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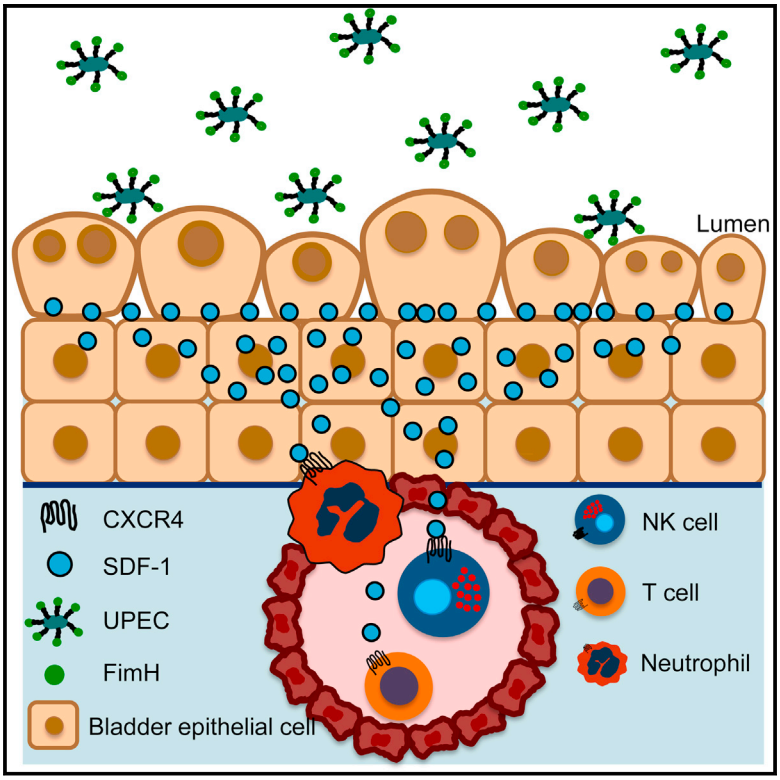
Stromal Cell-Derived Factor 1 Mediates Immune Cell Attraction upon Urinary Tract Infection

Graphical Abstract



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In Brief

Urinary tract infections (UTIs) are mainly caused by uropathogenic *Escherichia coli* (UPEC) and evoke a complex immune response. Isaacson et al. show that upon UPEC infection, bladder epithelial cells secrete the chemokine stromal cell-derived factor 1, triggering the migration and accumulation of immune cells at the site of infection.

Highlights

- Stromal-cell derived factor 1 (SDF-1) is secreted shortly after UPEC infection
- Natural killer cells, T cells, and neutrophils are recruited upon SDF-1 secretion
- UPEC's adhesin FimH mediates SDF-1 secretion by urinary epithelial cells

Isaacson et al., 2017, Cell Reports *20*, 40–47
July 5, 2017
http://dx.doi.org/10.1016/j.celrep.2017.06.034

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Stromal Cell-Derived Factor 1 Mediates Immune Cell Attraction upon Urinary Tract Infection

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http://dx.doi.org/10.1016/j.celrep.2017.06.034

SUMMARY

Urinary tract infection (UTI) is the most common type of bacterial infection in humans. Fifty percent of all women will experience at least one UTI in their lifetime, with uropathogenic *Escherichia coli* (UPEC) accounting for 80% of reported cases. UTI evokes a complex, well-timed immune response that is crucial for bacterial clearance. The majority of immune cells participating in the immune response are absent from the healthy bladder, and the mechanisms used to recruit them upon UTI are not fully understood. Here, we show that immediately after UPEC infection, bladder epithelial cells secrete stromal cell-derived factor 1 (SDF-1), initiating immune cell accumulation at the site of infection. SDF-1 blockade significantly reduced immune cell migration to the infected bladder, resulting in severe exacerbation of infection. We also show that FimH, the adhesin of type 1 fimbria, one of UPEC's virulence factors, is directly involved in the secretion of SDF-1 upon UTI.

