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Renal tubular fluid shear stress facilitates monocyte activation toward inflammatory macrophages

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Miravète M, Dissard R, Klein J, Gonzalez J, Caubet C, Pecher C, Pipy B, Bascands JL, Mercier-Bonin M, Schanstra JP, Buffin-Meyer B. Renal tubular fluid shear stress facilitates monocyte activation toward inflammatory macrophages. *Am J Physiol Renal Physiol* 302: F1409–F1417, 2012. First published March 9, 2012; doi:10.1152/ajprenal.00409.2011.—Modified urinary fluid shear stress (FSS) induced by variations of urinary fluid flow and composition is observed in early phases of most kidney diseases. Recently, we reported that renal tubular FSS promotes endothelial cell activation and subsequent adhesion of human monocytes, thereby suggesting that changes in urinary FSS can induce the development of inflammation (Miravète M, Klein J, Besse-Patin A, Gonzalez J, Pecher C, Bascands JL, Mercier-Bonin M, Schanstra JP, Buffin-Meyer B, *BBRC* 407: 813–817, 2011). Here, we evaluated the influence of tubular FSS on monocytes as they play an important role in the progression of inflammation in nephropathies. Human renal tubular cells (HK-2) were exposed to FSS 0.01 Pa for 30 min or 5 h. Treatment of human THP-1 monocytes with the resulting conditioned medium (FSS-CM) modified the expression of macrophage differentiation markers, suggesting differentiation toward the inflammatory M1-type macrophage. The effect was confirmed in freshly isolated human monocytes. In contrast to endothelial cells, the activation of monocytes by FSS-CM did not require TNF-α. Cytokine array analysis of FSS-CM showed that FSS modified secretion of cytokines by HK-2 cells, particularly by increasing secretion of TGF-β and by decreasing secretion of C-C chemokine ligand 2 (CCL2). Neutralization of TGF-β or CCL2 supplementation attenuated the effect of FSS-CM on macrophage differentiation. Finally, FSS-injured HK-2 cells expressed and secreted early biomarkers of tubular damage such as kidney injury molecule 1 and neutrophil gelatinase-associated lipocalin. In conclusion, changes in urinary FSS should now also be considered as potential insults for tubular cells that initiate/perpetuate interstitial inflammation.

renal tubular damage; mechanical insult; hydrodynamic forces; macrophage infiltration

INFLAMMATION IS A KEY AND early event in the progression of many renal diseases. The main infiltrating cells are monocyte-derived macrophages, and their accumulation in the tubular interstitium is well correlated with the progression of nephropathies (17). Monocyte recruitment in damaged tissue requires the contribution of the vascular endothelium, which synthesizes chemokines and adhesion molecules in response to inflammatory cytokines (47, 51). Subsequently, these recruited monocytes undergo differentiation toward macrophages that adopt various states of functional activation: *1*) classically activated (M1) macrophages that display mainly proinflammatory activities and *2*) alternatively activated (M2) with anti-inflammatory and tissue remodeling activities (34, 47, 51, 62).

Tubular cells in the kidney are continuously exposed to urinary fluid shear stress (FSS) generated by luminal urine flow. Over the last years, a number of reports have shown urinary FSS as a mechanical signal regulating renal tubule function (7, 16, 27, 64). Chronic variations in urinary FSS take place in most nephropathies (43, 50), and recently, we demonstrated in vitro that tubular FSS stimulated both tubular and endothelial TNF-α secretion, thereby facilitating monocyte adhesion to endothelial cells (43). Thus, modification of urinary FSS may potentially contribute to the inflammatory state involved in initiation/perpetuation of renal diseases.

Given the important role of the monocyte-derived macrophage activation state in the progression of inflammation, we studied the effect of tubular FSS on the monocyte phenotype. In addition, as tubular cells are first in line in many inflammatory renal diseases, we also evaluated the effect of FSS on expression of early biomarkers of tubular injury. For this purpose, we exposed human proximal tubular (HK-2) cells to low-intensity FSS (0.01 Pa), as to mimic the acute reperfusion phase in transplanted kidneys (43). We observed that FSS-stimulated tubular cells displayed increased kidney injury molecule 1 (KIM1) and neutrophil gelatinase associated lipocalin (NGAL) mRNA and protein levels, suggesting tubular cell damage. In addition, FSS-exposed HK-2 cells released factors, including increased TGF-β and reduced C-C chemokine ligand 2 (CCL2), which induced the differentiation of human monocytes into inflammatory macrophages. Thus, these results confirm the concept of urinary FSS as an early tubular aggressor that promotes the inflammatory process in renal pathologies.

MATERIALS AND METHODS

Reagents. Human neutralizing TNF-α and TGF-β antibodies were from R&D Systems (Minneapolis, MN). Recombinant CCL2 was from PeproTech (Neuilly-Sur-Seine, France).

Cells. HK-2 (immortalized human proximal tubular cells) and THP-1 cells (human leukemia cells with monocytic characteristics) were cultured at 37°C in 5% CO_2 atmosphere in DMEM (cat. no. 31966; InVitrogen, Rockville, MD) containing 10% FCS (InVitrogen). Peripheral blood mononuclear cells (PBMC) were isolated from the cytapheresis residues obtained from healthy donors by density gradient on Lymphoprep (AbCys, Paris, France) according to the manufacturer's instructions.

