The CX_3C-Chemokine Fractalkine in Kidney Diseases

M.J. Koziolek*, R. Vasko, C. Bramlage, G.A. Müller and F. Strutz

Department of Nephrology and Rheumatology, Georg-August-University Goettingen, 37075 Goettingen, Germany

Abstract: The chemokine CX_3C-L/FKN is expressed in both soluble and transmembrane/mucin hybrid forms, thus combining chemoattractant functions together with receptor/adhesion molecule properties. In contrast to other chemokine receptors, CX_3C-R is expressed not only on lymphoid cell populations, but also on several intrinsic cells including tubular epithelial cells and renal fibroblasts where it regulates various aspects of cell viability, matrix synthesis and degradation, migration, inflammation as well as oxidative stress.

In the kidney, the chemokines/receptor pair has been shown to play a role in nephrogenesis as well as in the pathogenesis primary and secondary nephropathies. In several animal models and human specimens with acute and chronic renal failure including allograft nephropathy, CX_3C-L/CX_3C-R has been shown to exert immune and non-immune mediated renal damages. A blockade of this chemokine system ameliorated acute and chronic renal damages, though the latter to a more robust extent.

There seems to a role of the CX_3C-L/CX_3C-R pair in mediating acute renal inflammation as well as in progressive chronic renal failure. However, functional studies are lacking for many aspects and further studies are necessary to better define the functional properties of CX_3C-L/FKN and its receptor.

Key Words: Acute renal failure, chemotaxis, chronic renal failure, Fractalkine, inflammation, nephrogenesis, renal transplantation, tubulointerstitial fibrosis.

INTRODUCTION

Chemokines comprise a large superfamily of low-molecular-weight proteins with chemotactic properties and a highly homologous three-dimensional structure. So far, about 50 chemokines have been described. They are produced by a wide variety of cells, including professional immune and tissue cells [1]. Based on the conserved cysteine motifs, chemokines are classified into C, CC, CXC, and CX_3C chemokines [2]. These cysteines can be separated by one or three additional amino acids (designated as X). Chemokines can be further classified according to function and regulation of expression as inflammatory or homeostatic. The inflammatory chemokines are up-regulated by pro-inflammatory stimuli and orchestrate innate and adaptive immune responses, such as regulation of T-cell differentiation. The homeostatic group of chemokines is constitutively expressed in certain tissues and may be responsible for basal leukocyte trafficking and formation of the architecture of lymphoid organs during immune surveillance [3]. Table **1** gives an overview over the chemokine system.

The biological activity of chemokines has been shown to be critically influenced by their association with glycosaminoglycans, tethered to proteoglycans on cell surfaces and in the extracellular matrix. The glycosaminoglycan interaction is thought to be responsible for the establishment of chemokine gradients over endothelial cells under vascular flow conditions [4]. With the exception of CXC-L16 and CX_3C-L/FKN, which are integral membrane proteins, all chemokines are secreted basic proteins, which bind to negatively charged glycosaminoglycans [5]. Chemokines are known to induce leukocyte migration, growth, and activation through seven transmembrane domain G protein-coupled cell-surface receptors on target cells. Chemokines exert their chemotactic functions by binding to chemokine receptors, coupled to a heterotrimeric G protein [5]. These receptors are also classified into C, CC, CXC, and CX_3C chemokine receptors [2]. However, there is a broad cross-reaction between several chemokines and chemokine receptors. Chemokine receptors can signal through different Gα-protein families, leading to distinct transduction pathways and biological effects [5]. Table **2** gives an overview over the expression of chemokine receptors in lymphoid cells and their binding ligands.

In the past, there have been several reports on the role of chemokines and their receptors in progression and resolution of renal disease, reviewed very nicely by Anders *et al.* in 2003 [3]. They have shown a broad interaction between glomerular, tubulointerstitial and infiltrating cells, involving chemokines in several phases of progression and resolution of kidney disease. Effective tissue repair is dependent on a well-orchestrated cellular response and on timely induction and suppression of chemokines in a locally restricted manner [6]. However, recent evidence suggests that chemokine signaling mediates actions beyond leukocyte chemotaxis and activation, regulating instead different processes such as angiogenesis and fibrous tissue deposition. Here we will focus on the role of CX_3C-L/CX_3C-R in kidney diseases, where growing data suggest possible roles of this chemokine/

*Address correspondence to this author at the Department of Nephrology and Rheumatology; Georg-August-University Goettingen; Robert-Koch-Str. 40; D-37075 Goettingen; Germany; Tel.: +49 551 39 6331; FAX: +49 551 39 8906; E-mail: mkoziolek@med.uni-goettingen.de

1389-5575/09 $55.00+.00

Table 1. Overview Over the Chemokine System (Modified According to [6a])

Systematic Name	Common Name	Receptor	Homeostatic (H) Inflammatory (I)	Remarks
C Chemokines				
XCL1	Lymphotactin, SCM-1α, ATAC	XCR1	H, I	
XCL2	Lymphotactin β, SCM-1β	XCR2	H, I	
CC Chemokines				
CCL1	I-309, TCA3, P500, SISe	CCR8	I	
CCL2	MCP-1, MCAF, SMC-CF, GDCF-2, HC11, TDCF	CCR2	I	
CCL3	MIP-1a, LD78a, AT464.1, G0S19-1, SCI,TY-5, L2G25B, MIP-1aS, SISa	CCR1, CCR5	I	
CCL3L1	LD78b, AT464.2, G0S19-2, MIP-1aP, SISb	CCR1, CCR5	I	
CCL3L2	LD78g, G0S19-3		I	
CCL3L3	CCL3L3		I	
CCL4	MIP-1b, AT744.1, Act-2, G-26, HC21, H400SISg, MAD-5	CCR5, CCR8	I	
CCL5	RANTES, SISd	CCR1, CCR3, CCR5	I	
(CCL6)	C10, MRP-1	unknown	unknown	mouse only?
CCL7	MCP-3, NC28, FIC, MARC	CCR1, CCR2, CCR3	I	
CCL8	MCP-2, HC14	CCR3, CCR5	I	
(CCL9/CCL10)	MRP-2, CCF18, MIP-1γ	CCR1	unknown	mouse only?
CCL11	Eotaxin	CCR3	I	
(CCL12)	MCP-5	CCR2	unknown	mouse only?
CCL13	MCP-4, NCC-1, Ckβ10	CCR2, CCR3	I	
CCL14	HCC-1, MCIF, Ckβ1, NCC-2, CCL	CCR1, CCR5	H	
CCL15	HCC-2, NCC-3, MIP-5, Lkn-1, MIP-1d, HMRP-2B	CCR1, CCR3	H	
CCL16	HCC-4, NCC-4, LEC, LMC, LCC-1, CKb12, Mtn-1	CCR1, CCR2	H	
CCL17	TARC, ABCD-2	CCR4	I, H	
CCL18	PARC, DC-CK1, MIP-4, AMAC-1, CKb7, DCCK1	unknown	H	
CCL19	MIP-3b, ELC, exodus-3, CKb11, Scya19-ps1, Scya19-ps2, Scya19-ps3	CCR7	H	
CCL20	MIP-3a, LARC, exodus-1, ST38, CKb4	CCR6	I, H	
CCL21	SLC, 6Ckine, exodus-2, TCA4, 6Ckine-ser (Scya21a), 6Ckine-leu (Scya21b), CKb9, 6CKBAC1, 6CKBAC2	CCR7	I, H	
CCL22	MDC, STCP-1, ABCD-1, DC/B-CK	CCR4	I, H	
CCL23	MPIF, MIP-3, MPIF-1, CKb8, CKb8-1	CCR1	I	
CCL24	eotaxin-2, MPIF-2, CKb6	CCR3	I	
CCL25	TECK, Ckb15	CCR9	H	
CCL26	eotaxin-3, IMAC, MIP-4a, TSC-1	CCR3	I	
CCL27	CTAK, ALP, skinkine, ILC, ESkine, PESKY	CCR10	H	
CCL28	MEC, CCK1	CCR3, CCR10	I, H	

(Table 1. Contd….)

Systematic Name	Common Name	Receptor	Homeostatic (H) Inflammatory (I)	Remarks
CXC Chemokines				
CXCL1	GROα, GRO1, MGSA-a, NAP-3	CXCR2>CXCR1	I	
CXCL2	GROβ, GRO2, MIP-2a, MGSA-b	CXCR2	I	
CXCL3	GROγ, GRO3, MIP-2b	CXCR2	I	
CXCL4	PF4	unknown	unknown	
CXCL5	ENA-78	CXCR2	I	
CXCL6	GCP-2, CKA-3	CXCR1, CXCR2	I	
CXCL7	NAP-2, CTAPIII, β-Ta, PEP, MDGF, LDGF, THBGB1, RTCK-1	CXCR2	I	
CXCL8	IL-8, MDNCF, NAP-1, 3-10C, MONAP, LUCT, AMCF-I, LYNAP, NAF, b-ENAP, GCP-1	CXCR1, CXCR2	I	
CXCL9	MIG, CRG-10,	CXCR3	I	
CXCL10	IP-10, CRG-2, mob-1, C7, gIP-10, IFI10	CXCR3	I	
CXCL11	I-TAC, β-R1, IP-9, H174	CXCR3	I	
CXCL12	SDF-1α, SDF-1β, SDF-1g, PBSF, TLSF-a, TLSF-b, TPAR1	CXCR4	H	
CXCL13	BCA-1, BLC, BLR1L, Angie, ANGIE2	CXCR5	H	
CXCL14	BRAK, NJAC, bolekine, Kec, MIP-2g, BMAC, KS1	unknown	I, H	
(CXCL15)	lungkine, CINC-2b-like, weche	unknown	unknown	
CXCL16	SRPSOX	CXCR6	I	
CXCL17	DMC, VCC-1, BC024561		unknown	
CX_3C Chemokine				
CX_3CL1	Fractalkine, Neurotactin, ABCD-3	CX_3CR	I, H	

Table 2. Overview Over the Expression of Chemokine Receptors in Lymphoid Cells and their Binding Ligands (Modified According to [6a])

Receptor	High Affinity Ligand	Functional Expression on Immune Cells
CXCR1	CXCL2,3,5,6,7,8	polymorphonuclear leukocytes , mast cells, monocytes, macrophages
CXCR2	CXCL1,2,3,5,6,7,8	polymorphonuclear leukocytes , mast cells, monocytes, macrophages
CXCR3	CXCL9,10,11	T-cells (Th1>Th2), B-cells, NK
CXCR4	CXCL12	most progenitor cells, T-cells, B-cells, polymorphonuclear leukocytes , monocytes, macrophages, dendritic cells
CXCR5	CXCL13	B-cells, memory T-cells
CXCR6	CXC16	memory T-cells
CCR1	CCL3,5,7,14,15,16,23	memory T-cells, monocyte
CCR2	CCL2,7,12,13	monocytes, DC, NK, T-cells, basophils, polymorphonuclear leukocytes
CCR3	CCL5,7,8,13,15,24,26	eosinophils, basophils, mast cells, T-cells (Th2>Th1)
CCR4	CCL17,22	T-cells (Th2>Th1)

(Table 2. Contd….)