INTRODUCTION

Urinary tract infections (UTIs) represent the most common type of extraintestinal *Escherichia coli* infection and the second most common type of bacterial infection in humans and are caused predominately by uropathogenic *E. coli* (UPEC) (Foxman, 2010). The majority of UTI cases can be treated with antibiotics, but a growing problem is the development of antibiotic-resistant strains, as well as growing sensitivity to recurring infections (Flores-Mireles et al., 2015). If left untreated, bladder infection (cystitis) can develop into kidney infection (pyelonephritis) and, in extreme cases, sepsis (Flores-Mireles et al., 2015).

In steady-state conditions, the bladder is inhabited by very few immune cells, most of which are mast cells (Choi et al., 2016) and tissue resident macrophages (Schiwon et al., 2014). UTI elicits an intriguing immune response that is responsible for fast and effective bacterial clearance and containment of the infection within the bounds of the urinary tract (Abraham and Miao, 2015). This battlefield has been closely studied. The host immune response to UPEC infection involves many immune cell types, which play different roles in the infection. Neutrophils, for example, are known to migrate to the site of infection, secrete matrix metalloproteinases (MMPs), and move through the infected bladder epithelium into the lumen (Schiwon et al., 2014). However, phagocytic cells are not the only cells participating in the immune response to UPEC infection. It was shown by our group that upon UTI, natural killer (NK) cells and T cells also migrate to the infected bladders. NK cells help reduce the bacterial loads via secretion of the cytokine tumor necrosis factor alpha (TNF-α) (Gur et al., 2013).

The site of inflammation also contains a range of signaling molecules, including various cytokines and chemokines. A few key signaling molecules have been singled out as important players in UTI resolution. CXCL2 (Schiwon et al., 2014) and interleukin 8 (IL-8) (Agace et al., 1993), for instance, have been shown to play a role in phagocytic cell recruitment to the infected bladder. That being said, the mechanisms by which neutrophils, T cells, and NK cells are attracted to the infected bladder are not fully understood.

Stromal cell-derived factor 1 (SDF-1, CXCL12) is a chemokine best known for its role in migration and homing of hematopoietic stem cells to the bone marrow (Ara et al., 2003). SDF-1 binds to its receptor, CXC-motif chemokine receptor 4 (CXCR4), a seven-transmembrane G-protein-coupled receptor (GPCR), and activates downstream signaling via the Ras/Raf/MEK/ERK pathway (Okabe et al., 2005). To the best of our knowledge, it has never been shown to have any role in UTI.

Type 1 fimbriae are a key virulence factor of UPEC (Mulvey et al., 2000). Expression of the type 1 fimbria is important for adherence as the first step of bacterial colonization (Mulvey et al., 2000). Its expression is dependent on its promoter, which can exist in one of two transcriptional states: the "on" state allows expression of the operon, while in the "off" state, bacteria do not express type 1 fimbria (Gunther et al., 2002). During the course of infection, bacteria can turn type 1 fimbria expression