HK-2 cell exposure to shear stress. Confluent HK-2 cells grown on gelatin-coated plastic slides were placed in 5% FCS-DMEM for 18 h

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before stimulation. Then slides were assembled into a homemade parallel plate flow chamber. The input of the chamber was connected to a flask containing 5% FCS-DMEM. Flow of medium culture was controlled by peristaltic pump for 30 min or 5 h, and the resulting supernatant was collected (2.7 ml or 27 ml, according to flow rate). The flow system was kept at 37°C in 5% CO_2. FSS (0.01 Pa) was based on the formula $6\mu Q/h^2l$ where μ is the fluid dynamic viscosity (0.7×10^{-3} Pa/s), Q is the flow rate (90 μl/min), h and l are, respectively, the flow channel thickness (0.205 mm) and width (10 mm). For the control condition, HK-2 cells were handled similarly but maintained in static conditions: in experiments where the effects of 30 min of FSS were evaluated, control HK-2 cells were maintained in static conditions for 30 min with 2.7 ml of 5% FCS-DMEM (e.g., the same volume of medium used in 30 min FSS experiments); to obtain controls for the 5 h FSS experiments, cells were incubated under static conditions for 5 h in 27 ml of culture medium. FSS did not modify the viability of HK-2 cells (data not shown), as evaluated by measuring adenylate kinase release (Lonza, Rockville, MD). In addition, FSS did not change the pH value of the culture medium (data not shown).

Analysis of mRNA expression. Total RNAs from cells were isolated using the RNeasy plus Mini kit (Qiagen) and analyzed by real-time PCR with SYBRGreen (Eurogentec, Liège, Belgium) and using ABI-PRISM 7900HT, as previously described (8). The mRNA copy number was calculated using the cycle threshold value and was normalized to 18S RNA.

Treatment of PBMC. PBMC were treated for 2 h (37°C in 5% CO_2 atmosphere) with supernatant from HK-2 cells exposed or not to FSS (1.25 ml of FSS-CM and static-CM, respectively). The nonadherent cells were removed by three PBS washes (Eurobio, Les Ulis, France), and the adherent cells, predominantly monocytes, were incubated for 24 h [which has been shown sufficient for LPS (M1 macrophage promoter) to induce TNF-α production by these cells (22, 40)] with static-CM or FSS-CM (1.25 ml). The viability of monocytes treated with static-CM or FSS-CM, assessed by adenylate kinase release (Lonza), was similar (data not shown).

