Expression and Targeting of CX$_3$CL1 (Fractalkine) in Renal Tubular Epithelial Cells

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The chemokine CX$_3$CL1 plays a key role in glomerulonephritis and can act as both chemoattractant and adhesion molecule. CX$_3$CL1 also is upregulated in tubulointerstitial injury, but little is known about the subcellular distribution and function of CX$_3$CL1 in renal tubular epithelial cells (RTEC). Unexpectedly, it was found that CX$_3$CL1 is expressed predominantly on the apical surface of tubular epithelium in human renal transplant biopsy specimens with acute rejection or acute tubular necrosis. For studying the targeting of CX$_3$CL1 in polarized RTEC, MDCK cells that expressed untagged or green fluorescent protein–tagged CX$_3$CL1 were generated. The chemokine was present on the apical membrane and in subapical vesicles. Apical targeting of CX$_3$CL1 was not due to signals that were conferred by its intracellular domain, to associations with lipid rafts, or to O-glycosylation but, rather, depended on N-linked glycosylation of the protein. With the use of fluorescence recovery after photobleaching, it was found that CX$_3$CL1 is immobile in the apical membrane. However, CX$_3$CL1 partitioned with the triton-soluble rather than -insoluble cellular fraction, indicating that it is not associated directly with the actin cytoskeleton or with lipid rafts. Accordingly, disruption of rafts through cholesterol depletion did not render CX$_3$CL1 mobile. For exploration of potential functions of apical CX$_3$CL1, binding of CX$_3$CR1-expressing leukocytes to polarized RTEC was examined. Leukocyte adhesion to the luminal surface was enhanced significantly when CX$_3$CL1 was present. These data demonstrate that CX$_3$CL1 is expressed preferentially on the apical membrane of RTEC and suggest a novel function for the chemokine in recruitment and retention of leukocytes in tubulointerstitial inflammation.


Renal tubulointerstitial inflammation is characterized by recruitment of circulating leukocytes to the site of injury. Traffic signals for leukocyte migration are provided by chemokines, a family of small molecular weight proteins (1). Among the chemokines, CX$_3$CL1 (fractalkine) has a unique role in the inflammatory cascade because of its structure: an extracellular chemokine domain and mucin stalk, tethered to a transmembrane region and a 37–amino acid cytoplasmic tail. Transmembrane CX$_3$CL1 is cleaved proximal to the membrane by metalloproteinases of the A disintegrin and metalloproteinase (ADAM) family to release a soluble species (2–5). Soluble CX$_3$CL1 acts as a chemoattractant, whereas the transmembrane protein acts as a cell adhesion molecule for monocytes, natural killer (NK) cells, and subsets of CD8$^+$ T cells, all of which express CX$_3$CR1, the receptor for CX$_3$CL1. In this way, CX$_3$CL1 and CX$_3$CR1 promote leukocyte infiltration at sites of injury (2,6,7). In vivo, CX$_3$CL1 is strongly implicated in inflammation, including allograft rejection and, most notably, atherosclerosis (8–13).

Numerous organs express CX$_3$CL1, including brain, lung, heart, intestine, and kidney (10,14–17). In humans and rodents, CX$_3$CL1 expression is prominent in renal glomerular disease, particularly in endothelial and mesangial cells (18–20). The role of CX$_3$CL1 in mediating glomerular injury is well described in animal models of systemic lupus erythematosus nephritis, mesangioproliferative glomerulonephritis, and crescentic glomerulonephritis (21–23). In these disease processes, blockade of either CX$_3$CL1 or CX$_3$CR1 prevents renal injury and leukocyte infiltration (21–23). Little is known, however, regarding the role of CX$_3$CL1 in tubulointerstitial renal disease. Expression of CX$_3$CL1 clearly is enhanced in tubulointerstitial injury, including that associated with tubular protein overload and acute allograft rejection (19,24,25). However, knowledge regarding the subcellular distribution and function of CX$_3$CL1 in renal tubular epithelial cells (RTEC) is rudimentary.

We demonstrate here that CX$_3$CL1 is present on the apical membrane of RTEC, where it is anchored firmly. We further demonstrate that CX$_3$CL1 is targeted to the apical membrane via N-glycosylation of the chemokine. The implications of apical targeting of CX$_3$CL1 for the pathogenesis of tubulointerstitial inflammation are discussed.