Receptor	High Affinity Ligand	Functional Expression on Immune Cells
CCR5	CCL3,4,5	progenitors, Th1-cells, monocytes, macrophages, dendritic cells
CCR6	CCL20	memory T-cells, dendritic cells
CCR7	CCL19,21	T-cells, B-cells, dendritic cells
CCR8	CCL1,4	Th2 Cells
CCR9	CCL25	α4b7+ T-cells, dendritic cells , macrophages
CCR10	CCL27	CLA+ T-cells
CCR11	CCL2,8,13	
CX_3CR1	CX_3CL1	polymorphonuclear leukocytes , monocytes, NK, T-cells, dendritic cells
XCR1	XCL1,XCL2	T-cells

receptor system in resident cell migration, viability and activation of pro-inflammatory and –fibrotic pathways [7, 8].

CX_3C-CHEMOKINE FRACTALKINE (CX_3C-L/FKN) AND ITS RECEPTOR CX_3C-R

CX_3C-L/FKN is one of two chemokines (the other being CXC-L16) which are expressed in both soluble and transmembrane/mucin hybrid forms, thus combining chemoattractant functions together with receptor/adhesion molecule properties [4]. CX_3C-L/FKN was identified as the sole member with a unique CX_3C motif, which is clustered on chromosome 16q13 [9] and was first described in 1997 [10].

CX_3C-L/FKN is synthesized as a 50-75 kDa precursor that is rapidly processed, presumably glycosylated, yielding the mature 100 kDa species. Direct apical trafficking of CX_3C-L/FKN is not conferred by the protein's cytoplasmic domain, O-glycosylation of the protein, or associations with lipid rafts but is determined by N-glycosylation. Thereby, transmembrane CX_3C-L/FKN is in a dynamic exchange with the intracytoplasmatic pool [11], but when reaching the cell surface it is anchored into the membrane and serves as a potent adhesion molecule [12]. The exact anchoring mechanism is currently not clear. It is not achieved by direct tethering to the actin cytoskeleton or by association with lipid rafts [13]. In the human renal tubular epithelial lines HK-2 and MDCK cells, an intracellular pool of CX_3C-L/FKN in subapical vesicles and basolateral intracellular portions was described [13]. Moreover, intracellular localization has been described within a specialized juxtanuclear compartment which is distinct from late endosomes/lysosomes and Golgi cisternae but which partially colocalizes with transferrin receptor-associated recycling endosomes in human endothelial ECV-400 cells [11]. In primary porcine aortic endothelial cells and fibroblastic cells, transfected with tagged CX_3C-L/FKN, it was seen to accumulate in the juxtanuclear location in a time-dependent fashion, providing evidence of dynamic exchange between the membrane and the intracytoplasmatic compartment. The intracellular CX_3C-L/FKN compartment partially co-localizes with syntaxin-13 as well as with vesicle-associated membrane protein 3 (VAMP-3), but not with VAMP-2, suggesting that intracellular CX_3C-L/FKN represents a subcompartment of recycling endosomes and that recycling may occur by SNARE (soluble N-ethylmaleimide factor attachment protein receptor) - mediated fusion of endosomal and plasmalemmal membranes [11]. The mature form can be cleaved from the cell surface to yield a soluble 85 kDa fragment encompassing the majority of the ectodomain. Cleavage of CX_3C-L/FKN occurs constitutively or inducibly. Constitutive cleavage occurs at low levels and is mediated by the metalloprotease ADAM-10. Basal expression of CX_3C-L/FKN may be relevant for positioning and survival of tissue-homing leukocytes, whereas the cleavage of soluble form is discussed in the context of inflammatory processes [14]. The N-terminal end of CX_3C-L/FKN can also be cleaved by a protease called tumor necrosis factor alpha (TNFα) converting enzyme (TACE, also called ADAM-17) [11, 12].

In contrast to other chemokine receptors, CX_3C-R expression has not only been described on lymphoid cell populations, but also on several intrinsic cells. In regard to immune cells, CX_3C-R is mainly expressed on monocytes/ macrophages, NK-, T- and dendritic cells [8, 15-17]. CX_3C-R has also been found in endothelial, mesenchymal (including stem cells) and epithelial cells of different organs [8, 18-22]. In addition to chemotactic and adhesive functions for immune cells [15, 16, 23, 24], it has been shown to regulate aspects of cell viability [19, 21, 25], matrix synthesis and degradation [19, 26], migration [8, 27, 28], inflammation [19, 26, 27, 29] and oxidative stress [30] in non-lymphoid tissues.

REGULATION OF THE CX_3C-L/CX_3C-R PAIR

A broad series of different stimuli have been shown to induce CX_3C-L/FKN. They can roughly be divided into pro-inflammatory and pro-fibrotic cytokines as well as metabolites of oxidative stress. In addition, cytotoxic agents as well as some hormones seem to regulate CX_3C-L/FKN synthesis. Table **3** summarizes the known regulators of CX_3C-L/FKN. Only little is known about the regulation of CX_3C-R. The sparse data show that again certain pro-inflammatory and -fibrotic mediators, but also metabolites of oxidative stress

Table 3. Known Stimuli of CX_3C-L/FKN

Stimuli	Cell	Upregulation (↑), Downregulation (↓)	Reference
TNF-α	Vascular smooth muscle cells, mesangial cells, proximal tubular epithelial cells, Dermal/cardiac/lung microvascular endothelial cells, vascular smooth muscle cells, Nasal fibroblasts/intestinal epithelial cells	↑	[19, 33, 41, 64, 66-69]
IL-1β	Mesangial cells, Proximal and distal tubular epithelial cells, Vein endothelial cells, dermal/cardiac/lung/intestinal epithelial cells microvascular endothelial cells, chondrocytes	↑	[19, 33, 41, 70, 71]
IL-4	Macrophages, nasal fibroblasts	↑	[69, 72]
IL-13	macrophages	↑	[72]
IFNγ	Vein endothelial cells	↑	[70]
sIL-6R	Vein endothelial cells	↓	[70]
TGF-β	Proximal and distal tubular epithelial cells	↑	(Koziolek M *et al.*, in review)
PDGF	Mesangial cells	↑	[33]
bFGF	Mesangial cells	↑	[33]
CTGF	Mesangial cells	↑	[55, 73]
pentoxifylline	Vascular smooth muscle cells	↓	[66]
pyrrolidine dithiocarbamate	Vascular smooth muscle cells	↓	[66]
curcumin	Mesangial cells	↓	[33, 67]
MG132	Mesangial cells	↓	[33, 67]
Calphostin c	Mesangial cells	↓	[67]
PD 98059	Mesangial cells	↓	[67]
phorbol myristate acetate	Mesangial cells	↑	[67]
Pathogenic, non-complexed anti-DNA antibodies	Mesangial cells	↑	[35]
Hydrogen peroxide	Proximal and distal tubular epithelial cells	↑	(Koziolek M *et al.*, in review)
hypoxia	Vein endothelial cells	↓	[12]
Albumin	Proximal tubular epithelial cells	↑	[45]
Shiga Toxin 2	Vein endothelial cells	↑	[42]
lipopolysaccharides	Dermal/cardiac/lung microvascular endothelial cells/intestinal epithelial cells	↑	[19, 41]
15-deoxy-Δ-12,14-prostaglandin-J_2	Vein endothelial cells	↓	[74]
heparin	Vein endothelial cells	↓	[75]
thrombin	Vein endothelial cells	↑	[76]
endothelin	proximal tubular epithelial cells	↑	[77]
glucocorticoids	Lung epithelial cells	↓	[78]
Antimycin A	Microvascular endothelial cells	↑	[43]
cisplatin	Tubular epithelial cells	↑	[44]
Fluticasone	airway smooth muscle	↑	[79]
calcitriol	airway smooth muscle	↓	[79]
human chorionic gonadotropin	Ovarian granulosa cells	↑	[80]

upregulate CX_3C-R expression. Mediators, known to regulate CX_3C-R expression are listed in Table **4**.

EXPRESSION AND FUNCTION OF THE CX_3C-L/CX_3C-R PAIR IN RENAL DEVELOPMENT

Gröne *et al.* investigated the expression of CX_3C-L/FKN and CX_3C-R in seven human fetal renal tissue samples, representing the time period of weeks 14 to 22 of gestation. CX_3C-L/FKN expression was observed within the nephrogenic blastema and was accentuated around S-shaped structures. In developing glomeruli, the mesangium stained strongly positive. Furthermore, venous endothelia, medial smooth muscle cells, and arterial endothelial cells also exhibited CX_3C-L/FKN expression. In the medulla, peritubular capillaries and distal tubules stained positively for CX_3C-L/FKN, whereas proximal tubules did not. The medulla and collecting ducts demonstrated positive basolateral staining. The CX_3C-L/FKN expressing stromal cells were not positive for the monocyte/macrophage marker CD68 [31]. Cells within the stroma of medulla and cortex stained also positive for CX_3C-R. These cells were found in and around glomeruli and comma and S-shaped structures. The CX_3C-R-positive mononuclear cells were characterized by relatively large oval nuclei and varyingly broad granule-free cytoplasm. These cells could not be colabeled by a series of antibody reagents directed against monocytes/macrophage antigens (CD68, MAC387, MRP8, and MRP14), or the stem cell antigen (CD34), so that the cellular source of CX_3C-R-expression remained unknown. A very weak but distinct staining for CX_3C-R was also seen in the media of preglomerular vessels and the mesangium [31].