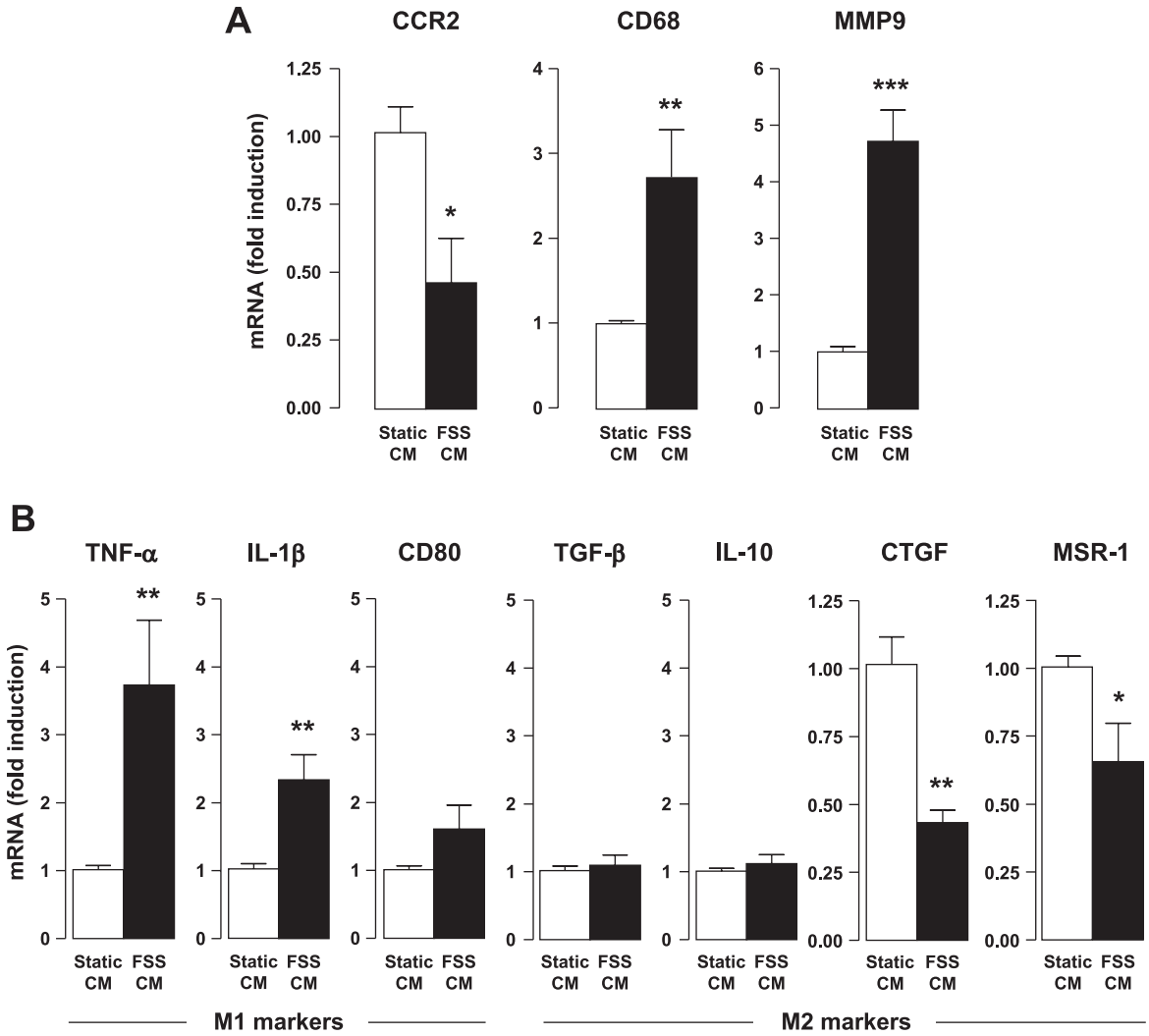

Fig. 1. Differentiation of THP-1 monocytes into M1 macrophages after treatment with conditioned medium from HK-2 renal tubular cells subjected to fluid shear stress (FSS). Confluent monolayers of HK-2 cells were submitted to FSS 0 (static) or FSS 0.01 Pa for 30 min, and conditioned medium (static-CM and FSS-CM, respectively) was collected to treat THP-1 cells for 48 h. *A*: monocyte/macrophage differentiation. *B*: macrophage polarization. The level of mRNA encoding for monocyte C-C chemokine receptor 2 (CCR2) or macrophage [CD68, matrix metalloproteinase 9 (MMP9)] markers and for M1 (TNF-α, IL-1β, CD80) or M2 [TGF-β, IL-10, connective TGF (CTGF) and macrophage scavenger receptor-1 (MSR-1)] polarization signatures were analyzed in THP-1 cells by real-time PCR. Results are expressed as the fold induction compared with static-CM. Data represent means ± SE of 4–7 experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. static-CM.

Release of TNF-α by isolated human monocytes. To measure TNF-α secreted by PBMC-derived-monocytes, fractions (20 μl) of supernatant, were collected every 2 h and immediately frozen to avoid potential degradation of secreted TNF-α over the entire time span of the experiment. At the end of the experiment, all fractions were pooled and TNF-α quantities were determined by ELISA (Pepro-Tech), as described by the manufacturer.

Antibody cytokine array. The cytokine expression profile in the supernatant of FSS activated-HK-2 cells was analyzed using the RayBiotech cytokine array VI (Raybiotech, Norcross, GA), as described by the manufacturer. Enhanced chemiluminescence (ECL) signals were captured on X-OMATBlue XBfilm (Kodak Scientific Imaging Film), and ECL signal intensity was determined by densitometry using ImageJ1.43u software. For each spot, the mean intensity of blank spots was subtracted, and the resulting difference was normalized to the mean intensity of positive spots.

KIM1 and NGAL protein release. To measure KIM1 and NGAL protein release by HK-2 cells subjected to FSS, medium was collected at the end of FSS exposure. NGAL or KIM1 protein was analyzed by ELISA (R&D Systems), as described by the manufacturer.

Statistical analysis. Data are expressed means ± SE. A Student's *t*-test and an ANOVA analysis with the post hoc Bonferroni test were performed to compare two groups or more, respectively. $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Conditioned medium of FSS subjected-tubular cells increases monocyte activation. In vivo, injured tubular cells secrete inflammatory cytokines/chemokines that facilitate recruitment of circulating monocytes in the interstitium and, subsequently, their differentiation into macrophages (23, 47). Therefore, we examined whether the molecules released by tubular cells subjected to FSS activate differentiation of monocytes into macrophages. For this, HK-2 cells were submitted to FSS 0 (static) or FSS 0.01 Pa for 30 min. This condition mimics in vitro the increase of urinary FSS that takes place during the reperfusion phase after the ischemia period in transplanted kidney (43). Indeed, during the ischemia period, urinary shear stress is absent since the nonirrigated nephrons do not filter plasma. However, when renal blood supply returns, glomerular filtration and subsequently urinary tubular fluid flow are restored, leading to abrupt induction of urinary shear stress. Urinary FSS does not reach physiological FSS [0.1 Pa (4, 15)] because animals subjected to ischemia-reperfusion (I/R) do not completely recover normal glomerular filtration rate (GFR) in the first hours following reperfusion (9, 10, 12, 67).