Materials and Methods

Cells and Constructs

HK-2 cells were a gift from Dr. András Kapus, (University of Toronto, Toronto, ON, Canada). MDCK cells were from American Type Culture Collection (Manassas, VA). MDCK cells that stably expressed sodium-hydrogen exchanger 3 (NHE3) tagged with hemagglutinin (HA) were provided by Dr. Sergio Grinstein (University of Toronto).
The preceding cells were cultured in DMEM and Ham’s F12 (Wisent, St-Bruno, Quebec, Canada) that contained 5% FCS. K562 erythroleukemia cells and K562 cells that stably expressed CX3CR1 (K562-CX3CR1) were from Dr. Dhavalkumar Patel (University of North Carolina, Chapel Hill, NC) and were cultured in RPMI supplemented with HEPES and 15% FCS. Primary human RTEC (Clonetics, Cambrex, Walkersville, MD) were a gift from Dr. Jim Scholey (University of Toronto) and were cultured according to the manufacturer’s instructions (26). CX3CL1 expression in these cells was verified by Western blotting (27). Human peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood from healthy volunteers (28).

DNA constructs for CX3CL1 or CX3CL1 tagged with green fluorescent protein (CX3CL1-GFP) were created as described previously (6,27,29). MDCK cells were stably transfected with these constructs using FuGENE (Roche, Indianapolis, IN) and selected in 500 μg/ml G418 (Wisent).

The predicted transmembrane portion of CX3CL1 ends at amino acid 360 (PSORT II). A mutant construct that lacked amino acids 361 to 397, CX3CL1–360, was generated by PCR using the common upstream primer 5'-GTGGAATTCTGCAGTCGACTC-3' and the downstream primer 5'-GCCGCCGGTCATGGCCACCCCCCGAGCAG-3'. In the downstream primer, stop codons and Nol sites were introduced. PCR products were cloned into TOPO vector (Invitrogen, Burlington, ON, Canada) and sequenced. Inserts were released by cutting with Nol and EcoRI and subcloned into HA-pcDNA3.1 (Invitrogen). MDCK cells were transfected using FuGENE and selected in 500 μg/ml G418 (MDCK-CX3CL1–360). DNA constructs that encoded GFP-tagged glycosyl phosphatidylinositol (GPI-GFP) and FcRIIa receptor (FcR-GFP) were a gift from Dr. S. Grinstein (University of Toronto) (30,31).

**Antibodies and Reagents**

The following reagents were used: Goat anti-human CX3CL1 directed against the extracellular chemokine region (R&D Systems, Minneapolis, MN) (19,32), goat anti-human CX3CL1 against the intracellular carboxy terminus (Santa Cruz Biotechnology, Santa Cruz, CA), anti-actin (Sressgen, Victoria, BC, Canada), and anti-HA ( Covance, Berkeley, CA), Cy3- and horseradish peroxidase-conjugated anti-goat IgG, anti-mouse IgG, and 18 nm gold-labeled anti-goat IgG were from Jackson ImmunoResearch Laboratories (Leica Microsystems, Toronto, ON, Canada), equipped with an Hamamatsu backthinned EM-CCD camera. Images were acquired using 100X oil immersion and the appropriate filters. Z stacks were constructed and images deconvolved using Velocity software (Improvement, Lexington, MA).

**PCR**

RNA was isolated from HK-2 cells using Trizol (Life Technologies, Burlington, ON, Canada), and reverse transcriptase-PCR for CX3CL1 was performed using oligo(dt) and specific primers (24). PCR products were size-fractionated on 1% agarose in TBE gels and stained with ethidium bromide.

**Immunoblotting**

SDS-PAGE and immunoblotting were performed using anti-CX3CL1 Ab (0.2 μg/ml) (27,34). Immunoreactive bands were visualized by enhanced chemiluminescence (Amersham Biosciences UK Limited, Buckinghamshire, UK) recorded on x-ray film. Detergent resistance was assessed as described previously (34). Triton-soluble and -insoluble components were compared with total cell lysate.

**Electron Microscopy**

Scanning electron microscopy (EM) was performed as described previously using anti-CX3CL1 Ab (1 μg/ml) and 18 nm of gold-labeled anti-goat IgG secondary antibody (34).

**Fluorescence Recovery after Photobleaching**

Experiments were performed as described previously (34,35). Briefly, the apical surface was brought into focus on a confocal microscope, and two 2.5-μm diameter areas of similar fluorescence intensity were selected per cell. After acquisition of baseline measurements, one defined area was photobleached irreversibly, and fluorescence of both areas was measured over time. Fractional fluorescence recovery of the bleached area was determined relative to prebleach measurements. The unbleached area was used to control for inadvertent bleaching during repeated image acquisition. Results are expressed as the mean fluorescence recovery for at least six experiments, with SEM.