Segerer *et al.* investigated the expression of CX_3C-R in 12 fetal human kidneys ranging in age from 58 to 112 days by immunohistochemistry. They found CX_3C-R expressing cells in a small, scattered population between developing nephrons as well as in developing glomeruli. These cells did neither follow typical patterns of cells forming specific parts of the developing nephron nor demonstrate an association with the stage of differentiation or the developing nephron. The distribution was similar to the distribution of CD56 positive cells, but there was also CX_3C-R positivity within stromal cells without further specification [32].

The presence of CX_3C-L/FKN in developing kidneys is thought to stimulate the attraction and adhesion of CX_3C-R-bearing cells from the blood flow in developing glomeruli, which may indicate an involvement in "fine tuning" of nephrogenesis [31].

EXPRESSION, REGULATION AND FUNCTION OF THE CX_3C-L/CX_3C-R PAIR IN PRIMARY GLOMERULOPATHIES

So far, there exists no data on CX_3C-L/FKN expression, regulation and function in human primary glomerulopathies. Segerer *et al.* investigated the CX_3C-R expression in minimal change disease, as a non-inflammatory disease without significant tubulointerstitial infiltrates, as well as in membranous nephropathy, focal and segmental glomerulosclerosis and collapsing glomerulopathy as glomerular diseases that usually show no prominent glomerular inflammatory cell influx but may have variable amounts of tubulointerstitial infiltrates and fibrosis depending on the disease stage [32]. In renal biopsies with minimal change GN the number of CX_3C-R positive cells was low and the distribution similar to the small number of CD3-positive T cells and CD68-positive monocyte/macrophages within the interstitium. In membranous nephropathy, focal and segmental glomerulosclerosis and collapsing glomerulopathy, the number and distribution of CX_3C-R positive cells also correlated well with that of CD3-positive T cells and CD68-positive monocyte/macrophages. In biopsies with crescentic nephropathy or membranoproliferative GN a similar pattern of CX_3C-R positive cells compared to the distribution of CD68+ monocytes/ macrophages was described in glomeruli. Within the tubulointerstitium CX_3C-R was found in areas with T-cell and monocyte/macrophage infiltration.

Chen and co-workers investigated the expression of CX_3C-L/FKN in the nephritic anti-Thy1.1 rat model. Glomerular CX_3C-L/FKN was significantly up-regulated, peaking at 2 h and sustaining into day 5 of the nephritis. A corresponding increase in urinary CX_3C-L/FKN protein was evident after day 1 of the nephritis, but became more prominent during the proliferative phase of mesangial cells (days 3–5). Thereby, activated mesangial cells were identified as the major source for urinary CX_3C-L/FKN. Incubation of cultured mesangial cells with TNF-α, IL-1ß, PDGF-AB or bFGF

Table 4. Known Regulators of CX_3C-R

Ligand	Cell	Upregulation (↑), Downregulation (↓)	Reference
MCP-1	Monocytes	↑	[81]
Hydrogen peroxide	Renal fibroblasts	↑	[8]
RANK-L	Osteoclast precursor cells	↓	[82]
TNF-α	Vascular smooth muscle cells	↑	[64]
TGF-β	Microglia	↑	[83]
lipopolysaccharides	Microglia	↓	[84]

significantly up-regulated CX_3C-L/FKN mRNA and protein expression, whereas pre-incubation with the NF-κB inhibitors curcumin or MG132 attenuated the induction [33], suggesting a regulatory function of early pro-inflammatory, but also pro–fibrotic cytokines.

RENAL EXPRESSION, REGULATION AND FUNCTION OF THE CX_3C-L/CX_3C-R PAIR IN SYSTEMIC DISORDERS

Yoshimoto S *et al.* analysed the expression and distribution of CX_3C-L/FKN in 49 renal biopsies from patients with different stages of lupus nephritis (class I: minimal mesangial glomerulonephritis, class II: mesangial proliferative glomerulonephritis, class III: focal proliferative glomerulonephritis, class IV: diffuse proliferative glomerulonephritis, class V: membranous glomerulonephritis, class VI: glomerulosclerosis). The presence of CX_3C-L/FKN was found in the mesangial area and/or along the capillary wall within the glomerulus, but not in glomeruli with lupus nephritis classes I and V. Conversely, CX_3C-L/FKN was present in the mesangial area in class II glomeruli and both the mesangial area and along the capillary wall in class III and IV glomeruli. In serial sections they showed CX_3C-L/FKN expression in α-smooth muscle actin+ and/or CD31+ cells, suggesting that mesangial and/or endothelial cells express CX_3C-L/FKN in glomeruli of patients with lupus nephritis. Mean CX_3C-L/FKN levels in glomeruli were significantly greater in classes III and IV glomeruli than in the other classes in quantitative real-time PCR after laser microdissection. In addition, there was a strong correlation between the glomerular CX_3C-L/FKN expression index and histopathologic activity index, the number of CD16+ monocytes/macrophages or CD3+ T-cell count. In 10 patients with class IV lupus nephritis a second renal biopsy was investigated 6 months after glucocorticoid and/or methylprednisolone pulse therapy. The glomerular CX_3C-L/FKN expression index showed a tendency to decrease, albeit not significantly, in contrast to a significant improvement of the histopathologic activity index and a decrease in $CD16^+$ monocytes/macrophages infiltration [34]. Qing X *et al.* found in an experimental model an upregulation of CX_3C-L/FKN in primary mesangial cells, derived from lupus-prone MRL/*lpr* mice, treated with pathogenic, non-complexed anti-DNA antibodies. The glomerular expression increased over time with the development of glomerular antibody deposition and active nephritis in MRL/*lpr* mice, suggesting a direct role of pathogenetic antibodies in CX_3C-L/FKN regulation [35].

Twenty-three patients with crescentic glomerulonephritis were evaluated in the study of Furuichi *et al.*, which were related to ANCA positive vasculitis (n=16), IgA nephropathy (n=5), lupus nephritis (n=1) or cryoglobulinemia (n=1). Additional, specimens from second biopsies were obtained from 11 patients after glucocorticoid therapy. CX_3C-L/FKN cells were detected in the interstitial endothelium, while they were not detected in glomeruli. The staining was localized in capillary vessels. The number of CX_3C-L/FKN-positive cells in the interstitium of human crescentic glomerulonephritis did not correlate with the pathological findings (atrophy, cell infiltration, fibrosis), but correlated with the number of CD16-positive cells in the interstitium before glucocorticoid therapy. The number of CX_3C-L/FKN-positive cells in the interstitium decreased after glucocorticoid therapy. This suggested, therefore, that CX_3C-L/FKN may mediate NK cell infiltration into the interstitium *in vivo* [36]. Tam *et al.* investigated the urinary CX_3C-L/FKN content in patients with ANCA-associated vasculitis with renal involvement in comparison to healthy control subjects, but found no differences between the investigated groups [37]. In rats with antiglomerular basement membrane crescentic glomerulonephritis induction of CX_3C-L/FKN mRNA was prominent on days 3 and 5 after antibody application, persisted through day 7, and started to subside by day 9. Immunofluorescence staining of CX_3C-L/FKN in the nephritic kidney showed a nonlinear pattern typical of glomerular endothelium and was only detected in nephritic glomeruli, but not in normal control kidneys. The time-course of CX_3C-R mRNA expression paralleled that of its ligand. CX_3C-R was expressed in inflammatory leukocytes isolated from nephritic glomeruli, which were identified as CD3+ T cells and ED1+ macrophages. The chemotaxis response was inhibited by application of anti-CX_3C-R antibody with a reduction of CD3+ T-cells and ED1+ macrophages in the glomeruli. The anti-CX_3C-R-treated group had minimal pathological damage in the kidneys and crescentic formation was virtually abolished. On a functional basis, anti-CX_3C-R-treatment improved proteinuria and serum creatinine [38].

There are no data on CX_3C-L/CX_3C-R in diabetic nephropathy in human, but in experimental animal model. Kikuchi *et al.* investigated the expression of CX_3C-L/CX_3C-R pair in streptozotocin-induced diabetic kidneys with or without the treatment with an angiotensin-converting enzyme inhibitor (ACE-I) or aminoguanidine after 8 weeks. CX_3C-L/FKN expression in untreated diabetic rats significantly increased compared with that of control rats. Intense staining of CX_3C-L/FKN was detected on capillary endothelial cells in the glomeruli and those of peritubular capillaries partially in mesangial lesions. CX_3C-L/FKN expression in diabetic rats treated with an ACE-I or aminoguanidine was significantly suppressed compared with untreated diabetic rats. CX_3C-R expression markedly increased in untreated diabetic rats compared with control rats. CX_3C-R was detected at the capillary lumen in the glomeruli in diabetic rats. A few infiltrating cells in glomeruli were also CX_3C-R positive. Pretreatment with the ACE-I or aminoguanidine reduced expression compared with untreated diabetic rats [39]. In another study, the same study group identified high glucose levels, AGE-BSA and TNFα, but not angiotensin II or mannitol, as inductors of CX_3C-L/FKN expression in normal Sprague–Dawley rat glomeruli [40].

In septic rats CX_3C-L/FKN was strongly expressed on renal endothelial cells, particularly at site of neutrophil infiltration. However, inhibition in leukocyte migration using supernatants of TNF-α-stimulated microvascular endothelials cells as chemoattractant was only achieved by antibodies directed against CXCR1, CXCR2 and CCR2 but not against CX_3C-R, suggesting that the preference for inflammation kidney in septic patients is not related to the expression or regulation of CX_3C-L/FKN [41].

Zanchi C *et al.* demonstrated an upregulation of CX_3C-L/FKN in HUVEC´s as well as in mice glomeruli in response to Shiga Toxin 2 stimulus, suggesting a role of

CX_3C-L/ CX_3C-R pair in haemolytic uremic syndrome associated renal microvascular dysfunction [42].

EXPRESSION, REGULATION AND FUNCTION OF THE CX_3C-L/CX_3C-R PAIR IN TUBULOINTERSTITIAL INJURY

There exist no studies on CX_3C-L/CX_3C-R-system in acute renal failure (ARF) in human. Oh DJ *et al.* induced a reversible acute ischemic ARF in mice using renal artery clamping in the presence or absence of a prior specific anti-CX_3C-R antibody administration. 24 hours after reperfusion, samples were taken for analyses. CX_3C-L/FKN protein expression in the kidney increased markedly in ischemic ARF, predominantly in endothelial cells, whereas the other compartments were not described. In *in vitro* analyses they found a marked upregulation of CX_3C-L/FKN after stimulation of mouse microvascular endothelial cells with antimycin A, simulating a hypoxia chemically. Administration of the anti-CX_3C-R antibody (1 h before induction of ischemia) protected against ARF, as determined by renal function and histology. Renal function (as determined by serum creatinine) improved as did the acute tubular necrosis score in CX_3C-R antibody-treated ARF. Administration of the anti-CX_3C-R antibody in ischemic ARF had no effect on apoptosis of tubular cells or number of neutrophil infiltration, but significantly reduced the number of CD11b-positive macrophages in the kidney during ischemic ARF [43].