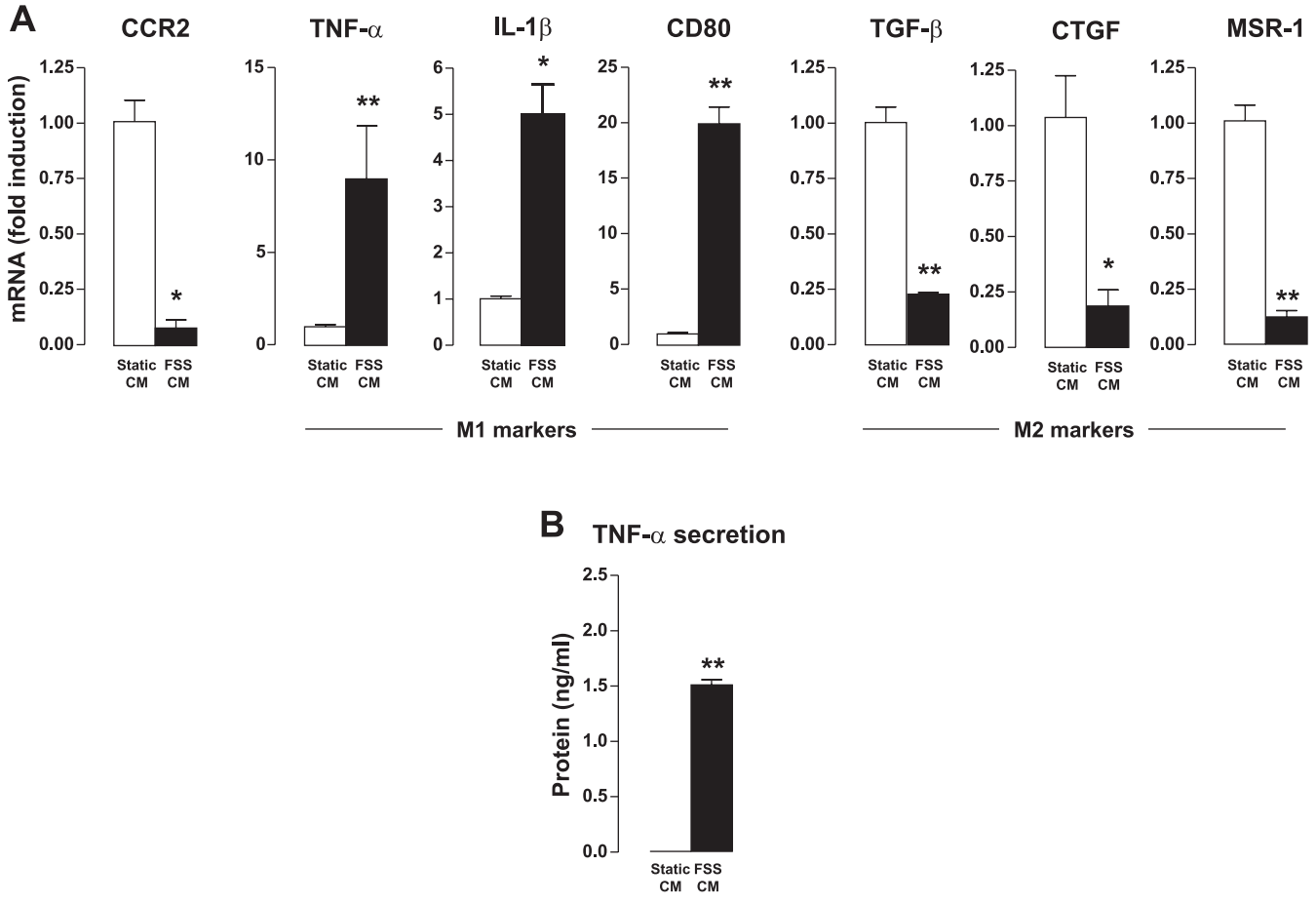

Fig. 2. M1 polarization of freshly isolated human monocyte macrophages in response to FSS-CM. Confluent monolayers of HK-2 cells were submitted to FSS 0 (static) or FSS 0.01 Pa for 30 min, and conditioned medium (static-CM and FSS-CM, respectively) was collected to treat peripheral blood mononuclear cells (PBMC) for 24 h. *A*: monocyte and macrophage mRNA expression levels. *B*: TNF-α protein secretion. The level of mRNA encoding for monocyte (CCR2), M1 macrophage (TNF-α, IL-1β, CD80) or M2 macrophage (TGF-β, CTGF, MSR-1) markers was analyzed in PBMC, as described in Fig 1. For TNF-α protein content analysis, a 20 μl aliquot of monocyte supernatant was collected every 2 h and frozen at −20°C. At the end of the experiment, aliquots were pooled and analyzed by ELISA. TNF-α released by PBMC was calculated as TNF-α present in pooled aliquots minus TNF-α measured at *time 0* of incubation. Data represent means ± SE of 3–4 experiments. *$P < 0.05$, **$P < 0.001$ vs. static-CM.

Table 1. *HK-2 cells exposed to prolonged fluid shear stress (FSS) continue to activate monocytes*

	Static-lateCM	FSS-lateCM
CCR2	1.03 ± 0.14	0.23 ± 0.11**
MMP9	1.03 ± 0.15	4.55 ± 1.27*
M1 markers		
TNF-α	1.00 ± 0.01	2.56 ± 1.00
IL-1β	1.05 ± 0.20	3.06 ± 0.92
M2 markers		
CTGF	1.00 ± 0.01	0.46 ± 0.07***
MSR-1	1.05 ± 0.18	0.43 ± 0.11*

Confluent monolayers of HK-2 renal tubular cells were submitted to FSS 0 (static) or FSS 0.01 Pa for 5 h, and culture medium (static-lateCM and FSS-lateCM, respectively) was collected for the last 30 min to treat THP-1 cells (48 h). mRNA levels of monocyte (CCR2, MMP9) and macrophage markers (TNF-α, IL-1β, CTGF and MSR-1) were evaluated by real-time PCR, and results are the fold induction compared with static-CM. CCR2, C-C chemokine receptor 2; MMP9, matrix metalloproteinase 9; CTGF, connective TGF; MSR-1, macrophage scavenger receptor-1. Data represent means ± SE of 4 experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. static-CM.

Conditioned medium from static- or FSS HK-2 cells (static-CM and FSS-CM, respectively) was collected and used, without dilution or supplementation, to treat THP-1 monocytes for 48 h [time required for phorbol myristate acetate (PMA, reference compound) to induce monocyte differentiation (11, 25, 48, 68)]. Monocyte/macrophage differentiation was evaluated by quantifying mRNA expression of C-C chemokine receptor 2 (CCR2) as a marker for monocytes, and CD68 and matrix metalloproteinase 9 (MMP9) as markers for differentiation toward macrophages (5, 13, 19, 32, 53). Interestingly, FSS-CM decreased CCR2 and increased CD68 and MMP9 mRNA expression (Fig. 1*A*), suggesting that THP-1 cells acquired a macrophage-like phenotype.

Monocyte-derived macrophages undergo specialization into either inflammatory and pathogenic M1 macrophages or anti-inflammatory and protective M2 macrophages (47). We therefore studied the effect of FSS-CM on M1/M2 polarization of THP-1 cells by measuring cytokine and surface receptor expression. We chose TNF-α, IL-1β, and CD80 as markers for the M1 phenotype and TGF-β, IL-10, connective TGF (CTGF) and macrophage scavenger receptor-1 (MSR-1) as markers for the M2 phenotype (5, 39, 47). FSS-CM induced upregulation of inflammatory cytokines TNF-α and IL-1β without changing expression of CD80. FSS-CM also downregulated CTGF and MSR-1 mRNA levels but did not modify TGF-β and IL-10 expression (Fig. 1*B*). These results suggest that tubular cells exposed to FSS promote differentiation of monocytes into, mainly, M1 macrophages.