**Adhesion Assays**

Experiments were performed as described elsewhere with minor modifications (10). Briefly, MDCK-CX3CL1-GFP cells were grown to confluence on 25-mm coverslips and pretreated with 10 μM TAPI-2 for 2 h to maximize cell surface expression of CX3CL1. K562-CX3CR1 cells were labeled with cholera toxin B-555, and 1 × 10⁶ cells were added to wells of either MDCK or MDCK-CX3CL1-GFP cells with gentle rocking at 10 cycles/min. Nonadherent cells were washed away, and remaining cells were fixed and mounted onto slides. Using a Leica deconvolution microscope, we examined at least 50 high-power fields (×63) to count the number of adhered cells. Results are the mean of three separate experiments with SEM and were compared using paired t test. Cells also were examined using a spinning disk confocal microscope.

**Results**

**CX3CL1 Is Expressed in RTEC**

To define the functions of CX3CL1 in RTEC, we first examined CX3CL1 expression in five human renal allograft biopsy specimens. Three specimens had histologic diagnosis of acute
CX3CL1 expression in renal tubules, particularly on the apical surface (Figure 1, A and B). In addition to being expressed on tubular epithelium, modest CX3CL1 expression was noted within the glomeruli and on vascular endothelium (data not shown). This is in keeping with a previously published report (19). To verify the specificity of staining, we omitted primary anti-CX3CL1 Ab from some experiments. When the secondary Ab alone was used, very little background immunofluorescence was observed (data not shown). At our institution, donor preimplantation renal transplant biopsies are not performed routinely and therefore could not be used as an additional negative control. However, using the same anti-CX3CL1 Ab as the one used in our study, other investigators previously reported minimal immunohistochemical expression of CX3CL1 in normal renal biopsy tissue (19,32). We then examined expression and subcellular distribution of CX3CL1 in HK-2 cells, a human RTEC line. Using reverse transcriptase–PCR, we detected CX3CL1 mRNA (Figure 1C). To verify CX3CL1 protein expression, we performed immunofluorescence staining and again found the chemokine predominantly at the apical surface (Figure 1D).

CX3CL1 Is Expressed in the Apical Membrane of RTEC

Because HK-2 cells, unlike other tubular epithelial lines, grow in flattened monolayers, we adopted another approach, stably expressing full-length CX3CL1 or CX3CL1-GFP in MDCK cells, a renal cell line with cuboidal morphology that is typical of epithelial cells, facilitating distinction between apical and basolateral surfaces. Using Western analysis and anti-CX3CL1 Ab, MDCK-CX3CL1 and MDCK-CX3CL1-GFP cells each displayed a single band of appropriate molecular weight (Figure 2A). For assessment of the subcellular localization of CX3CL1, MDCK-CX3CL1-GFP cells (Figure 2B) were fixed and incubated with anti-CX3CL1 Ab, which labeled only the apical surface (Figure 2C). For visualization of intracellular CX3CL1, the same cells then were permeabilized and incubated with anti-CX3CL1 Ab followed by a different secondary Ab. This confirmed an intracellular pool of CX3CL1 in subapical vesicles (Figure 2D). To confirm that any labeling of MDCK-CX3CL1-GFP cells with anti-CX3CL1 Ab was specific for CX3CL1, we incubated untransfected MDCK cells with the same Ab. In this instance, no immunofluorescence staining was observed (data not shown). To control further for any background staining of Cy3- or Cy5-conjugated secondary Ab used, we also performed experiments in which the primary Ab was omitted. Once again, incubation with secondary Ab alone yielded no immunofluorescence labeling (data not shown). Collectively, these results demonstrate that CX3CL1 is expressed on the apical plasma membrane as well as within subapical vesicles.