Lu LH *et al.* observed CX_3C-L/FKN expression in a mouse model of cisplatin-induced ARF one to three days after induction. The protein expression of CX_3C-L/FKN in whole kidney was significantly increased on days 1, 2, and 3 after cisplatin administration, which was predominantly expressed on endothelial cells. Again, there was no description of CX_3C-L/FKN expression in other renal compartments. This upregulation in endothelial cells was dedicated to a direct effect of cisplatin as ascertained by *in vitro* analysis. Administration of the anti-CX_3C-R antibody (1 h after administration of cisplatin) was not sufficient to prevent the rise in serum creatinine or BUN. CX_3C-$R^{-/-}$ mice were not protected from Cisplatin induced ARF as determined by serum creatinine and BUN [44].

Donadelli R *et al.* investigated the effects of proteinuria on tubular CX_3C-L/FKN expression *in vitro* and *in vivo*. Human proximal tubular cells were incubated with human serum albumin, which induced a dose-dependent increase in CX_3C-L/FKN mRNA (up to 2.8-fold) associated with increased levels of both membrane-bound and soluble forms of the protein. Pre-treatment with the unspecific NF-κB inhibitor pyrrolidine dithiocarbamate partially abrogated CX_3C-L/FKN induction by albumin as was the case with the use of the p38 MAPK inhibitor SB202190. These results were confirmed in mouse model with protein-overload proteinuria. Pre-treatment with an antibody against the CX_3C-L/FKN receptor CX_3C-R partially, but not significantly abolished the peritubular accumulation of F4/80 positive mononuclear cells. Renal function was ameliorated after treatment with the anti-CX_3C-R antibody [45].

Renal proximal tubular epithelial cells were treated with TNF-α and IFN-γ and supernatants were used as chemoattractants towards resting and activated peripheral blood T-lymphocytes. Blocking CX_3C-R reduced chemotaxis of these cells by only 29%, considerably less compared to blocking of CCR5 or CXCR3, suggesting that the majority of T-lymphocyte recruitment is not mediated by the CX_3C-L/ CX_3C-R system [46].

In ARF, probably discrepant data exist showing protective effects of blocking CX_3C-L/CX_3C-R-system in ischemia induced ARF on the hand but no effects in cisplatin toxicity.

EXPRESSION, REGULATION AND FUNCTION OF THE CX_3C-L/CX_3C-R PAIR IN KIDNEY TRANSPLANTATION

Durkan AM *et al.* investigated the expression and cellular distribution of CX_3C-L/FKN in human renal biopsies with acute rejection and acute tubular necrosis. They detected a CX_3C-L/FKN expression in renal tubules, particularly on the apical surface, and a modest expression within the glomeruli and on vascular endothelium. On functional basis, this apical CX_3C-L/FKN mediated an enhanced adhesion of leukocytes to CX_3C-L/FKN-expressing renal tubular epithelial cells [13].

Increased expression of the CX_3C-L/CX_3C-R-pair was observed in chronic allograft nephropathy compared to hyperacute rejection, acute rejection and normal kidneys in the interstitium and tubular epithelial cell basolateral membranes. CX_3C-L/FKN colocalized with the expression of vascular endothelial growth factor (VEGF) and CX_3C-R in the tubulointerstitium. Furthermore, the distribution of CX_3C-R and VEGF corresponded to the distribution of both T cells and monocytes/macrophages. These expression patterns were thought that CX_3C-L/FKN is a strong candidate for directing mononuclear cell infiltration in human renal chronic allograft rejection which may further stimulate myofibroblast proliferation and thus interstitial fibrosis [47]. Pietryk MC *et al.* investigated CX_3C-L/FKN by quantitative real-time PCR in acute renal allograft rejection in the compartiments glomeruli, tubules and vessels after microdissection. They found a significant increase in tubules, glomeruli and vascular parts compared to controls [48]. Peng and coworkers found that the levels of urinary CX_3C-L/FKN of patients with acute tubular necrosis and chronic allograft nephropathy were significantly lower than those of patients with acute rejection. Besides, the more serious the rejection was, the higher the level of urinary CX_3C-L/FKN became [49].

The effects of immunosuppressive therapy were analyzed by Cao *et al.* They showed that the expression of CX_3C-L/CX_3C-R-pair in grafted kidneys was significantly lower among the mycophenolic acid mofetil (MMF) treated group than the cyclosporine A (CsA) or the control group using immunohistochemistry and quantitative real-time PCR. The downregulatory effects of MMF on the expression of the CX_3C-L/CX_3C-R-pair in chronic allograft nephropathy may play a role in delaying progression toward graft failure [50].

In summary, there were discrepant data on CX_3C-L/FKN levels with an increased tubulointerstitial expression in chronic allograft nephropathy compared to acute rejection on the one hand and elevated urinary CX_3C-L/FKN levels in

acute renal allograft rejection but significant lower in chronic allograft nephropathy on the other hand.

EXPRESSION, REGULATION AND FUNCTION OF THE CX_3C-L/CX_3C-R PAIR IN CHRONIC RENAL FAILURE AND RENAL FIBROSIS

Chemokines cooperate with profibrotic cytokines in the development of fibrosis by recruiting myofibroblasts, macrophages and other key effector cells to sites of tissue injury. A large number of chemokine signalling pathways are involved in the mechanism of fibrogenesis. Some of them were identified and some are suspected as direct profibrotic mediators [51].

There are several studies available demonstrating a possible role of CX_3C-L/CX_3C-R-pair in tubulointerstitial fibrosis. Our own studies demonstrated increased CX_3C-R expression in human renal fibrotic kidneys in comparison with non-fibrotic, non-inflammatory nephropathies. CX_3C-R was not only detected in mononuclear, tubular epithelial and dendritic cells but also in α-smooth muscle actin and vimentin-positive interstitial myofibroblasts in fibrotic kidneys (Fig. (**1**)).

In vitro, hydrogen peroxide, but neither pro-inflammatory nor pro-fibrotic, was identified as an inducer of CX_3C-R in human renal fibroblasts. This increased CX_3C-R expression was associated with an enhancement of migration of renal fibroblasts [8]. These data were corroborated by extrarenal findings, where a CX_3C-R expression in skin fibroblasts was suspected to directly mediate recruitment, activation and heterotypic adhesion between macrophage and fibroblast, macrophage and endothelial cell, and/or fibroblast and endothelial cells. In wounded CX_3C-R knockout mice a reduced α-smooth muscle actin and collagen deposition in skin was found [52]. Moreover, in the liver CX_3C-R was present on primary hepatic stellate cells and CX_3C-L/FKN stimulation lead to a suppression of tissue inhibitor of metalloproteinase (TIMP)-1 mRNA, and CX_3C-R genotypes were associated with TIMP-1 mRNA expression in hepatitis C virus (HCV)-infected liver. This made CX_3C-L/CX_3C-R pair susceptible for hepatic fibrosis in HCV infection [53]. In which mechanisms of fibrogenesis CX_3C-L/CX_3C-R pair are involved, are not clear in detail.

In the chronic folic acid nephropathy (FAN) mouse model, CX_3C-L/FKN expression was upregulated within the endothelial cell layer and tubular compartment (Fig. (**2**)), starting in the earliest investigated stadiums with acute renal failure, but remained elevated or even increased until chronic damage with tubulointerstitial fibrosis was detectable. We identified the pro-inflammatory cytokines IL-1β and TNFα, the pro-fibrotic cytokine TGF-β as well as the reactive oxygen species hydrogen peroxide as inductors of CX_3C-L/FKN in mouse proximal and distal tubular epithelial cell lines. Moreover, CX_3C-L/FKN exerted effects on aspects of fibrogenesis in renal fibroblasts as it interacted with cell viability and matrix degradation. Moreover, CX_3C-L/FKN induced an autoinduction (Koziolek M *et al.*, in review).

An upregulation of CX_3C-L/FKN by a pro-fibrotic milieu was corroborated by the findings of Kruse *et al.*. In this study mouse kidneys were irradiated with single doses of 16 Gy, and protein and mRNA levels of CX_3C-L/FKN and PE-CAM-1 were examined after 10 to 40 weeks. Increased CX_3C-L/FKN immunoreactivity was seen at glomerular sites 30 to 40 weeks after irradiation. This CX_3C-L/FKN expression was strongly associated with the presence of leukocytes surrounding the Bowman's capsule of the same glomeruli. No significant changes in mRNA levels of CX_3C-L/FKN

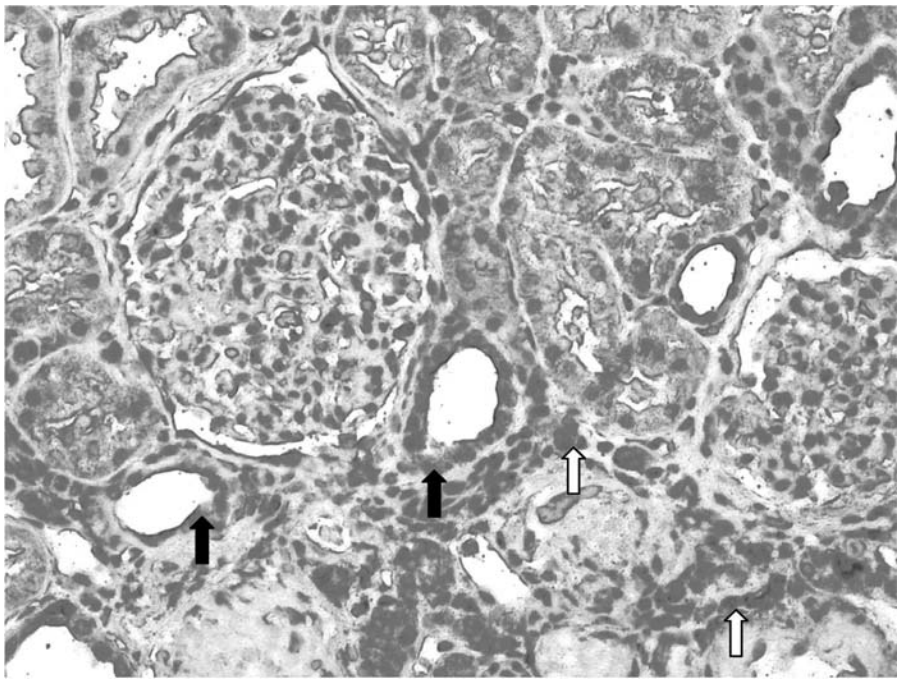

Fig. (1). CX_3C-R expression in fibrotic human kidney. CX_3C-R was detected in tubular epithelial cells (black arrow) as well as interstitial cells (white arrows) which were differentiated as myofibroblasts, dendritic cells and macrophages by double immunofluorescence. Magnification x 400.