The effect of tubular FSS on macrophage polarization was also evaluated using freshly isolated human monocytes. For this, PBMC were incubated for 2 h with conditioned medium from HK-2 cells, to select monocytes on their ability to adhere on plastic. Compared with static-CM, FSS-CM did not modify the number of adherent monocytes (data not shown). However, adherent monocytes treated for 24 h with FSS-CM displayed increased TNF-α, IL-1β, and CD80 (M1 macrophage) and decreased CCR2 (monocyte marker), TGF-β, CTGF, and MSR-1 (M2 macrophage) mRNA levels (Fig. 2*A*). In addition, this effect was associated with increased secretion of TNF-α protein (Fig. 2*B*), thereby confirming the role of tubular FSS in

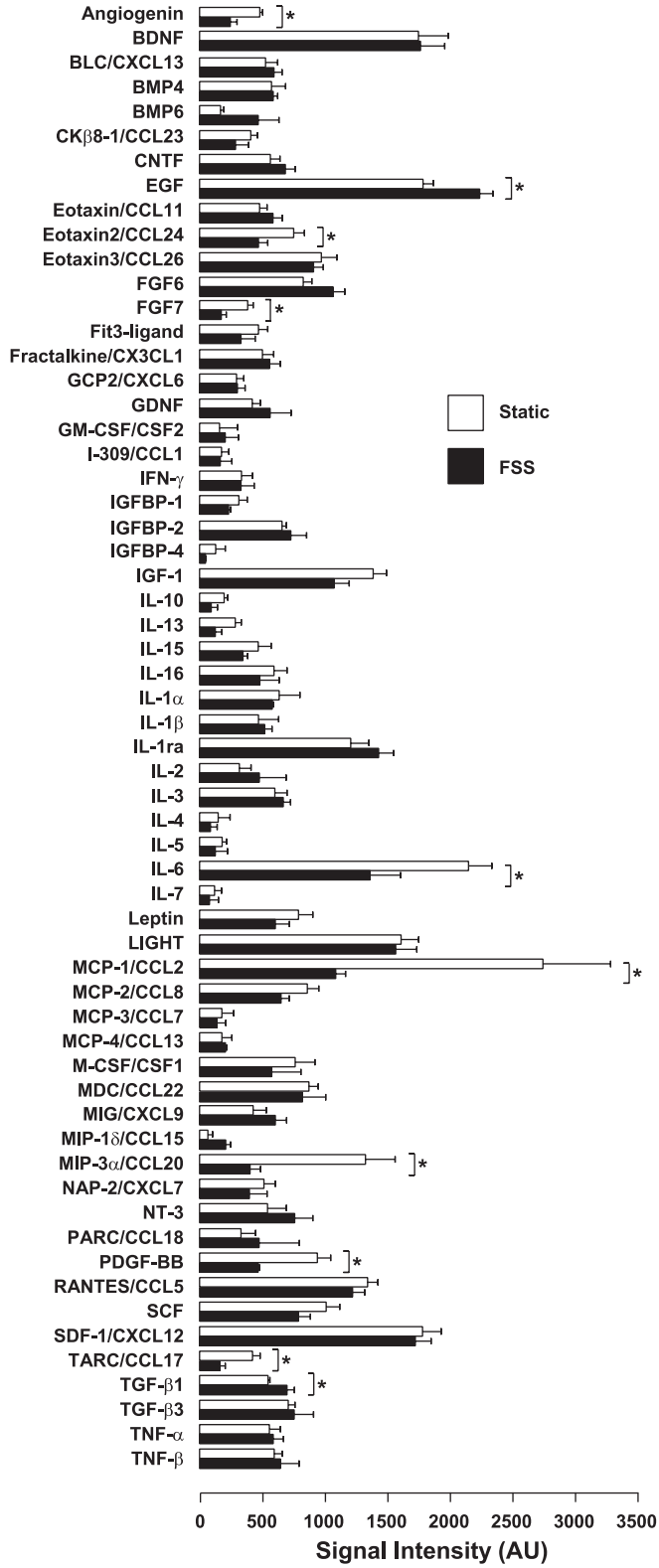

Fig. 3. Cytokine antibody-array profiling of FSS-exposed HK-2 supernatant. Confluent monolayers of HK-2 cells were submitted to FSS 0 (static) or FSS 0.01 Pa for 30 min, and the level of cytokine protein was analyzed using a cytokine antibody array. For each membrane, results are the signal intensity mean of 2 duplicate spots. Data represent means ± SE of 4 experiments. *$P < 0.05$ vs. FSS 0.

the orientation of monocyte-derived macrophages toward the M1-type in freshly isolated human monocytes.

Finally, to verify whether prolonged tubular FSS exposure continues to activate monocytes, HK-2 cells were submitted to 0.01 Pa FSS for 5 h and the effects of conditioned medium (static-lateCM and FSS-lateCM, respectively) on THP-1 cells were studied. FSS-CM continued to promote monocyte/macrophage differentiation, as shown by reduced CCR2 and increased MMP9 mRNA expression in THP-1 cells (Table 1). In addition, the preferential macrophage orientation toward the M1 phenotype was maintained after longer periods of exposure to FSS since FSS-CM induced decreased CTGF and MSR-1 mRNA expression. TNF-α and IL-1β expression, however, was not significantly increased (Table 1). In the context of in vivo transplantation, renal grafts undergo temporary blood deprivation followed by the return of blood supply. In parallel, modifications of glomerular filtration and subsequently of urinary fluid circulation in tubules occur, leading to transitory changes in FSS (halted, followed by abruptly induced FSS) on tubular cells. I/R injury is associated with delayed graft function, which affects long-term transplant evolution (45), and interstitial macrophage infiltration contributes to persistent inflammation in both acute lesions and long-term development of fibrosis (28, 35). Although ischemia in I/R is a critical factor in organ transplantation, reperfusion causes severe local oxidative stress and inflammation leading to additional tissue lesions (18, 63). Present results suggest that urinary FSS induces tubular secretion of inflammatory molecules, leading to stimulation of monocytes/macrophages and subsequent inflammation. In this manner, increased urinary FSS may participate in related damage observed after renal reperfusion.