In MDCK-CX3CL1-GFP cells, GFP is attached to the cytoplasmic tail of the chemokine. Because the tagged construct was recognized by the Ab directed against the extracellular domain of CX3CL1, the protein expressed must traverse the apical membrane (Figure 2, B and E). We also noted a second pool of GFP, present in the basolateral membrane (Figure 2B). After permeabilization, the basolateral pool of GFP could be labeled by an anti-CX3CL1 Ab directed against the intracellular domain of the chemokine (data not shown). We believe that basolateral GFP represents the intracellular portion of CX3CL1 that remains after the extracellular segment has been proteolytically released. Accordingly, Western analysis of MDCK-CX3CL1-GFP cells using anti-GFP Ab consistently revealed two separate bands. The molecular masses of these bands correlated with full-length CX3CL1-GFP fusion protein (Figure 2A) or a fusion protein corresponding to the intracellular region of CX3CL1 together with GFP (35 kD; data not shown). To ensure that the GFP tag did not alter subcellular traffic of CX3CL1, we examined MDCK cells that expressed the untagged chemokine and found a similar distribution (Figure 2F).

We used scanning EM to confirm that CX3CL1 is expressed on the apical membrane of RTEC. As shown in Figure 2G, CX3CL1 is associated with microvilli on the apical surface.

CX3CL1 Is Targeted to the Apical Membrane by N-Glycosylation

We next investigated the signals that target CX3CL1 to the apical membrane. Apical sorting of transmembrane proteins can be determined by signals that are conferred by the cytoplasmic tail of the protein (36,37), by O- or N-glycosylation (38), or by membrane anchoring of the protein through GPI or directly to lipid rafts (39). We examined each of these potential mechanisms.
CX3CL1 Is Anchored to the Apical Membrane in RTEC

To determine whether CX3CL1 is anchored in the luminal surface, we used fluorescence recovery after photobleaching to study the mobility characteristics of apical CX3CL1-GFP in MDCK-CX3CL1-GFP cells (see Materials and Methods) (Figure 4A, A [before bleaching], B [immediately after bleaching], and C [5 min after bleaching]). As a control, MDCK cells were transfected with a DNA construct that encoded GPI-GFP, which is freely mobile in the apical plasma membrane. A region of the apical membrane was bleached similarly, and the recovery of fluorescence was observed over time (Figure 4D through F). CX3CL1-GFP recovered with significantly slower kinetics than GPI-GFP (Figure 4G). For CX3CL1-GFP, the bleached area recovered to 10.5 ± 4.3% of prebleached fluorescence intensity by 4 min, compared with 61.1 ± 11.9% for GPI-GFP (P < 0.01). As an additional control, MDCK cells were transfected with DNA that encoded the single transmembrane protein FcR-GFP. When fluorescence recovery after photobleaching was performed similarly, the bleached area in the membrane recovered significantly faster than CX3CL1-GFP, with 4-min recovery of 51.5 ± 6.1% for FcR-GFP (P < 0.01; Figure 4G). These data demonstrate that the ability of CX3CL1 to diffuse laterally within the apical membrane is impeded, and, therefore, transmembrane CX3CL1 is immobile.

Some single-pass transmembrane proteins are tethered to the apical membrane by direct association with lipid rafts (42). To determine whether CX3CL1 is anchored in such a manner, we disrupted lipid rafts by treating cells with MβCD. However, this treatment did not mobilize CX3CL1 in the apical membrane (Figure 4G). To study whether apical CX3CL1 is immobile because it is tethered directly to the actin cytoskeleton, we next assessed potential associations of CX3CL1 with lipid rafts. We treated cells with MβCD to extract cholesterol, thereby disrupting lipid rafts (40). To ensure that MβCD treatment was effective, we measured cholesterol spectrophotometrically (41). MβCD depleted total cellular cholesterol by 58% (P < 0.05; Figure 3B) but had no effect on apical targeting of CX3CL1 (Figure 3C).

We next examined glycosylation of CX3CL1 as a determinant of apical targeting. O-glycosylation was inhibited with benzyl-2-acetamido-2-deoxy-α-D-galacto-pyranoside. Again, CX3CL1 targeting to the apical membrane remained intact (Figure 3D). To inhibit N-glycosylation of CX3CL1, we treated cells with tunicamycin. This treatment resulted in intracellular retention of CX3CL1 with virtually no expression observed on the apical surface (Figure 3E). To verify that the observed effects were not due to nonspecific disruption of apical protein traffic, we evaluated the effects of tunicamycin on NHE3, a protein that does not require N-glycosylation for its apical targeting. As expected, tunicamycin treatment did not affect the normal apical distribution of NHE3 (Figure 3F).

Collectively, these data indicate that the signals that direct apical trafficking of CX3CL1 are not conferred by the protein’s cytoplasmic domain, O-glycosylation of the protein, or associations with lipid rafts. Rather, apical localization of CX3CL1 is determined by N-glycosylation.