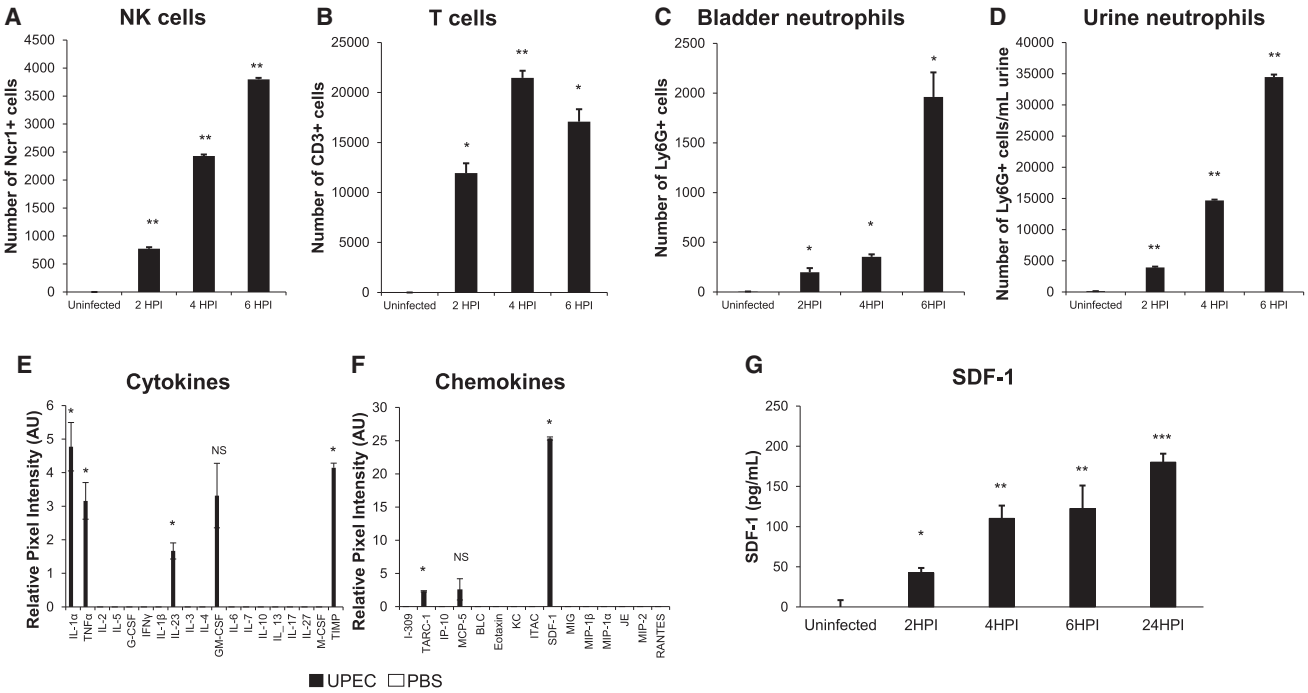

Figure 1. Immune Cells, Chemokines, and Cytokines in the Infected Bladder

(A–D) Immune cell accumulation. Number of NK cells (A), T cells (B), and neutrophils (C) accumulating in UPEC-infected bladders and urine (D) as early as 2 hr post-inoculation (HPI). n = 6 mice in each group. Figure shows one representative experiment out of three performed. *$p < 0.05$, **$p < 0.01$.

(E and F) Chemokine/ cytokine microarray performed on total lysates of bladders inoculated with UPEC or PBS. Cytokine (E) and chemokine (F) expression levels are presented as arbitrary units measured by densitometry. n = 6 mice in each group. Figure shows one representative experiment out of four performed. *$p < 0.01$.

(G) SDF-1 levels in infected bladders extracted at different time points after infection as measured by enzyme linked immunosorbent assay (ELISA). n = 5 mice in each group. *$p \leq 0.01$, **$p < 0.005$, ***$p \leq 0.001$.

Data are presented as mean ± SD.

on and off as the promoter is situated on an invertible element (Gunther et al., 2002). FimH is the adhesin part of the type 1 fimbria structure (Schilling et al., 2001). As a part of bladder colonization, FimH binds mannose on urinary epithelial glycoproteins, allowing bacteria to escape clearance by urination (Schilling et al., 2001).

Here, we show that UPEC infection causes immediate, FimH-dependent SDF-1 secretion followed by immune cell accumulation in the bladder. We show that interruption to the SDF-1/CXCR4 axis results in a significant decrease in immune cell recruitment to the site of infection. We also demonstrate that neutrophils and NK cells are crucial for the eradication of UPEC from infected bladders.