Other pathophysiological situations associate increased urinary FSS and renal lesions. For example, after reduction of renal mass (during subtotal nephrectomy in animal models or following cancers or trauma in human), residual nephrons compensate renal ablation by increased GFR (26). This adaptation is considered beneficial at the level of renal function since it minimizes the reduction on total GFR. However, elevated GFR also leads to damage of the remnant nephrons (6, 26) involving inflammation, with coordinated regulation of CCL2, TGF-β, TNF-α, and IL-1β, macrophage recruitment (54), and fibrosis (1, 6). Although the in vitro comparison of static versus FSS cannot be extrapolated to the situation of nephrectomy, it is tempting to speculate that part of tubular lesions induced by glomerular hyperfiltration in this situation may be caused by increased urinary FSS and its ability to trigger inflammation. Further experiments comparing physiological versus increased FSS should be performed to validate this hypothesis.

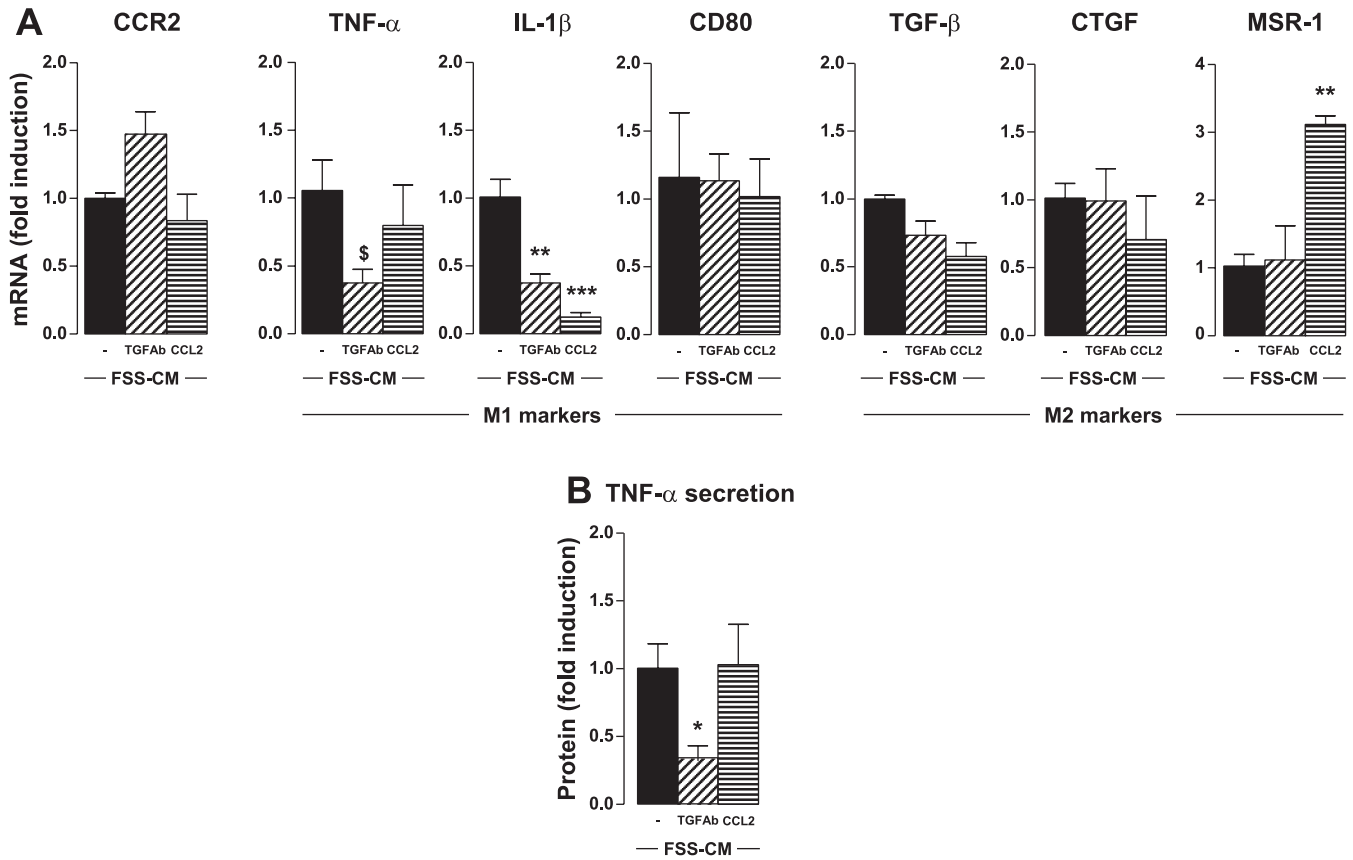

Fig. 4. TGF-β blockade and CCL2 addition attenuate FSS-CM-induced monocyte activation. Confluent monolayers of HK-2 cells were exposed to FSS 0.01 Pa for 30 min. Culture medium (FSS-CM) was collected and preincubated for 1 h with a TGF-β antibody (5 μg/ml, TGFAb) or recombinant CCL2 (40 ng/ml, CCL2). PBMC were then treated for 24 h with FSS-CM ± TGFAb or CCL2. *A*: monocyte and macrophage mRNA expression levels. *B*: TNF-α protein secretion. The level of mRNA encoding for monocyte (CCR2), M1 macrophage (TNF-α, IL-1β, CD80), or M2 macrophage (TGF-β, CTGF, MSR-1) markers and the TNF-α protein content analysis were analyzed in PBMC, as described in Fig 2. Results are expressed as the fold induction compared with FSS-CM alone, and data represent means ± SE of 3–4 experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $\$P = 0.057$ vs. FSS-CM alone.