To determine whether the cytosolic tail of CX3CL1 mediates apical targeting, we generated a mutant construct that lacked the cytoplasmic domain (CX3CL1–360). We examined the distribution of CX3CL1 in MDCK cells that stably expressed “tailless” CX3CL1 but found that the protein still was directed to the apical membrane (Figure 3A). We then assessed potential associations of CX3CL1 with lipid rafts. We treated cells with MβCD to extract cholesterol, thereby disrupting lipid rafts (40). To ensure that MβCD treatment was effective, we measured cholesterol spectrophotometrically (41). MβCD depleted total cellular cholesterol by 58% (P < 0.05; Figure 3B) but had no effect on apical targeting of CX3CL1 (Figure 3C).

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fractionated cellular proteins between two distinct fractions—triton soluble and triton insoluble—as described previously (33). In polarized epithelial cells, proteins that were anchored directly to the actin cytoskeleton preferentially remain in the triton-insoluble fraction. We found, however, that CX3CL1 largely was soluble in triton solution, suggesting that it is not tethered directly to the actin cytoskeleton (Figure 4H). These data also confirm that CX3CL1 is not associated with lipid rafts (Figures 3C and 4G). Collectively, these data indicate that after sorting to the apical surface, CX3CL1 is held immobile there, but this is not achieved by direct tethering to the actin cytoskeleton or by association with lipid rafts.

**CX3CL1 Expressed on the Apical Surface of RTEC Promotes Adhesion of Leukocytes**

CX3CL1 can act as a cell adhesion molecule, facilitating the binding of leukocytes (7,43). We therefore hypothesized that CX3CL1 might perform a similar function when expressed on
the apical surface of RTEC. Under the low-shear conditions used, there was minimal binding of control K562 leukocytes to either MDCK or MDCK-CX3CL1-GFP cells (data not shown). We found significant binding of K562-CX3CR1 leukocytes to MDCK-CX3CL1-GFP cells (Figure 5, A and B). When CX3CL1 was present on the apical membrane, leukocyte adhesion was almost three times greater than when CX3CL1 was absent ($P < 0.001$; Figure 5C).

To verify that any observed responses were not an idiosyncratic feature of transformed cell lines, we performed the same experiments using primary, untransfected cells. Human PBMC...
were isolated from heparinized blood of healthy volunteers. PBMC contain multiple leukocyte subsets, including CD8+ T lymphocytes, monocytes, and NK cells, all of which express the CX3CR1 receptor. PBMC efficiently adhered to the apical surface of monolayers of primary human RTEC. When primary RTEC were preincubated with function-blocking anti-CX3CL1 Ab, binding of PBMC was inhibited significantly (Figure 5D). Collectively, these data suggest that CX3CL1 expressed on the luminal surface of RTEC promotes adhesion of leukocytes that bear the complementary receptor CX3CR1. Our results are in keeping with previous reports in which blockade of CX3CL1/CX3CR1 impaired binding of THP-1 monocytic cells to primary proximal RTEC (32).

**Discussion**

The expression and the function of CX3CL1 in endothelial cells have been well described (6,7,27,43). In the kidney, an important role for CX3CL1 has been found in diseases that involve glomerular inflammation and endothelial injury (19,20,22,23). CX3CL1 also is upregulated in renal tubulointerstitial inflammation, most notably in acute allograft rejection (19), but the specific localization of the chemokine (apical versus basolateral) has been somewhat unclear (32). The purpose of these studies, therefore, was to ascertain the subcellular distribution and targeting of CX3CL1 in polarized tubular epithelial cells. Unexpectedly, we found that transmembrane CX3CL1 was expressed predominantly on the apical surface of RTEC. This pattern of expression mirrors that found in epithelial cells of biliary ductules (44) but differs markedly from that of intestinal epithelial cells, where CX3CL1 largely is expressed in the basolateral membrane and in regions of intercellular contact (45,46). In the intestine, as in the kidney, expression of CX3CL1 is highly enhanced in disease states that are marked by active inflammation (14,45,47).