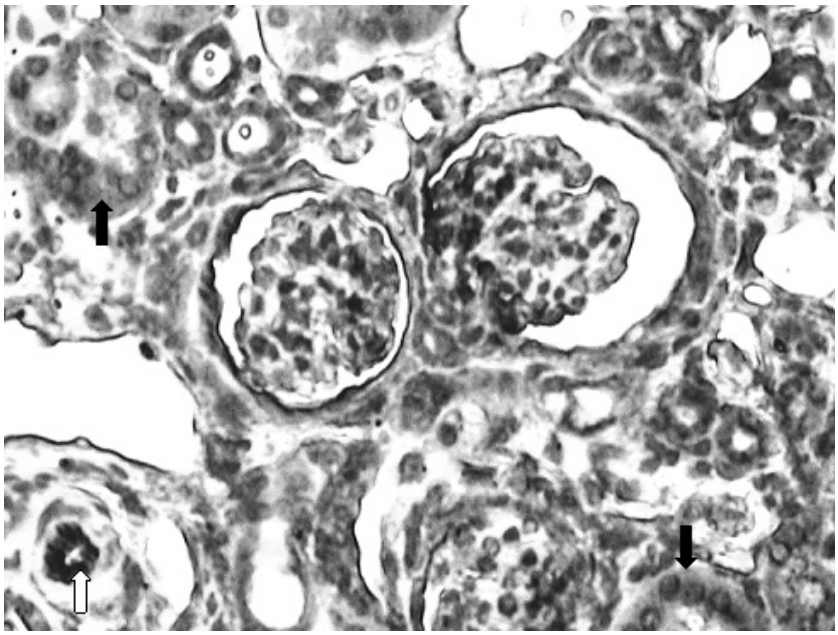

Fig. (2). CX_3C-L/FKN expression in folic acid nephropathy. CX_3C-L/FKN is expressed in tubular epithelial cells (black arrow) and endothelial cell lining of arterioles and arteries (white arrow). Moreover, CX_3C-L/FKN is found in epithelial cells of Bowman´s capsule. Magnification x 400.

were seen in whole kidney extracts after irradiation. However, expression levels were not determined for isolated glomeruli. The results suggest that CX_3C-L/FKN may be an important mechanism of leukocyte trafficking in the development of a radiation induced inflammatory response [54]. Moreover, another study demonstrated that connective tissue growth factor (CTGF), a potential profibrotic cytokine, increased the mRNA and soluble expression of CX_3C-L/FKN, in a time-dependent manner in cultured mesangial cells. LXA_4 downregulated the mRNA expressions of CX_3C-L/FKN in mesangial cells stimulated by CTGF. Pretreatment of the cells with PD98059 or LY294002, or PDTC, partially but significantly decreased the CTGF-induced increments of CX_3C-L/FKN in supernatants, indicating that synthesis of CX_3C-L/FKN, was NF-κB- and/or MAPK-dependent [55].

Furuichi K *et al.* investigated the expression and role of the CX_3C-L/CX_3C-R system in a mouse kidney ischemia-reperfusion injury model in time-course from 24 hours until 14 days after induction. CX_3C-L/FKN immunoreactivity was detected mainly on endothelial cells throughout the kidney in sham-operated mice but by 24 hours after ischemia-reperfusion was redistributed to the outer medulla where it could also be detected in association with infiltrating cells and tubular epithelial cells. Increased expression persisted throughout the course of the experiment but began to wane by day 7 after injury. Expression of CX_3C-R mRNA was extremely low in sham-operated mice but was markedly increased after ischemia-reperfusion injury, peaking at 48 hour in wild-type mice and remaining high throughout the 2-week course of the experiment. This co-localized with infiltrating cells. TGF-ß expression did not differ significantly between wild-type versus CX_3C-R-deficient mice. PDGF levels were reduced by 100% and 80% at 24 and 48 hours after injury, respectively, in CX_3C-$R^{-/-}$ mice relative to wild-type controls. However, there were no differences in the expression profile of other chemokines (CCL-2, CCL3, CXCL9) or receptors (CCR1, CXCR2, CCR4, CXCR3) comparing wild-type versus CX_3C-R-deficient mice. CX_3C-R neutralization by injection of a specific antibody resulted in significant and specific reduction in the area of fibrosis after injury, measured either by trichrome-stained collagen fibers or α-sm actin-staining area as it reduced $F4/80^+$ cell recruitment. Renal function, as determined by serum creatinine, decreased by ~50% from baseline by 48 hours after injury in both wild-type mice and CX_3C-R-deficient mice. To test injured kidney function directly, they analyzed serum creatinine at day 14 after injury in mice that underwent right nephrectomy at day 12 after injury. The results showed a ~400% increase in serum creatinine of injured wild-type mice relative to sham-operated wild-type mice. In CX_3C-R-deficient mice creatinine was reduced by ~25% at this time point [7].

Progressive nephropathies are characterized by both a highly enhanced glomerular permeability to proteins, in turn leading to proteinuria, and concomitant tubulointerstitial damage. Donadelli *et al.* investigated the effects of high albumin concentrations on proximal tubular cell (HK-2) expression of CX_3C-L/FKN. Albumin caused a dose- und time-dependent increase of membrane-bound and soluble CX_3C-L/FKN with a maximal expression after 24 hours incubation which might be NF-κB and p38 MAPK phosphorylation dependent. However, in proteinuric mice treated with anti-CX_3C-R antibody, the number of F4/80+ interstitial infiltrates tended to decrease, albeit this decrease failed to reach significance [45].

Conversely, the uremic solute *para*-cresol (4-methylphenol, *p*-cresol) inhibited the increase in CX_3C-L/FKN mRNA by 55% as well as soluble CX_3C-L/FKN production

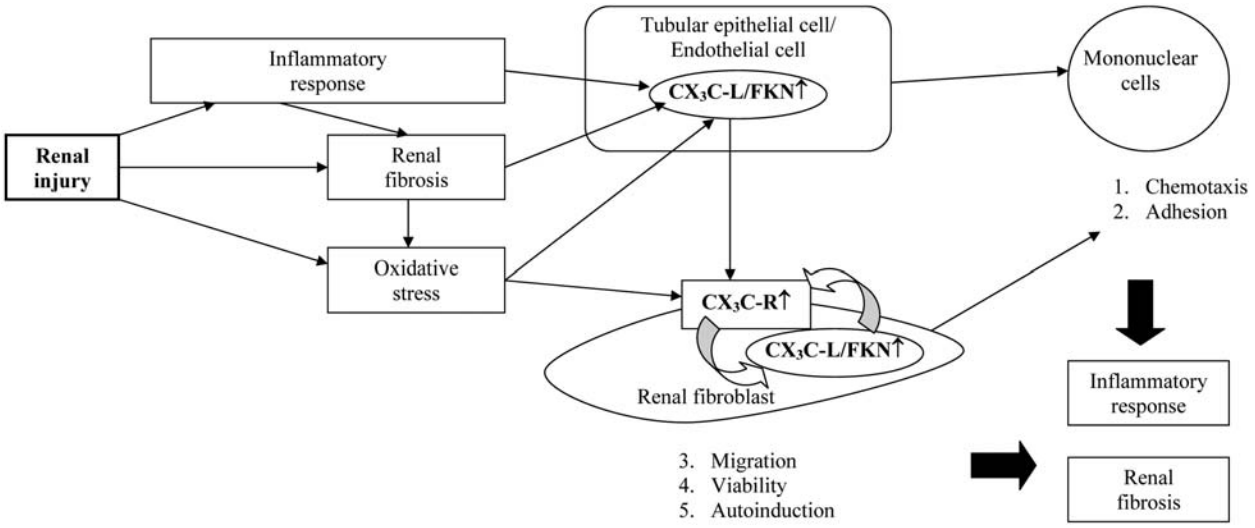

Fig. (3). Possible role of CX_3C-L/CX_3C-R-system in renal inflammation and fibrosis. CX_3C-L/CX_3C-R is induced and activated in response to inflammatory and oxidative stress as well as pro-fibrotic mediators. Otherwise, CX_3C-L/CX_3C-R itself activates pro-inflammatory and –fibrotic pathways.

by IL-1ß-stimulated HUVECs. This could have a direct inhibitory effect on leukocyte transendothelial migration and may play a role in the immune dysfunction of uremic patients [56].

Additional, there are some examples in extrarenal tissue which also demonstrated a possible role of CX_3C-L/CX_3C-R pair in fibrogenesis. These studies included investigations in systemic sclerosis [57], cardiac fibrosis [58], chronic pancreatitis [59], or atherosclerosis [60]. The exact mechanisms by which the CX_3C-L/CX_3C-R pair influences fibrogenesis are not clear in detail, but there seems to be a role in regulation of cell viability, cell migration and regulation of extracellular matrix. Stimulation of trophoblasts with CX_3C-L/FKN revealed significant changes in alpha-catenin (CTNNA1), extracellular matrix protein 1 (ECM1), osteopontin (SPP1), integrin alpha 6 (ITGA6), matrix metalloproteinase 12 (MMP12), and integrin beta 5 (ITGB5) expression. This intervening of CX_3C-L/CX_3C-R pair in regulation of adhesion molecules and extracellular matrix suggests a role in cell migration [61] as it was in synovial fibroblasts of patients with osteoarthritis [62]. This was further supported by data showing an increased actin polymerization in dendritic cells following CX_3C-L/FKN stimulation [63]. In atherosclerotic lesion of human coronary arteries CX_3C-R was expressed in vascular smooth muscle cells. On a functional basis, it was shown that CX_3C-L/FKN acts as a mediator of smooth muscle cell migration, cell-cell adhesion, cellular proliferation and matrix accumulation rather than mediating inflammatory cell recruitment in atherosclerosis [27, 64, 65].