RESULTS

Immune Cells Migrate to Infected Bladders following SDF-1 Secretion

We previously demonstrated that NK and T cells are present in bladders of mice infected with UPEC (Gur et al., 2013; Mobley et al., 1990). We examined the kinetics of NK and T cell accumulation 24 and 48 hr following UTI induction and observed a decline in NK and T cell numbers ~96 hr post-inoculation (HPI) (Gur et al., 2013). We now wanted to examine the immediate kinetics of immune cell accumulation at the site of infection during the first 6 hr. For this purpose, we used the immune competent $Ncr1^{gfp/+}$ mice generated by our group, in which NK cells are labeled with GFP and can easily be detected (Gazit et al., 2006). Intraurethral catheterization was used to inoculate bladders of 6- to 8-week-old C57BL/6 $NCR1^{gfp/+}$ female mice with the UPEC strain CFT073 (acute pyelonephritis isolate) (Mobley et al., 1990). NK cell, T cell, and neutrophil accumulation was monitored at early time points post-inoculation. At each time point, urine was collected and urinary bladders were harvested. As can be seen, NK cells, T cells, and neutrophils appear at the site of infection (the bladder) as early as 2 HPI (Figures 1A–1C). As previously reported, (Haraoka et al., 1999), neutrophils (Figure 1D), but not NK or T cells (data not shown), were detected in the urine of infected mice at the same time point (Figure 1D).

To identify the chemokine responsible for immune cell recruitment into the infected bladder, we used a mouse cytokine and chemokine antibody array. 6- to 8-week-old mice were intraurethrally injected with CFT073 or PBS, bladders were excised, and tissue proteins were extracted. Several cytokines and chemokines were elevated (Figures 1E and 1F, respectively), including TNF-α, which is essential for bacterial clearance, as previously shown by our group (Gur et al., 2013). Some cytokines

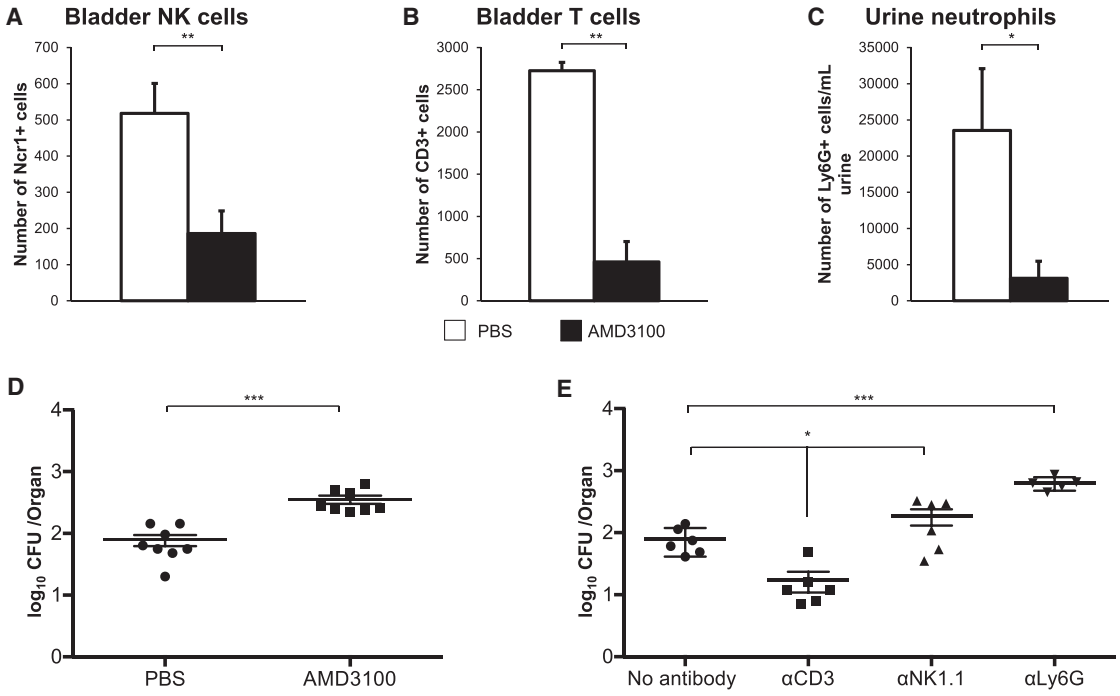

Figure 2. A CXCR4 Antagonist Reduces Immune Cell Accumulation in the Bladder, Resulting in Exacerbation of Infection

(A–C) Number of NK cells (A), T cells (B), and neutrophils (C) in the bladder (A and B) and urine (C) 24 hr after bladder inoculation with UPEC, in the presence or absence of AMD3100. n = 7 mice per group. The figure represents an average of three experiments performed. *$p < 0.05$, **$p \leq 0.01$.

