Chassin et al. http://www.jem.org/cgi/content/full/jem.20071032/DC1

SUPPLEMENTAL MATERIALS AND METHODS

siRNA studies. Experiments were performed using predesigned siRNAs (HP GenomeWide; QIAGEN) using a target DNA sequence from the *Tlr4* gene (5′-AATTCTCCGAACGTGTCACGT-3′; sense, UUCUCCGAACGUGUCACGUdTdT; antisense, ACGUGACACGUUCGGAGAAdTdT). A universal negative control siRNA (target DNA sequence: AATTCTCCGAACGTGTCACGT; sense, UUCUCCGAACGUGUCACGUdTdT; antisense, ACGUGACACGUUCGGAGAAdTdT) was also used. Single-strand sense and antisense RNA nucleotides were annealed (90°C for 1 min followed by 37°C for 1 h) to generate an RNA duplex according to the manufacturer's instructions. $mpkCCD_{cl4}$ cells were trypsinized, transferred to 24-well plates (35,000 cells per well), and, 24 h later, 8.4 ng (0.6 pmol) of siRNA duplex and 2 μl of INTERFERin (Polyplus-transfection) preincubated in serum-free medium for 10 min were added to each well. The final concentration of siRNA was 1 nM. No cytotoxic effects were observed during the incubation. The complexes were then removed, and the cells were rinsed twice in PBS. The cells were incubated for 48 h at 37°C under their normal growing conditions. Gene silencing was monitored by quantitative real-time PCR (see the following section).

Retrograde infection studies on mice pretreated with dDAVP or SR121463B. Sets of C3H/HeN and C3H/HeOuJ mice (six mice per group) were given either dDAVP (1 ng/μl/h) or sterile PBS (1 μl/h) via an osmotic minipump (1003 D; Alzet) for 3 d (six mice per group) while being on normal water intake. 24 h after the subcutaneous abdominal insertion of the osmotic pump, mice were anesthetized again and infected with 50 μl of an HT7 bacterial suspension (10^8 bacteria) in sterile PBS introduced via the transurethral route into the bladder, as previously described (Chassin, C., J.M. Goujon, S. Darche, L. du Merle, M. Bens, F. Cluzeaud, C. Werts, E. Ogier-Denis, C. Le Bouguenec, D. Buzoni-Gatel, and A. Vandewalle. 2006. *J. Immunol.* 177:4773–4784). In separate sets of experiments, SR121463B (0.03 mg/kg body weight [i.e., 0.004 μmol/kg] or 0.3 mg/kg body weight [i.e., 0.04 μmol/kg]) or vehicle only (DMSO diluted 1:10^4 in isotonic saline) was administered intraperitoneally (100 μl) to normal C3H/HeN mice 6 h before and 14 h after the transurethral inoculation of 50 μl of an HT7 bacterial suspension (10^8 bacteria), as described. In all cases, urine and blood samples were collected 24 h after the bacterial inoculation, and the mice were killed. The kidneys were removed aseptically. The two halves of one kidney were fixed or quick frozen in liquid nitrogen before use. The contralateral kidney was homogenized, diluted in sterile PBS, and plated on LB agar plates to count the number of CFU. Urine and plasma osmolality were determined on 50-μl samples using an automatic microosmometer apparatus (Thermo Fisher Scientific). While Lps^n and Lps^d mice were receiving a normal water supply, they were also acclimatized to metabolic cages to estimate urine volume. Urine was collected under oil every 24 h before (control condition) and after dDAVP infusion via the insertion of osmotic pumps.

Table S1. Effects of continuous infusion of dDAVP on urine volume in untreated and dDAVP-treated C3H/HeN (*Lps*^{n}) and C3H/HeJ (*Lps*^{d}) mice housed in metabolic cages

	Body weight (g)	Urine volume (ml/d)	
		−dDAVP	+dDAVP
C3H/HeN	20.8 ± 1.5	0.97 ± 0.13	0.45 ± 0.09*
C3H/HeJ	19.6 ± 0.6	0.74 ± 0.05	0.42 ± 0.1*

Values are means ± SE from six to eight individual measurements performed under each condition tested. *, P < 0.05 versus −dDAVP values.

Table S2. Effects of dDAVP on plasma and urine osmolality analyzed in naive and *E. coli*–infected mice

	Mouse strain	Naive mice	HT7-infected mice	
		−dDAVP	−dDAVP	+dDAVP
Plasma osmolality (mOsm/kg)	C3H/HeN	287 ± 15	288 ± 17	160 ± 3*
	C3H/HeJ	284 ± 11	277 ± 6	178 ± 2*
Urine osmolality (mOsm/kg)	C3H/HeN	2,070 ± 23	2,152 ± 49	3,555 ± 119*
	C3H/HeJ	2,027 ± 40	2,065 ± 44	3,376 ± 95*

Plasma and urine osmolality were analyzed in naive C3H/HeN (*Lps*^{n}) and C3H/HeJ (*Lps*^{d}) mice, and in mice continuously infused with either PBS (−dDAVP) or dDAVP (+dDAVP; 1 ng/h) and then inoculated with 50 µl of an HT7 bacterial suspension (10^{8} bacteria per mouse). Values are means ± SE from six to eight individual measurements performed under each condition tested. *, P < 0.05 versus −dDAVP values.

Table S3. Plasma and urine osmolality analyzed in HT7-infected C3H/HeN mice pretreated with SR121463B

	HT7-infected C3H/HeN mice		
		SR121463B	
	None	0.03 mg/kg	0.3 mg/kg
Plasma osmolality (mOsm/kg)	269 ± 6	278 ± 15	302 ± 6*
Urine osmolality (mOsm/kg)	2,083 ± 151	2,023 ± 304	2,322 ± 52

Values are means ± SE from six individual measurements performed in each group of animals tested. *, P < 0.05 versus none values.