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Distinct roles of Mac-1 and its counter-receptors in neonatal obstructive nephropathy

B Lange-Sperandio[1], K Schimpgen[1], B Rodenbeck[1], T Chavakis[2,3], A Bierhaus[2], P Nawroth[2], B Thornhill[4], F Schaefer[1] and RL Chevalier[4]

[1]Department of Pediatrics, University of Heidelberg, Heidelberg, Germany; [2]Department of Internal Medicine I, University of Heidelberg, Heidelberg, Germany; [3]Experimental Immunology Branch, NCI, National Institutes of Health, Bethesda, Maryland, USA and [4]Department of Pediatrics, University of Virginia, Charlottesville, Virginia, USA

Urinary tract obstruction during renal development leads to tubular atrophy and interstitial fibrosis. Inflammatory macrophages are crucial in this process, and β_2-integrins play a major role in leukocyte recruitment. We investigated the role of β_2-integrins and their major counter-receptors (intercellular adhesion molecule-1 (ICAM-1), receptor for advanced glycation endproducts (RAGE), junctional adhesion molecule (JAM)-C) in obstructive nephropathy in neonatal mice. Two-day-old β_2-integrin-deficient mice (Mac-1$^{-/-}$ and LFA-1$^{-/-}$(deficient for leukocyte function-associated antigen-1)) and wild-type mice (C57BL/6) underwent unilateral ureteral obstruction (UUO) or sham operation. After 1, 5 or 12 days of obstruction, renal macrophage infiltration and tubulointerstitial damage were quantitated. Tissue abundance of Mac-1 and its ligands ICAM-1, RAGE and JAM-C was examined by Western blot and immunoprecipitation. Deficiency of either integrin was associated with reduced early macrophage invasion into the obstructed kidney. After 12 days of UUO, macrophage infiltration and tubulointerstitial injury were reduced only in Mac-1$^{-/-}$ but not in LFA-1$^{-/-}$ mice. Besides ICAM-1, an upregulation of two novel Mac-1 ligands, RAGE and JAM-C were observed, however, with distinct time courses. We conclude that β_2-integrins mediate macrophage infiltration in UUO. Mac-1 is the predominant leukocyte integrin involved in leukocyte recruitment after obstruction. ICAM-1 and its new ligands RAGE and JAM-C are sequentially activated in UUO. Blocking of Mac-1 and its ligands may confer synergistic renoprotective effects in neonatal obstructive nephropathy.

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Correspondence: *B Lange-Sperandio, Department of Pediatrics, University of Heidelberg, INF 150, 69120 Heidelberg, Germany. E-mail: baerbel.lange-sperandio@med.uni-heidelberg.de*

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Congenital obstructive nephropathy is a major cause of kidney failure in infants and children.[1] Chronic unilateral ureteral obstruction (UUO) leads to interstitial inflammation, interstitial fibrosis and tubular atrophy.[2,3] Central to these events is the acute macrophage infiltration of the tubulointerstitium, which is preceded by local upregulation of macrophage chemokines and adhesion molecules.[4–11] Macrophages contribute to the tubulointerstitial injury by releasing proinflammatory cytokines and cytotoxic substances,[12,13] by inducing apoptosis in tubular cells[14] and by activating interstitial fibroblasts.[15,16] Although the selective infiltration of the interstitium by macrophages in UUO is well established,[17,18] the molecular mechanisms underlying the recruitment of these immune cells in UUO are poorly defined. In particular, although the expression of β_2-integrins and their major endothelial ligand intercellular adhesion molecule-1 (ICAM-1) has been investigated in UUO,[19,20] there are no comprehensive studies assessing the functional role of these molecules in the pathogenesis of UUO.