CONCLUSION

Taken together, the CX_3C-L/CX_3C-R pair does play a role in renal development as well as in acute and chronic, glomerular and tubulointerstitial kidney diseases. Summarizing these results, there seems to be role of CX_3C-L/CX_3C-R pair in mediating acute inflammation, but beyond this possibly also in the progression of tubulointerstitial fibrosis. In addition to chemotactic and adhesive functions for immune cells, it has been shown to regulate aspects of cell viability, migration of resident cells and inflammation in the kidney. A blockade of this chemokine system ameliorated acute and chronic renal damages, though the latter to a more robust extent. (Fig. **3**) summarizes the hypothetical role of CX_3C-L/CX_3C-R-system in progressive renal failure. However, functional studies are lacking for many aspects and further studies are necessary to better define the functional properties of CX_3C-L/FKN and its receptor.

Abbreviations:

ACE-I	=	Angiotensin converting enzyme inhibitor
ADAM	=	A disintegrin and metalloprotease
AGE-BSA	=	Advanced glycosylated endproducts – bovine serum albumin
ARF	=	Acute renal failure
α-sm actin	=	Alpha smooth muscle actin
BUN	=	Blood urea nitrogen
CD	=	Cluster of differentiation
Col I	=	Collagen type I
CsA	=	Cyclosporine A
CX_3C-L/FKN	=	Fractalkine
CX_3C-R	=	Fractalkine receptor
CTGF	=	Connective tissue growth factor
EMT	=	Epithelial mesenchymal transition
FGF-2	=	Fibroblast growth factor 2
FN	=	Fibronectin
FSP-1	=	Fibroblast specific protein 1
GN	=	Glomerulonephritis

HCV	=	Hepatitis C virus
HRP	=	Horseradish peroxidase
HUVEC	=	Human umbilical vein endothelial cell
Ig	=	Immunoglobulin
IL-1β	=	Interleukin-1β
LPS	=	Lipopolysaccharide
MMF	=	Mycophenolic acid mofetil
MRP	=	Myeloid-related protein
NF-κB	=	Nuclear factor of kappa light chain in B cells
PECAM	=	Platelet endothelial cell adhesion molecule
PDGF	=	Platetelet derived growth factor
PDTC	=	Pyrrolidine dithiocarbamate
SD	=	Standard deviation
SNARE	=	Soluble N-ethylmaleimide factor attachment protein receptor
THP	=	Tamm-horsfall protein
TIMP	=	Tissue inhibitor of metalloproteinase
TGF-ß	=	Transforming growth factor-ß
TNF-α	=	Tumour necrosis factor α
VAMP	=	Vesicle-associated membrane protein
VEGF	=	Vascular endothelial growth factor

REFERRENCES

[1] Holdsworth, S.R.; Kitching, A.R.; Tipping, P.G. Chemokines as therapeutic targets in renal disease. *Curr. Opin. Nephrol. Hypertens.,* **2000**, *9,* 505-511.

[2] Nanki, T.; Imai, T.; Nagasaka, K.; Urasaki, Y.; Nonomura, Y.; Taniguchi, K.; Hayashida, K.; Hasegawa, J.; Yoshie, O.; Miyasaka, N. Migration of CX3CR1-positive T cells producing type 1 cytolytic and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. *Arthritis Rheumatol.,* **2002**, *46,* 2878-2883.

[3] Anders, H.J.; Vielhauer, V.; Schlöndorff, D. Chemokines and chemokine receptors are involved in the resolution or progression of renal disease. *Kidney Int.,* **2003**, *63,* 401-415.

[4] Braunersreuther, V.; Mach, F.; Steffens, S. The specific role of chemokines in atherosclerosis. *Thromb. Haemost.,* **2007**, *97,* 714-721.

[5] Viola, A.; Luster, A.D. Chemokines and their receptors: drug targets in immunity and inflammation. *Annu. Rev. Pharmacol. Toxicol.,* **2008**, *48,* 171-197.

[6] (a) Olson, T.S.; Ley, K. Chemokines and chemokine receptors in leukocyte trafficking. *Am. J. Physiol*., **2002**, *281*(1), R7-R298. (b) Frangogiannis, N.G. Chemokines in ischemia and reperfusion. *Thromb. Haemost.,* **2007**, *97,* 738-747.

[7] Furuichi, K.; Gao, J.L.; Murphy, P.M. Chemokine receptor CX3CR1 regulates renal interstitial fibrosis after ischemia-reperfusion injury. *Am. J. Pathol.,* **2006**, *169,* 372-387.

[8] Koziolek, M.J.; Schmid, H.; Cohen, C.; Blaschke, S.; Hemmerlein, B.; Zapf, A.; Müller, G.A.; Strutz, F. Potential role of fractalkine receptor (CX3C-R) expression in human renal fibrogenesis. *Kidney Int.,* **2007**, *72,* 599-607.

[9] Nomiyama, H.; Imai, T.; Kusuda, J.; Miura, R.; Callen, D.F.; Yoshie, O. Human chemokines fractalkine (SCYD1), MDC (SCYA22) and TARC (SCYA17) are clustered on chromosome 16q13. *Cytogenet. Cell. Genet.,* **1998**, *81,* 10-11.

[10] Bazan, J.F.; Bacon, K.B.; Hardiman, G.; Wang, W.; Soo, K.; Rossi, D.; Greaves, D.R.; Zlotnik, A.; Schall, T.J. A new class of membrane-bound chemokine with a CX_3C motif. *Nature,* **1997**, *385,* 640-644.

[11] Liu, G.Y.; Kulasingam, V.; Alexander, R.T.; Touret, N.; Fong, A.M.; Patel, D.D.; Robinson, L.A. Recycling of the membrane anchored chemokine, CX3CL1. *J. Bol. Chem.,* **2005**, *280*, 19858-19866.

[12] Imaizumi, T.; Matsumiya, T.; Fujimoto, K.; Okamoto, K.; Cui, X.; Ohtaki, U.; Yoshida, H.; Satoh, K. Interferon-γ stimulates the expression of CX3CL1/fractalkine in cultured human endothelial cells. *Tohoku J. Exp. Med.,* **2000**, *192,* 127-139.

[13] Durkan, A.M.; Alexander, R.T.; Liu, G.Y.; Rui, M.; Femia, G.; Robinson, L.A. Expression and targeting of CX3CL1 (fractalkine) in renal tubular epithelial cells. *J. Am. Soc. Nephrol.,* **2007**, *18,* 74-83.

[14] Ludwig, A.; Weber, C. Transmembrane chemokines: Versatile 'special agents' in vascular inflammation. *Thromb. Haemost.,* **2007**, *97,* 694-703.

[15] Fong, A.M.; Robinson, L.A.; Steeber, D.A.; Tedder, T.F.; Yoshie, O.; Imai, T.; Patel, D.D. Fractalkine and CX_3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. *J. Exp. Med.,* **1998**, *188,* 1413-1419.

[16] Imai, T.; Hieshima, K.; Haskell, C.; Baba, M.; Nagira, M.; Nishimura, M.; Kakizaki, M.; Takagi, S.; Nomiyama, H.; Schall, T.J.; Yoshie, O. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediated both leukocyte migration and adhesion. *Cell,* **1997**, *91,* 521-530.

[17] Foussat, A.; Coulomb-L´Hermine, A.; Gosling, J.; Krzysiek, R.; Durand-Gasselin, I.; Schall, T.; Balian, A.; Richard, Y.; Galanaud, P.; Emilie, D. Fractalkine receptor expression by T lymphocyte subpopulations and *in vivo* production of fractalkine in human. *Eur. J. Immunol.,* **2000**, *30,* 87-97.

[18] Efsen, E.; Grappone, C.; DeFranco, R.M.; Milani, S.; Romanelli, R.G.; Bonacchi, A.; Caligliuri, A.; Failli, P.; Annunziato, F.; Pagliai, G.; Pinzani, M.; Laffi, G.; Gentilini, P.; Marra, F. Up-regulated expression of fractalkine and its receptor CX3CR1 during liver injury in humans. *J. Hepatol.,* **2002**, *37,* 39-47.

[19] Brand, S.; Sakaguchi, T.; Gu, X.; Colgan, S.P.; Reinecker, H.C. Fractalkine-mediated signals regulate cell-survival and immune-modulatory responses in intestinal epithelial cells. *Gastroenterology,* **2002**, *122,* 166-177.

[20] Wong, B.W.; Wong, D.; McManus, B.M. Characterization of fractalkine (CX3CL1) and CX3CR1 in human coronary arteries with native atherosclerosis, diabetes mellitus, and transplant vascular disease. *Cardiovasc. Pathol.,* **2002**, *11,* 332-338.

[21] Perros, F.; Dorfmüller, P.; Souza, R.; Durand-Gasselin, I.; Godot, V.; Capel, F.; Adnot, S.; Eddahibi, S.; Mazmanian, M.; Fadel, E.; Hervé, P.; Simonneau, G.; Emilie, D.; Humbert, M. Fractalkine-induced smooth muscle cell proliferation in pulmonary hypertension. *Eur. Respir. J.,* **2007**, *29,* 937-943.

[22] Ji, J.F.; He, B.P.; Dheen, S.T.; Tay, S.S. Interacion of chemokines and chemokine receptors mediated migrationb of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. *Stem Cells,* **2004**, *22,* 415-427.

[23] Goda, S.; Imai, T.; Yoshie, O.; Yoneda, O.; Inoue, H.; Nagano, Y.; Okazaki, T.; Imai, H.; Bloom, E.T.; Domae, N.; Umehara, H.: CX_3C-chemokine, fractalkine-enhanced adhesion of THP-1 cells to endothelial cells through integrin-dependent and -independent mechanisms. *J. Immunol.,* **2000**, *164,* 4313-4320.

[24] Haskell, C.A.; Cleary, M.D.; Charo, I.F. Unique role of the chemokine domain of fractalkine in cell capture. *J. Biol. Chem.,* **2000**, *275,* 34183-34189.

[25] Matsubara, T.; Ono, T.; Yamanoi, A.; Tachibana, M.; Nagasue, N. Fractalkine-CX3CR1 axis regulates tumor cell cycle and deteriorates prognosis after radical resection for hepatocellular carcinoma. *J. Surg. Oncol.,* **2007**, *95,* 241-249.