(D) Bacterial loads in the bladder were assessed by a colony-forming unit (CFU) assay in the presence or absence of AMD3100, 24 hr following UPEC inoculation. Data are presented as mean ± SEM; n = 9 mice per group. Figure shows one representative experiment out of three performed. *$p < 0.005$.

(E) NK cells, T cells, and neutrophils were depleted prior to bladder inoculation with UPEC. Bacterial loads were assessed at 24 HPI using a CFU assay. n = 6 mice in each group. *$p < 0.0001$. Data are represented as mean ± SEM.

Unless stated otherwise, data are presented as mean ± SD.

that were known to be upregulated in UTI, such as IL-6 (Hunstad et al., 2005), were not detected in the array. This is probably due to poor capture of antibodies against these cytokines in the array. The most significant elevation was detected for SDF-1 (CXCL12), which increased ~25-fold (Figure 1F).

We next evaluated SDF-1 secretion kinetics in the bladders. We infected 6- to 8-week-old C57BL/6 NCR1$^{gfp/+}$ female mice with CFT073, harvested bladders at various time points, and performed an ELISA on the protein extracts to determine the levels of SDF-1. SDF-1 was absent from uninfected bladders, and its expression was detected at high levels as early as 2 HPI (Figure 1G), when immune cells start to accumulate in the bladder (Figures 1A–1C), and increased gradually with time (Figure 1G).

A CXCR4 Antagonist and Anti-SDF-1 Antibody Reduces Immune Cell Accumulation in the Bladder, Resulting in Exacerbation of Infection

SDF-1 was first characterized as the chemokine that mediates hematopoietic stem cell homing to the bone marrow (Ara et al., 2003). Its receptor, CXCR4, is expressed on a variety of cells, including NK cells, T cells, and neutrophils (Karin, 2010). To test whether SDF-1 indeed attracts immune cells into the bladder, we used the CXCR4 antagonist AMD3100 (Broxmeyer et al., 2005). Injection of AMD3100 resulted in a significant reduction of immune cells in infected bladders (Figures 2A–2C). Similar results were obtained when we used anti-SDF-1 neutralizing antibody, with the exception of urine neutrophils, that were still significantly decreased compared to the control (Figures S1A–S1C). To test whether or not this phenomenon has an effect on severity of infection, represented by bacterial loads in the bladders, we harvested bladders of mice infected with CFT073, with or without treatment with AMD3100. We found that reduced number of immune cells accumulating in the infected bladder results in exacerbation of the infection, mirrored by a significant elevation in bacterial load (Figure 2D). Similar results were obtained using the anti-SDF-1 neutralizing antibody (Figure S1D).

We next wanted to check which population of the immune cell migrating to the bladder upon infection was the most crucial for fighting infection and reducing bacterial load. We singly depleted NK cells, T cells, and neutrophils (using anti-NK1.1, anti-CD3, and anti-Ly6G monoclonal antibodies [mAbs], respectively) and measured the severity of infection. The depletion was verified by fluorescence-activated cell sorting (FACS) (Figures S1E–S1G). We saw that while T cell depletion did not affect the severity of infection (on the contrary, infection was surprisingly less pronounced), NK cell depletion had a moderate effect, and neutrophil depletion had the most dramatic effect (Figure 2E).