We next examined how CX3CL1 is sorted to the apical membrane. In polarized cells, several signals can sort transmembrane proteins to either the basolateral or the apical membrane. The sorting determinant may lie in the cytoplasmic, transmembrane, or extracellular domain of the protein. Sorting signals in the cytoplasmic tail include PDZ domains and dileucine motifs, used by the cystic fibrosis transmembrane regulator and the IgG Fc receptor, respectively (36,37). By deleting the cytoplasmic tail of CX3CL1, we excluded an apical targeting signal in this region. Other transmembrane proteins, such as Thy-1, apically target through direct or indirect associations with lipid rafts (48). However, when we disrupted any potential association with rafts by extracting cholesterol, we found no change in the distribution of CX3CL1.

Carbohydrate modification of the protein backbone also may direct trafficking of transmembrane proteins, as is the case for sucrase isomaltase, which requires O-glycosylation for apical targeting (38). CX3CL1 has 26 O-glycosylation sites in the mucin stalk. However, when we inhibited O-glycosylation, CX3CL1 still trafficked to the apical membrane. For other proteins, such as endolyn and the glycine transporter GLYT2, N-glycosylation is the crucial determinant of apical targeting (49,50). CX3CL1 has a single N-glycosylation site, located in the chemokine domain (2). Our studies indicate that glycosylation of this site is the key signal that directs CX3CL1 to the apical membrane.

We postulated that after translocating to the apical membrane, CX3CL1 would become anchored there, positioning it to act as a cellular adhesion molecule (7,10,43,51). Firm anchoring of the chemokine within the membrane would allow it to tether passing cells that express the receptor CX3CR1. Our experiments indicate that CX3CL1 indeed is immobile, similar to another adhesion molecule, CD44 (42,52).

We next considered how CX3CL1 might be physically anchored to the apical membrane. Some adhesion molecules, including CD44, are tethered to the cell membrane by direct association with lipid rafts. We therefore examined the effect of cholesterol depletion and disruption of rafts on apical tethering of CX3CL1. Disruption of lipid rafts did not increase the lateral mobility of CX3CL1. Moreover, CX3CL1 segregated with the detergent-soluble, rather than -insoluble, cellular fraction, further refuting a direct association with lipid rafts. These data also suggest that CX3CL1 is not anchored directly to the actin cytoskeleton. A recent model of membrane fluidity proposed that transmembrane proteins might be fenced into discrete regions of the membrane (53). Such a mechanism might account for the limited lateral mobility of CX3CL1. Alternatively, CX3CL1 could be anchored indirectly to the actin cytoskeleton by one or more triton-soluble adaptor proteins (54).

Although our studies demonstrate that CX3CL1 is targeted and anchored within the apical membrane, the key question is what role it might play there. CX3CL1 in intestinal epithelium has been shown to recruit leukocytes in inflammation and to regulate cell survival and wound repair (7,45,55). In intestinal epithelium, CX3CL1 is expressed predominantly on the basolateral surface, where it is in direct contact with CX3CR1-expressing host leukocytes, including macrophages, lymphocytes, and dendritic cells. In the lamina propria, basolateral CX3CL1 tethers dendritic cells to intestinal epithelium. Adherent dendritic cells then form transepithelial protrusions, which they extend into the gut lumen, facilitating immunosurveillance and defense against enteroinvasive bacteria (46). In contrast, no such immunosurveillance is known to occur within the kidney or the intrahepatic biliary tree. In biliary ductular epithelial cells, CX3CL1 is expressed both apically and basolaterally (44,47). CX3CL1 on the apical surface of regenerating biliary epithelial cells has been implicated in repair of the biliary tree after acute injury, presumably by recruiting hepatic stem cells, which express CX3CR1 (47). In RTEC, a similar role has been proposed for CD44. CD44 normally is expressed on the basolateral membrane, where it participates in cell–matrix interactions. However, after renal tubulointerstitial injury, CD44 is expressed apically, where it is thought to promote regeneration and repair (56,57).

**Conclusion**

We have found that CX3CL1 on the apical membrane promotes tethering of leukocytes that bear the complementary receptor. During tubulointerstitial renal inflammation, tight junctions break down, potentially allowing leukocytes to es-
cape into the urinary space. We postulate that apical CX3CL1 facilitates recruitment and retention of these leukocytes, redirecting them to the site of injury. In this manner, CX3CL1 may play an important role in tubulointerstitial nephritis, pyelonephritis, and renal allograft rejection, disease processes in which expression of CX3CL1 is highly upregulated. As with biliary epithelial cells, CX3CL1 may also participate in regeneration of tubules after inflammatory injury. Further work is needed to ascertain the in vivo functions of CX3CL1 located on the apical membrane of RTEC.

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Disclosures

None.

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