[26] Blaschke, S.; Koziolek, M.J.; Schwarz, A.; Middel, A.; Schwarz, G.; Hummel, K.M.; Müller, G.A. Proinflammatory role of fractalkine (CX_3CL1) in rheumatoid arthritis. *J. Rheumatol.,* **2003**, *30*(9), 1918-1927.

[27] Lucas, A.D.; Bursill, C.; Guzik, T.J.; Sadowski, J.; Channon, K.M.; Greaves, D.R. Smooth muscle cells in human atherosclerosis plaques express the fractalkine receptor CX_3CR1 and undergo

chemotaxis to the chemokine fractalkine (CX_3CL1). *Circulation,* **2003**, *108,* 2498-2504.

[28] Maciejewski-Lenoir, D.; Chen, S.; Feng, L.; Maki, R.; Bacon, K.B. Characterization of fractalkine in rat brain cells: migratory and activation signals for CX3CR-1-expressing microglia. *J. Immunol.,* **1999**, *163,* 1628-1635.

[29] Zujovic, V.; Benavides, J.; Vigé, X.; Carter, C.; Taupin, V. Fractalkine modulates TNF-alpha secretion and neurotoxicity induced by microglial activation. *Glia,* **2000**, *29,* 305-315.

[30] Schäfer, A.; Schulz, C.; Fraccarollo, D.; Tas, P.; Leutke, M.; Eigenthaler, M.; Seidl, S.; Heider, P.; Ertl, G.; Massberg, S.; Bauersachs, J. The CX3C chemokine fractalkine induces vascular dysfunction by generation of superoxide anions. *Arterioscler. Thromb. Vasc. Biol.,* **2007**, *27,* 55-62.

[31] Gröne, H.J.; Cohen, C.D.; Gröne, E.; Schmidt, C.; Kretzler, M.; Schlöndorff, D.; Nelson, P.J. Spatial and temporally restricted expression of chemokines and chemokine receptors in the developing kidney. *J. Am. Soc. Nephrol.,* **2002**, *13,* 957-967.

[32] Segerer, S.; Hughes, E.; Hudkins, K.L.; Mack, M.; Goodpaster, T.; Alpers, C.E. Expression of the fractalkine receptor (CX_3CR1) in human kidney diseases. *Kidney Int.,* **2002**, *62,* 488-495.

[33] Chen, Y.M.; Hu-Tsai, M.I.; Lin, S.L.; Tsai, T.J.; Hsieh, B.S. Expression of CX3CL1/fractalkine by mesangial cells *in vitro* and in acute anti-Thy1 glomerulonephritis in rats. *Nephrol. Dial. Transplant.,* **2003**, *18,* 2505-2514.

[34] Yoshimoto, S.; Nakatani, K.; Iwano, M.; Asai, O.; Samejima, K.; Sakan, H.; Terada, M.; Harada, K.; Akai, Y.; Shiiki, H.; Nose, M.; Saito, Y. Elevated levels of fractalkine expression and accumulation of CD16+ monocytes in glomeruli of active lupus nephritis. *Am. J. Kidney Dis.,* **2007**, *50,* 47-58.

[35] Qing, X.; Zavadil, J.; Crosby, M.B.; Hogarth, M.P.; Hahn, B.H.; Mohan, C.; Gilkeson, G.S.; Bottinger, E.P.; Putterman, C. Nephritogenic anti-DNA antibodies regulate gene expression in MRL/lpr mouse glomerular mesangial cells. *Arthritis Rheumatol.,* **2006**, *54,* 2198-2210.

[36] Furuichi, K.; Wada, T.; Iwata, Y.; Sakai, N.; Yoshimoto, K.; Shimizu, M.; Kobayashi, K.; Takasawa, K.; Kida, H.; Takeda, S.; Matsushima, K.; Yokoyama, H. Upregulation of fractalkine in human crescentic glomerulonephritis. *Nephron,* **2001**, *87,* 314-320.

[37] Tam, F.W.; Sanders, J.S.; George, A.; Hammad, T.; Miller, C.; Dougan, T.; Cook, H.T.; Kallenberg, C.G.; Gaskin, G.; Levy, J.B.; Pusey, C.D. Urinary monocyte chemoattractant protein-1 (MCP-1) is a marker of active renal vasculitis. *Nephrol. Dial. Transplant.,* **2004**, *19,* 2761-2768.

[38] Feng, L.; Chen, S.; Garcia, G.E.; Xia, Y.; Siani, M.A.; Botti, P.; Wilson, C.B.; Harrison, J.K.; Bacon, K.B. Prevention of crescentic glomerulonephritis by immunoneutralization of the fractalkine receptor CX_3CR1. Rapid Communication. *Kidney Int.,* **1999**, *56,* 612-620.

[39] Kikuchi, Y.; Ikee, R.; Hemmi, N.; Hyodo, N.; Saigusa, T.; Namikoshi, T.; Yamada, M.; Suzuki, S.; Miura, S. Fractalkine and its receptor, CX3CR1, upregulation in streptozotocin-induced diabetic kidneys. *Nephron Exp. Nephrol.,* **2004**, *97,* e17-25.

[40] Kikuchi, Y.; Imakiire, T.; Hyodo, T.; Kushiyama, T.; Higashi, K.; Hyodo, N.; Suzuki, S.; Miura, S. Advanced glycation end-product induces fractalkine gene upregulation in normal rat glomeruli. *Nephrol. Dial. Transplant.,* **2005**, *20,* 2690-2696.

[41] Beck, G.Ch.; Ludwig, F.; Schulte, J.; van Ackern, K.; van der Woude, F.J.; Yard, B.A. Fractalkine is not a major chemoattractant for the migration of neutrophils across microvascular endothelium. *Scand. J. Immunol.,* **2003**, *58,* 180-187.

[42] Zanchi, C.; Zoja, C.; Morigi, M.; Valsecchi, F.; Liu, X.Y.; Rottoli, D.; Locatelli, M.; Buelli, S.; Pezzotta, A.; Mapelli, P.; Geelen, J.; Remuzzi, G.; Hawiger, J. Fractalkine and CX3CR1 mediate leukocyte capture by endothelium in response to shiga toxin. *J. Immunol.,* **2008**, *181,* 1460-1469.

[43] Oh, D.J.; Dursun, B.; He, Z.; Lu, L.; Hoke, T.S.; Ljubanovic, D.; Faubel, S.; Edelstein, C.L. Fractalkine receptor (CX3CR1) inhibition is protective against ischemic acute renal failure in mice. *Am. J. Physiol. Renal Physiol.,* **2008**, *294,* F264-271.

[44] Lu, L.H.; Oh, D.J.; Dursun, B.; He, Z.; Hoke, T.S.; Faubel, S.; Edelstein, C.L. Increased macrophage infiltration and fractalkine expression in cisplatin-induced acute renal failure in mice. *J. Pharmacol. Exp. Ther.,* **2008**, *324,* 111-117.

[45] Donadelli, R.; Zanchi, C.; Morigi, M.; Buelli, S.; Batani, C.; Tomasoni, S.; Corna, D.; Rottoli, S.; Benigni, A.; Abbate, M.; Remuzzi, G.; Zoja, C. Protein overload induces fractalkine upregulation in proximal tubular cells through nuclear factor kappa B- and p38 mitogen-activated protein kinase-dependent pathways. *J. Am. Soc. Nephrol.,* **2003**, *13,* 2436-2446.

[46] Cockwell, P.; Calderwood, J.W.; Brooks, C.J.; Chakravorty, S.J.; Savage, C.O. Chemoattraction of T cells expressing CCR5, CXCR3 and CX3CR1 by proximal tubular epithelial cell chemokines. *Nephrol. Dial. Transplant.,* **2002**, *17,* 734-744.

[47] Cao, G.; Lu, Y.; Gao, R.; Xin, Y.; Teng, D.; Wang, J.; Li, Y. Expression of fractalkine, CX3CR1, and vascular endothelial growth factor in human chronic renal allograft rejection. *Transplant. Proc.,* **2006**, *38,* 1998-2000.

[48] Pietrzyk, M.C.; Banas, B.; Wolf, K.; Rummele, P.; Woenckhaus, M.; Hoffmann, U.; Kramer, B.K.; Fischereder, M. Quantitative gene expression analysis of fractalkine using laser microdissection in biopsies from kidney allografts with acute rejection. *Transplant. Proc.,* **2004**, *36,* 2659-2661.

[49] Peng, W.; Chen, J.; Jiang, Y.; Wu, J.; Shou, Z.; He, Q.; Wang, Y.; Chen, Y.; Wang, H. Urinary fractalkine is a marker of acute rejection. *Kidney Int.,* **2008**, *74,* 1454-1460.

[50] Cao, G.; Lu, Y.; Gao, R.; Xin, Y.; Teng, D.; Wang, J.; Wang, L.; Li, Y. Comparison of cyclosporine versus mycophenolate mofetil on expression of fractalkine and CX3CR1 in chronic allograft nephropathy. *Transplant. Proc.,* **2006**, *38,* 2234-2236.

[51] Wynn, T.A. Cellular and molecular mechanisms of fibrosis. *J. Pathol.,* **2008**, *214,* 199-210.

[52] Ishida, Y.; Gao, J.L.; Murphy, P.M. Chemokine receptor CX3C-R mediates skin wound healing by promoting macrophage and fibroblast accumulation and function. *J. Immunol.,* **2008**, *180,* 569-579.

[53] Wasmuth, H.E.; Zaldivar, M.M.; Berres, M.L.; Werth, A.; Scholten, D.; Hillebrandt, S.; Tacke, F.; Schmitz, P.; Dahl, E.; Wiederholt, T.; Hellerbrand, C.; Berg, T.; Weiskirchen, R.; Trautwein, C.; Lammert, F. The fractalkine receptor CX3CR1 is involved in liver fibrosis due to chronic hepatitis C infection. *J. Hepatol.,* **2008**, *48,* 208-215.

[54] Kruse, J.J.; Bomhoff-Wijdenes, I.F.; Poele, J.A.; Stewart, F.A. Fractalkine: an important candidate for directing periglomerular leukocyte accumulation in irradiated mouse kidneys. *Acta Oncol.,* **2007**, *46,* 945-950.

