. J Immunol 2008;181:6546–6556.
7 Topham PS, Csizmadia V, Soler D, Hines D, Gerard CJ, Salant DJ, Hancock WW: Lack of chemokine receptor CCR1 enhances Th1 responses and glomerular injury during nephrotoxic nephritis. J Clin Invest 1999;104:1549–1557.
8 Anders HJ, Vielhauer V: Identifying and validating novel targets with in vivo disease models: guidelines for study design. Drug Discov Today 2007;12:446–451.
9 Mosser DM, Edwards JP: Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008;8:958–969.
10 Yang SH, Kim SJ, Kim N, Oh JE, Lee JG, Chung NH, Kim S, Kim YS: Nkt cells inhibit the development of experimental crescentic glomerulonephritis. J Am Soc Nephrol 2008;19:1663–1671.

11 Wolf D, Hochegger K, Wolf AM, Rumpold HF, Gastl G, Tilg H, Mayer G, Gunsilius E, Rosenkranz AR: CD4+CD25+ regulatory t cells inhibit experimental anti-glomerular basement membrane glomerulonephritis in mice. J Am Soc Nephrol 2005;16:1360–1370.
12 Lin SL, Nowlin BT, Duffield JS: Bone marrow ly6chi monocytes are recruited to injured kidney and differentiate into ly6clo profibrotic macrophages (abstract). J Am Soc Nephrol 2009;19:SA-FC376.
13 Steinmetz OM, Velden J, Kneissler U, Marx M, Klein A, Helmchen U, Stahl RA, Panzer U: Analysis and classification of B-cell infiltrates in lupus and anca-associated nephritis. Kidney Int 2008;74:448–457.
14 Togel F, Isaac J, Hu Z, Weiss K, Westenfelder C: Renal SDF-1 signals mobilization and homing of CXCR4-positive cells to the kidney after ischemic injury. Kidney Int 2005; 67:1772–1784.
15 Wang A, Fairhurst AM, Tus K, Subramanian S, Liu Y, Lin F, Igarashi P, Zhou XJ, Batteux F, Wong D, Wakeland EK, Mohan C: CXCR4/CXCL12 hyperexpression plays a pivotal role in the pathogenesis of lupus. J Immunol 2009;182:4448–4458.
16 Sayyed S, Hägele H, Kulkarni OP, Endlich K, Segerer S, Eulberg D, Klussmann S, Anders HJ: Podocytes express the homeostatic chemokine SDF-1/CXCL12 which contributes to glomerulosclerosis, podocyte loss, and albuminuria in type 2 diabetes. Diabetologia 2009;52:2445–2454.
17 Graham GJ: D6 and the atypical chemokine receptor family: novel regulators of immune and inflammatory processes. Eur J Immunol 2009;39:342–351.
18 Mantovani A, Bonecchi R, Locati M: Tuning inflammation and immunity by chemokine sequestration: decoys and more. Nat Rev Immunol 2006;6:907–918.
19 Pruenster M, Mudde L, Bombosi P, Dimitrova S, Zsak M, Middleton J, Richmond A, Graham GJ, Segerer S, Nibbs RJ, Rot A: The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. Nat Immunol 2009; 10:101–108.
20 Zarbock A, Schmolke M, Bockhorn SG, Scharte M, Buschmann K, Ley K, Singbartl K: The Duffy antigen receptor for chemokines in acute renal failure: a facilitator of renal chemokine presentation. Crit Care Med 2007;35:2156–2163.
21 Vielhauer V, Allam R, Lindenmeyer MT, Cohen CD, Draganovici D, Mandelbaum J, Eltrich N, Nelson PJ, Anders HJ, Pruenster M, Rot A, Schlondorff D, Segerer S: Efficient renal recruitment of macrophages and T cells in mice lacking the Duffy antigen/receptor for chemokines. Am J Pathol 2009;175:119–131.