The recruitment of leukocytes from the circulation and their subsequent influx into surrounding tissues at sites of inflammation or injury requires multistep adhesive and signalling events, including selectin-mediated capture and rolling, leukocyte activation, integrin-mediated firm adhesion and their subsequent transendothelial migration.[21] During firm adhesion of leukocytes to the endothelium, members of the β_2-integrin family, leukocyte function-associated antigen-1 (LFA-1) ($\alpha L\beta_2$, CD11a/CD18) and Mac-1 ($\alpha M\beta_2$, CD11b/CD18) on the leukocyte surface, interact with endothelial counter-receptors such as ICAM-1 and surface-associated fibrinogen. LFA-1 and Mac-1 are exclusively expressed on leukocytes. Whereas LFA-1 contributes to leukocyte rolling by stabilizing the transient attachment, Mac-1 contributes to emigration from the vessel, suggesting that LFA-1 and Mac-1 serve sequential rather than parallel functions.[22,23] In UUO, expression of ICAM-1 has been shown by immunohistochemistry on tubular epithelial cells, interstitial cells and endothelial cells.[24] However, in ICAM-1 antisense oligonucleotide-treated mice, macrophage infiltration following UUO was reduced but not eliminated, suggesting that additional β_2-integrin counter-receptors may

be operative in leukocyte recruitment.[25] Recently, receptor for advanced glycation endproducts (RAGE), a multiligand receptor on vascular cells centrally involved in inflammatory processes,[26] was identified as a new β_2-integrin counterligand. RAGE binds to Mac-1 but not to LFA-1.[27] RAGE is expressed at low levels in normal tissues but becomes upregulated at inflammatory sites where its ligands accumulate.[28–30] Other ligands for the β_2-integrins include the family of junctional adhesion molecules (JAMs). JAMs are a family of three glycoproteins (JAM-A, -B, -C) participating in junction assembly, platelet activation and leukocyte transmigration.[31–33] JAMs localize to intercellular junctions of polarized endothelial and epithelial cells, but are also expressed on leukocytes and platelets. JAM-C is expressed in vascular endothelial cells, including high endothelial venules, in epithelial cells and platelets.[34] JAM-C binds to Mac-1 but not to LFA-1 and has a direct role in leukocyte transmigration.[35,36]

Because most glomeruli continue to form postnatally in mice, renal development in the neonatal mouse is analogous to renal development in the human fetus. For this reason, surgical ligation of one ureter in newborn mice is a model to study the effects of urinary tract obstruction on renal development. The present study was designed to characterize the role of the β_2-integrins and their ligands (ICAM-1, RAGE, JAM-C) in UUO in newborn mice.

RESULTS

Normal kidney morphology in LFA-1$^{-/-}$ and Mac-1$^{-/-}$ mice

LFA-1$^{-/-}$ and Mac-1$^{-/-}$ mice appeared healthy and developed without apparent defects. The kidneys from the LFA-1$^{-/-}$ and Mac-1$^{-/-}$ mutants grossly presented similar to those from wild-type (WT) mice. Light microscopic examinations of the kidney showed normal structure of the kidney, that is, glomeruli, tubulointerstitium and blood vessels (not shown). Obstructed kidneys of LFA-1$^{-/-}$, Mac-1$^{-/-}$ and WT mice presented with hydronephrosis at 1, 5 and 12 days after obstruction. The degree of tubulointerstitial injury was dependent on the β_2-integrin deficiency and the duration of obstruction. Whereas macrophage infiltration and tubular apoptosis were noted from day 1 after UUO, tubular atrophy and interstitial fibrosis were present from day 5 onwards.

Monocyte/macrophage recruitment

UUO resulted in a significant and progressive increase in interstitial macrophage infiltration in all obstructed WT, LFA-1$^{-/-}$ and Mac-1$^{-/-}$ kidneys when compared to sham-operated controls and unobstructed intact opposite kidneys (Figure 1a–c, 2a and b). β_2-Integrin-deficient mice showed a significant decrease in interstitial macrophage infiltration into the obstructed kidney when compared to WT; both Mac-1$^{-/-}$ and LFA-1$^{-/-}$ demonstrated a decrease by 97% at day 1 after UUO (WT 112.7 ± 20, LFA-1$^{-/-}$ 3.0 ± 0.9, Mac-1$^{-/-}$ 2.7 ± 0.7; $P < 0.001$). At 5 days after obstruction, Mac-1$^{-/-}$ demonstrated a decrease by 88% ($P < 0.001$) and LFA-1$^{-/-}$ showed a decrease by 74% ($P < 0.001$) when compared to WT (Figure 2a). Mac-1$^{-/-}$ still showed a significant reduction in macrophage infiltration at 12 days after UUO (decrease by 78%, $P < 0.05$) (Figure 1c).