Profiling of inflammation-related factors in FSS-CM. We subsequently investigated the molecular mechanisms involved in FSS-CM-mediated monocyte activation. We first evaluated the contribution on TNF-α since *1*) we previously demonstrated that TNF-α mediates endothelial cell activation induced by tubular FSS (43) and *2*) TNF-α is known to promote monocyte differentiation toward inflammatory M1 macrophages (38, 39, 47). However, TNF-α neutralization by a specific antibody did not modify the monocyte response to FSS-CM since neither CCR2 nor CD68, MMP9, TNF-α, and IL-1β mRNA expression were changed (data not shown). These results indicate that, in contrast to endothelial cell activation (43), FSS-CM-induced monocyte stimulation does not require TNF-α.

In the next step, we aimed to identify new molecules involved in the effects of FSS-activated HK-2 cells. For this, we analyzed FSS-CM of HK-2 cells using cytokine antibody arrays. We confirmed our previous result showing that FSS did not modify TNF-α secretion by HK-2 cells (43) (Fig. 3). However, FSS significantly reduced tubular release of chemokines (MCP-1/CCL2, TARC/CCL17, MIP-3α/CCL20, and eotaxin-2/CCL24), growth factors (FGF and PDGF-BB), IL-6, and soluble enzyme angiogenin, while it concomitantly increased the release of EGF and TGF-β (Fig. 3). Thus, FSS rearranges the chemokine/cytokine secretion pattern, and this effect could be associated (see below) with increased monocyte activation toward M1 macrophages.

Usually, M1 polarization is induced in vitro by IFN-γ supplemented with inflammatory cytokines (e.g., TNF-α, IL-1) (21, 47, 65), and M2 polarization is obtained in response to IL-4 and IL-13 (M2a) or IL-10 and TGF-β (M2c) (21). These usual stimuli are probably not involved in polarization of monocyte-derived macrophages in our experiments since no significant variation of IFN-γ, TNF-α, IL-1β, or IL-4, IL-10, and IL-13 release from HK-2 cells was observed after FSS. However, FSS modified the expression of other cytokines, which could explain the shift toward the M1 phenotype. Indeed, CCL2 and IL-6 can induce blood monocyte activation into M2 macrophages (49, 52, 56), while CCL17/TARC inhibits their activation in M1 macrophages (31) and the expression of these three cytokines is decreased in response to FSS. In addition, we also observed an increase in TGF-β release. Yet, although considered as a powerful inflammation suppressor, TGF-β is equally recognized for its proinflammatory properties

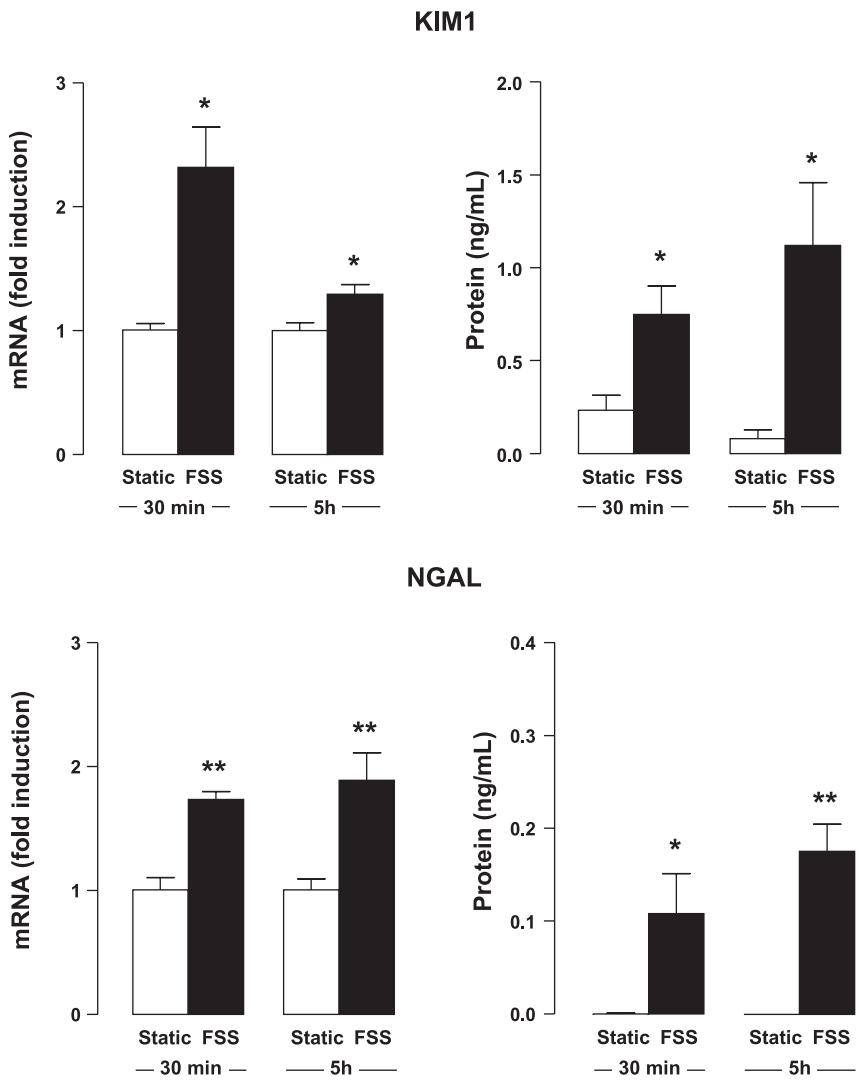

Fig. 5. HK-2 cells subjected to FSS express biomarkers of kidney injury. Confluent monolayers of HK-2 cells were exposed to FSS 0 (static) or FSS 0.01 Pa for 30 min or 5 h, and tubular kidney injury molecule 1 (KIM1) and neutrophil gelatinase-associated lipocalin (NGAL) mRNA and protein secretion were evaluated. mRNA expression was analyzed by real-time PCR, and results are expressed as the fold induction compared with static. To quantify protein released by HK-2 cells, ELISA was performed either in culture medium (CM) collected after 30 min of FSS, or in a pool of CM aliquots (50 μl), collected every 30 min and frozen at −20°C. Data represent means ± SE of 4 experiments. *$P <$ 0.05, **$P < 0.001$ vs. FSS 0.