[55] Wu, S.H.; Wu, X.H.; Lu, C.; Dong, L.; Zhou, G.P.; Chen, Z.Q. Lipoxin A4 inhibits connective tissue growth factor-induced production of chemokines in rat mesangial cells. *Kidney Int.,* **2006**, *69,* 248-256.

[56] Faure, V.; Cerini, C.; Paul, P.; Berland, Y.; Dignat-George, F.; Brunet, P. The uremic solute p-cresol decreases leukocyte transendothelial migration *in vitro*. *Int. Immunol.,* **2006**, *18,* 1453-1459.

[57] Hasegawa, M.; Sato, S.; Echigo, T.; Hamaguchi, Y.; Yasui, M.; Takehara, K. Up regulated expression of fractalkine/CX3CL1 and CX3CR1 in patients with systemic sclerosis. *Ann. Rheum. Dis.,* **2005**, *64,* 21-28.

[58] Husberg, C.; Nygård, S.; Finsen, A.V.; Damås, J.K.; Frigessi, A.; Oie, E.; Wæhre, A.; Gullestad, L., Aukrust, P.; Yndestad, A.; Christensen, G. Cytokine expression profiling of the myocardium reveals a role for CX3CL1 (fractalkine) in heart failure. *J. Mol. Cell. Cardiol.,* **2008**, *45*, 261-269.

[59] Ito, T. Can measurement of chemokines become useful biological and functional markers of early-stage chronic pancreatitis? *J. Gastroenterol.,* **2007**, *42,* 72-77.

[60] Saederup, N.; Chan, L.; Lira, S.A.; Charo, I.F. Fractalkine deficiency markedly reduces macrophage accumulation and atherosclerotic lesion formation in CCR2-/- mice: evidence for independent chemokine functions in atherogenesis. *Circulation,* **2008**, *117,* 1642-1648.

[61] Hannan, N.J.; Salamonsen, L.A. CX3CL1 and CCL14 regulate extracellular matrix and adhesion molecules in the trophoblast: potential roles in human embryo implantation. *Biol. Reprod.,* **2008**, *79,* 58-65.

[62] Klosowska, K.; Volin, M.V.; Huynh, N.; Chong, K.K.; Halloran, M.M.; Woods, J.M. Fractalkine functions as a chemoattractant for osteoarthritis synovial fibroblasts and stimulates phosphorylation of mitogen-activated protein kinases and Akt. *Clin. Exp. Immunol.,* **2009**, *156,* 312-319.

[63] Dichmann, S.H.Y.; Purlis, D.; Rheinen, H.; Gebicke-Härter, P.; Norgauer, J. Fractalkine induces chemotaxis and actin polymerization in human dendritic cells. *Inflamm. Res.,* **2001**, *50,* 528-533.

[64] Chandrasekar, B.; Mummidi, S.; Perla, R.P.; Bysani, S.; Dulin, N.O.; Liu, F.; Melby, P.C. Fractalkine (CX_3CL1) stimulated by nuclear factor kappa B (NF-κB)-dependent inflammatory signals induces aortic smooth muscle cell proliferation through an autocrine pathway. *Biochem. J.,* **2003**, *373,* 547-558.

[65] Combadière, C.; Potteaux, S.; Gao, J.L.; Esposito, B.; Casanova, S.; Lee, E.J.; Debré, P.; Tedgui, A.; Murphy, P.M.; Mallat, Z. Decreased atherosclerotic lesion formation in CX3CR1/apolipoprotein E double knockout mice. *Circulation,* **2003**, *107,* 1009-1016.

[66] Chen, Y.M.; Tu, C.J.; Hung, K.Y.; Wu, K.D.; Tsai, T.J.; Hsieh, B.S. Inhibition by pentoxifylline of TNFα-stimulated fractalkine production in vascular smooth muscle cells: evidence for mediation by NF-κB down regulation. *Br. J. Pharmacol.,* **2003**, *238,* 950-958.

[67] Chen, Y.M.; Lin, S.L.; Chen, C.W.; Chiang, W.C.; Tsai, T.J.; Hsieh, B.S. Tumor necrosis factor-alpha stimulates fractalkine production by mesangial cells and regulates monocyte transmigration: down-regulation by cAMP. *Kidney Int.,* **2003**, *63,* 474-486.

[68] Patel, A.; Jagadesham, V.P.; Porter, K.E.; Scott, D.J.; Carding, S.R. Characterisation of fractalkine/CX3CL1 and fractalkine receptor (CX3CR1) expression in abdominal aortic aneurysm disease. *Eur. J. Vasc. Endovasc. Surg.,* **2008**, *36,* 20-27.

[69] Yoshikawa, M.; Makajima, T.; Matsumoto, K.; Okada, N.; Tsukidate, T.; Iida, M.; Otori, N.; Haruna, S.; Moriyama, H.; Imai, T.; Saito, H. TNF-α and IL-4 regulate expression of fractalkine (CX_3CL1) as a membrane-anchored proadhesive protein and soluble chemotactic peptide on human fibroblasts. *FEBS Lett.,* **2004**, *561,* 105-111.

[70] Matsumiya, T.; Imaizumi, T.; Fujimoto, K.; Cui, X.; Shibata, T.; Tamo, W.; Kumagai, M.; Tanji, K.; Yoshida, H.; Kimura, H.; Satoh, K. Soluble Interleukin-6 receptor α inhibits the cytokine-induced Fractalkine/CX3CL1 expression in human vascular endothelial cells in culture. *Exp. Cell. Res.,* **2001**, *269,* 35-41.

[71] Sandell, L.J.; Xing, X.; Franz, C.; Davies, S.; Chang, L.W.; Patra, D. Exuberant expression of chemokine genes by adult human articular chondrocytes in response to IL-1beta. *Osteoarthritis Cartilage,* **2008**, *16*, 1560-1571.

[72] Greaves, D.R.; Häkkinen, T.; Lucas, A.D.; Liddiard, K.; Jones, E.; Quinn, C.M.; Senaratne, J.; Green, F.R.; Tyson, K.; Boyle, J.; Shanahan, C.; Weissberg, P.L.; Gordon, S.Y. Linked chromosome 16q13 chemokines, macrophage-derived chemokine, fractalkine, and thymus and activation-regulated chemokine, are expressed in human atherosclerotic lesions. *Arterioscler. Thromb. Vasc. Biol.,* **2001**, *21,* 923-929.

[73] Wu, S.H.; Lu, C.; Dong, L.; Chen, Z.Q. Signal transduction involved in CTGF-induced production of chemokines in mesangial cells. *Growth Factors,* **2008**, *26*, 192-200.

Received: 15 April, 2009 **Revised: 30 June, 2009** **Accepted: 02 July, 2009**

[74] Imaizumi, T.; Matsumiya, T.; Tamo, W.; Shibata, T.; Fujimoto, K.; Kumagai, M.; Yoshida, H.; Cui, X.F.; Tanji, K.; Hatakeyama, M.; Wakabayashi, K.; Satoh, K. 15-Deoxy-D12,14-prostaglandin J2 inhibits CX3CL1/fractalkine expression in human endothelial cells. *Immunol. Cell. Biol.,* **2002**, *80,* 531-536.

[75] Hatakeyama, M.; Imaizumi, T.; Tamo, W.; Yamashita, K.; Yoshida, H.; Fukuda, I.; Satoh, K. Heparin inhibits IFN-gamma-induced fractalkine/CX3CL1 expression in human endothelial cells. *Inflammation,* **2004**, *28,* 7-13.

[76] Popovic, M.; Laumonnier, Y.; Burysek, L.; Syrovets, T.; Simmet, T. Thrombin-induced expression of endothelial CX3CL1 potentiates monocyte CCL2 production and transendothelial migration. *J. Leukoc. Biol.,* **2008**, *84*, 215-223.

[77] Gerstung, M.; Roth, T.; Dienes, H.P.; Licht, C.; Fries, J.W. Endothelin-1 induces NF-kappaB via two independent pathways in human renal tubular epithelial cells. *Am. J. Nephrol.,* **2007**, *27,* 294-300.

[78] Bhavsar, P.K.; Sukkar, M.B.; Khorasani, N.; Lee, K.Y.; Chung, K.F. Glucocorticoid suppression of CX3CL1 (fractalkine) by reduced gene promoter recruitment of NF-κB. *FASEB J.,* **2008**, *22.*

[79] Banerjee, A.; Damera, G.; Bhandare, R.; Gu, S.; Lopez-Boado, Y.S.; Panettieri, R.A. Jr.; Tliba, O. Vitamin D and glucocorticoids differentially modulate chemokine expression in human airway smooth muscle cells. *Br. J. Pharmacol.,* **2008**, *155*, 84-92.

[80] Zhao, P.; De, A.; Hu, Z.; Li, J.; Mulders, S.M.; Sollewijn Gelpke, M.D.; Duan, E.K.; Hsueh, A.J. Gonadotropin stimulation of ovarian fractalkine expression and fractalkine augmentation of progesterone biosynthesis by luteinizing granulosa cells. *Endocrinology,* **2008**, *149,* 2782-2789.

[81] Green, S.R.; Han, K.H.; Chen, Y.; Almazan, F.; Charo, I.F.; Miller, Y.I.; Quehenberger, O. The CC chemokine MCP-1 stimulates surface expression of CX3CR1 and enhances the adhesion of monocytes to fractalkine/CX3CL1 *via* p38 MAPK. *J. Immunol.,* **2006**, *176,* 7412-7420.

[82] Saitoh, Y.; Koizumi, K.; Sakurai, H.; Minami, T.; Saiki, I. RANKL-induced down-regulation of CX3CR1 *via* PI3K/Akt signaling pathway suppresses Fractalkine/CX3CL1-induced cellular responses in RAW264.7 cells. *Biochem. Biophys. Res. Commun.,* **2007**, *364,* 417-422.

[83] Chen, S.; Luo, D.; Streit, W.J.; Harrison, J.K. TGF-β1 upregulates CX_3CR1 expression and inhibits fractalkine-stimulated signaling in rat microglia. *J. Neuroimmunol.,* **2002**, *133,* 46-55.

[84] Boddeke, E.W.; Meigel, I.; Frentzel, S.; Biber, K.; Renn, L.Q.; Gebicke-Härter, P. Functional expression of the fractalkine (CX3C) receptor and its regulation by lipopolysaccharide in rat microglia. *Eur. J. Pharmacol.,* **1999**, *374,* 309-313.