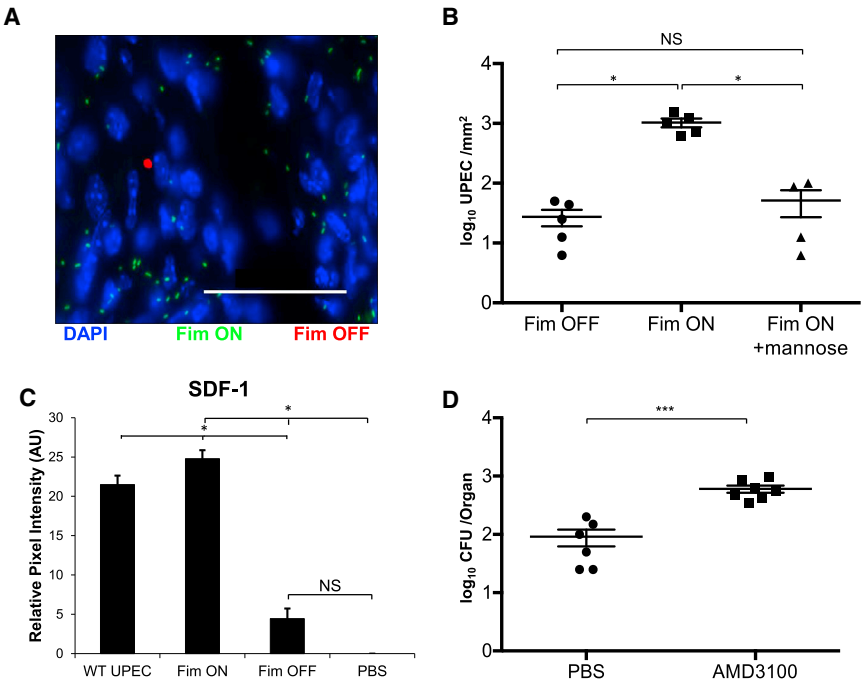

Figure 3. UPEC Type 1 Fimbria-Mediated Adhesion Is Mannose Dependent

(A and B) Representative images (A) and quantitative analysis (B) of GFP-expressing CFT073 Fim ON (in the presence or absence of mannose) and Cherry red-expressing CFT073 Fim OFF in mouse bladder sections. Each point represents the mean of counted bacteria in three arbitrarily selected fields in a single slide. Scale bar, 50 μm. n = 6 mice per group. *$p < 0.005$.

(C) Chemokine/cytokine microarray with total lysates of WT UPEC, Fim ON and Fim OFF mutants, or PBS inoculated bladders of C57BL/6 female mice 24 HPI. SDF-1 expression levels are presented as arbitrary units measured by densitometry. n = 6 mice per group. *$p < 0.05$.

(D) Bacterial loads in infected bladders were determined using a CFU assay 24 HPI with the Fim ON mutant with or without AMD3100. Figure shows one representative experiment out of three performed. Data are presented as mean ± SEM; ***$p = 2 \times 10^{-4}$.

Data are presented as mean ± SD.

Type 1 Fimbria Is Involved in Adhesion to the Urinary Epithelium in a Mannose-Dependent Manner

To identify bacterial factors that might be responsible for immune cell attraction to the bladder, we initially concentrated on the type 1 fimbria of UPEC, as this is one of the most important virulence factors in bacteria. We used two mutants: Fim ON and Fim OFF (Gunther et al., 2002).

We generated a Fim ON mutant expressing GFP and a Fim OFF mutant expressing mCherry fluorescent protein and tested their binding to mouse urinary epithelium using confocal microscopy. We found that the Fim OFF mutant is impaired in binding to the urinary epithelium, while the Fim ON mutant displayed increased binding (Figure 3A, quantified in Figure 3B); the same results were obtained with the opposite labeling (Fim ON expressing mCherry and Fim OFF expressing GFP; Figures S2A and S2B). This increased binding ability was abolished when the Fim ON mutant was blocked with mannose prior to introduction to the urinary epithelium (Figure 3B).