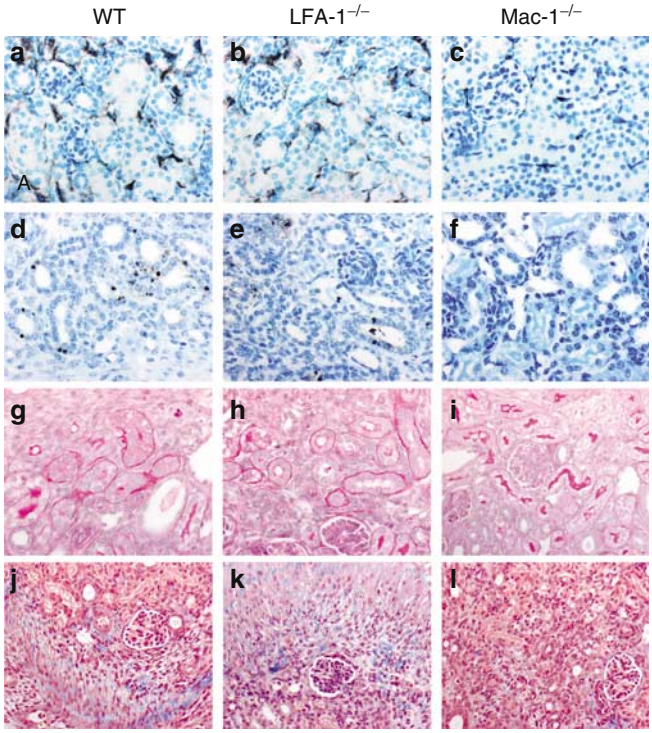

Figure 1 | Representative photomicrographs of obstructed kidneys (UUO). Renal sections were stained for macrophage infiltration (F4/80 antibody) at 12 days after UUO in control mice (**a**), β_2-integrin-deficient LFA-1$^{-/-}$ mice (**b**) and Mac-1$^{-/-}$ mice (**c**); original magnification $\times 400$. Mac-1$^{-/-}$ kidneys showed significantly fewer macrophages (black). Tubular apoptosis (TUNEL) at 12 days after obstruction in control mice (**d**), LFA-1$^{-/-}$ mice (**e**) and Mac-1$^{-/-}$ mice (**f**); original magnification $\times 400$. Mac-1$^{-/-}$ kidneys showed fewer TUNEL-positive tubular cells. Periodic acid Schiff staining identified the thickened, irregular tubular basement membrane characteristic of tubular atrophy in: control mice (**g**), LFA-1$^{-/-}$ mice (**h**) and Mac-1$^{-/-}$ mice (**i**) at day 12 after UUO. Renal sections were stained with Masson's trichrome to detect interstitial collagen (blue) in: WT mice (**j**), LFA-1$^{-/-}$ mice (**k**) and Mac-1$^{-/-}$ mice (**l**) at day 12 following UUO; original magnification $\times 250$.

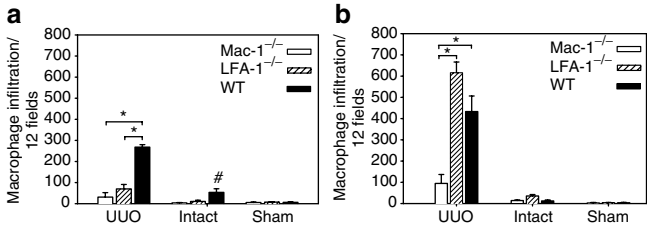

Figure 2 | Relative area of macrophage infiltration, identified by F4/80 antibody (a) 5 days and (b) 12 days after operation in Mac-1$^{-/-}$, LFA-1$^{-/-}$ and WT mice. Twelve fields were analyzed at a magnification of $\times 400$. Intact = intact opposite kidney, UUO = obstructed kidney, Sham = sham-operated control. $^{*}P < 0.05$. $^{\#}P < 0.05$ versus sham-operated control.