in certain conditions (46). In particular, in vitro treatment of monocytes with TGF-β stimulated TNF-α, IL-1β, and MMP9 release and decreased MSR-1 expression (14, 41, 55, 61, 66), thus promoting their maturation into M1 macrophages (3). Taken together, a decreased release of CCL2, IL-6, and CCL17 associated with an increased secretion of TGF-β may explain the preferential differentiation of THP-1 cells into inflammatory macrophages in response to FSS stimulated-HK-2 cells.

TGF-β and CCL2 are cytokines involved in the effects of FSS-CM. The array data identified a number of cytokines/chemokines as candidates for the FSS-CM-induced monocyte differentiation. We analyzed the role of TGF-β and CCL2 in the effects of FSS-CM on monocyte differentiation. For this, fresh monocytes were treated for 24 h with FSS-CM alone or with FSS-CM supplemented with either a neutralizing antibody directed against TGF-β or a recombinant CCL2. The two compounds did not change the viability of FSS-CM-treated monocytes (data not shown). As shown in Fig. 4, TGF-β neutralization attenuated the monocyte response to FSS-CM. Indeed, compared with FSS-CM alone, TGF-β antibody tended to increase CCR2 ($P = 0.10$) and decreased TNF-α and IL-1β mRNA levels (Fig. 4*A*). It also reduced TNF-α secretion (Fig. 4*B*), but it did not modify CD80, TGF-β, CTGF, and MSR-1 expression (Fig. 4*B*). CCL2 supplementation also reduced FSS-CM-induced monocyte activation since rCCL2 reduced IL-1β and increased MSR-1 mRNA expression without modifying CCR2, TNF-α, CD80, TGF-β, and CTGF expression (Fig. 4*A*).

Together, these results suggest that both upregulated secretion of TGF-β and downregulated release of CCL2 could play a role in the activation of monocytes into M1 macrophages by FSS-exposed HK-2 cells. The fact that the expression of not all macrophage markers was modified by TGF-β blockade or CCL2 supplementation strongly indicates that other mediators, in addition to TGF-β and CCL2, released by FSS-treated HK-2 contribute to monocyte activation.

In an in vivo context, monocyte migration toward injured sites and subsequent inflammation require a tissue environment rich in chemokines (38). On one hand, chemokines are produced by endothelial cells (47, 51); we have previously demonstrated that tubular cells exposed to FSS secrete mediators (not identified) that activate endothelial cells, increasing release of chemokine CCL2 and facilitating monocyte adhesion (43). Thus, elevated urinary FSS in nephropathies could induce basolateral tubular secretion of molecules that enter the interstitium and signal to endothelial cells to present chemokines to circulating monocytes (29, 38, 51). On the other hand, chemotactic molecules can also originate from other sources (47, 51); we observed that FSS applied in vitro to HK-2 (e.g., tubular) cells stimulated release of EGF and TGF-β, two cytokines known to exhibit monocyte chemotactic activity (37, 61, 66). It is therefore possible in vivo, upon changes in FSS, that these secreted chemotactic molecules are internalized at the abluminal surface of endothelial cells and by transcytosis are presented at the luminal side to circulating monocytes, as previously hypothesized (36, 42, 47, 51).

Tubular cells exposed to FSS express biomarkers of kidney injury. The fact that tubular cells secrete inflammatory mediators in response to FSS suggests that increased FSS represents an aggression for the renal tubule. So as a last step in this study, we evaluated the effect of FSS 0.01 Pa (30 min and 5 h) on tubular expression of KIM1 and NGAL, two well-established markers of renal tubular damage that are markedly upregulated in proximal tubules of most renal diseases including renal transplants (44, 57, 59, 60). Very interestingly, HK-2 cells both exhibited enhanced mRNA expression and protein secretion of KIM1 and NGAL after treatment with both short and long FSS (Fig. 5), thereby confirming the contribution of FSS to tubular injury.

The literature points to several candidates that can be involved in the primary induction of the tubular lesions, such as *1*) increased intratubular protein and growth factors during persistent proteinuria (24), *2*) tubular hypoxia induced by postglomerular microvasculature dysfunction (20), *3*) pressure-induced compression or stretching of tubular cells in obstructive pathologies (33), *4*) high glucose environment as a result from diabetes (58), or *5*) toxic substances (e.g., drugs, metals) exposure (2, 30). Our study suggests that FSS should now also be considered as a potential insult for tubular cells and, consequently, as a new actor in the progression of renal pathologies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.M., J.-L.B., M.M.-B., J.P.S., and B.B.-M. conception and design of research; M.M., R.D., C.P., J.P.S., and B.B.-M. performed experiments; M.M., R.D., C.P., J.P.S., and B.B.-M. analyzed data; M.M., R.D., J.K., J.G., C.C., B.P., J.-L.B., M.M.-B., J.P.S., and B.B.-M. interpreted results of experiments; M.M., R.D., J.K., J.G., C.C., C.P., B.P., J.-L.B., M.M.-B., J.P.S., and B.B.-M. approved final version of manuscript; J.K., J.-L.B., M.M.-B., J.P.S., and B.B.-M. drafted manuscript; J.K., J.-L.B., M.M.-B., J.P.S., and B.B.-M. edited and revised manuscript; J.P.S. and B.B.-M. prepared figures.

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