Next, we wanted to test whether or not type 1 fimbria plays a role in SDF-1 secretion upon infection, which leads, in turn, to recruitment of immune cells. We again used the mouse cytokine and chemokine antibody array, this time on protein extracts from bladders of 6- to 8-week-old female mice inoculated with the wild-type (WT) strain, the two mutant strains, or PBS. SDF-1 levels were elevated in mice infected with the WT strain, as shown above (Figure 1F). Interestingly, the Fim ON mutant caused a significant increase of SDF-1 secretion compared to the WT UPEC. In contrast, SDF-1 was secreted in very low levels when mice were inoculated with the Fim OFF mutant (Figure 3C). We also wanted to determine the effects of the CXCR4 antagonist AMD3100 on mice infected with the Fim mutants. The Fim ON mutant successfully colonized the bladders, but when we injected the mice with AMD3100, the infection was exacerbated, as shown by the significant elevation in bacterial loads post-treatment (Figure 3D).

FimH Is Involved in SDF-1 Secretion from Bladder Epithelial Cells upon UPEC Infection

Type 1 fimbria is a complex protein structure that is crucial for UPEC's adhesion to urinary epithelium. FimH is the adhesive tip of the type 1 fimbria, and its ligands are mannose moieties on uroepithelial glycoproteins (Schilling et al., 2001). We next wanted to determine whether FimH by itself is sufficient for the induction of SDF-1 secretion and immune cell migration. 6- to 8-week-old female mice were intraurethrally injected with recombinant FimH (rFimH) or PBS. After 2 hr, bladders were extracted and SDF-1 levels were measured using ELISA. Injection of rFimH alone was sufficient to induce SDF-1 secretion and immune cell migration into the bladder (Figures 4A and 4B, respectively). Blocking of rFimH prior to injection with D-mannose almost completely abolished SDF-1 secretion (Figure 4A).

Finally, we wanted to identify the cells responsible for the massive SDF-1 secretion detected shortly after UPEC infection. Uroplakin IIIa is a glycoprotein coating the surface of polynucleated umbrella cells, which are the superficial epithelial layer of the bladder (facing the lumen). Underneath the umbrella cells lie immature urinary epithelial cells. Immunofluorescent imaging of mouse bladders stained for SDF-1 before and 2 HPI of CFT073 showed that the urinary epithelium is responsible for SDF-1 secretion. Both umbrella and immature urinary epithelial cells are the source of the chemokine (Figure 4C). In the absence of bacteria, SDF-1 cannot be detected in the bladder tissue (Figure 4C).

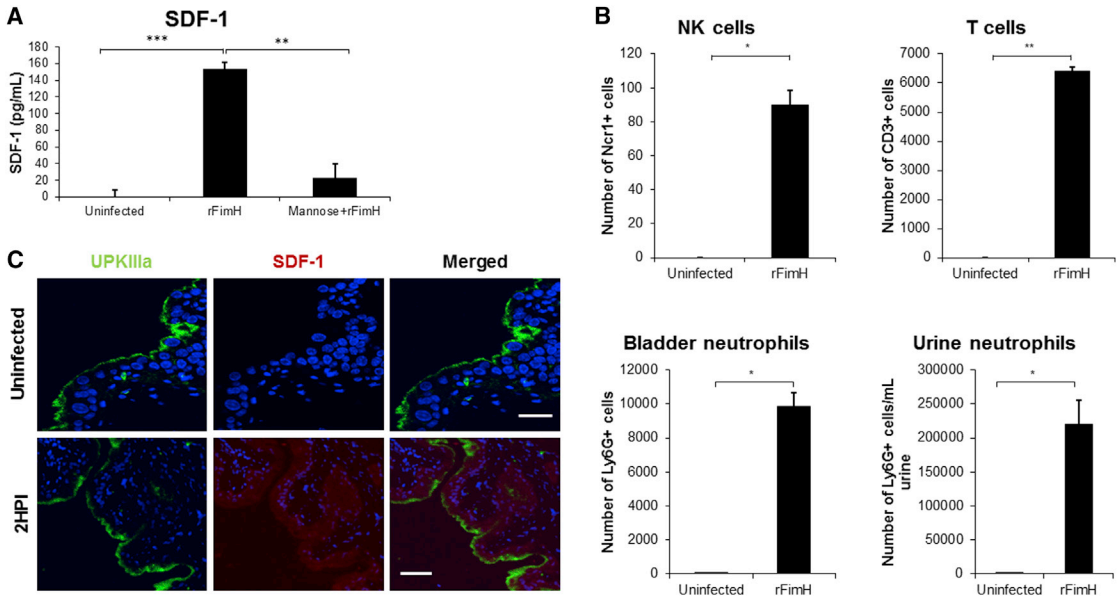

Figure 4. FimH Is Involved in SDF-1 Secretion Upon UPEC Infection

(A) SDF-1 levels in infected bladders extracted after 2 hr post-injection of recombinant FimH (rFimH) or rFimH that was blocked with D-mannose prior to injection, as measured by ELISA. n = 5 mice in each group. **p = 0.01, ***p < 0.001.

(B) Accumulation of NK cells and T cells in the bladder and neutrophils in the bladder and urine 6 hr following rFimH bladder injection. n = 5 mice in each group. *p < 0.05, **p < 0.005.

(C) Paraffin-embedded sections of infected (2 HPI) and uninfected mouse bladders were stained with anti-SDF-1 antibody and anti-mouse uroplakin IIIa antibody. Scale bar, 50 μm. Images were captured by confocal microscopy. At least ten fields were scanned per slide. Three slides were prepared for each condition. Data are presented as mean ± SD.

DISCUSSION

Here, we show a mechanism of immune cell recruitment upon UTI that is mediated by the chemokine SDF-1. Out of the array of candidates checked, SDF-1 was the chemokine most significantly elevated during UTI. We showed that SDF-1 is secreted at a very early time point, and by 2 HPI, it reaches particularly high levels in the bladder. For technical reasons, we could not look at a time point earlier than 2 HPI, but judging by the levels of SDF-1 at the 2-hr time point, it seems that its secretion starts even before that, shortly after infection.

SDF-1 attracted NK cells, T cells, and neutrophils, all of which are immune cells known to express its receptor, CXCR4 (Karin, 2010). Indeed, interfering with the SDF-1/CXCR4 interaction using the CXCR4 antagonist AMD3100 and neutralizing anti-SDF-1 antibody significantly reduced accumulation of these immune cells at the site of infection. The neutralizing anti-SDF-1 antibody was less efficient in blocking neutrophil migration. We hypothesize that this is due to the large amounts of neutrophils accumulating in infected bladders and to its reduced efficiency compared to AMD3100.

From all the cells that migrate to the site of infection, T cells are exceptional, as they belong to the adaptive immune system. Classically, T cells arrive at sites of infection after interacting with antigen-presenting cells (APCs) that have migrated to the adjacent lymph node from the site of infection. Because of the early arrival of T cells, we postulate that T cells arrive to the infected bladder as a result of non-specific migration, suggesting migration that is not mediated by an interaction with an APC. This hypothesis is supported by the observation that T cells do not seem to play a significant role in the eradication of infection and bacterial clearance, as their depletion did not result in exacerbation of the infection. On the contrary, T cell depletion resulted in fewer bacteria in the bladder, but the reasons for this are not completely understood. We showed that neutrophils play a cardinal role in bacterial clearance, as supported by studies previously performed by other groups (Abraham and Miao, 2015; Agace et al., 1995; Ingersoll et al., 2008; Loughman and Hunstad, 2011; Mora-Bau et al., 2015; Shahin et al., 1987; Zec et al., 2016) Finally, we showed that NK cells also play an important role in the resolution of the infection.

Neutrophils are phagocytic cells that are known to secrete MMPs, especially MMP9, in the context of UTIs (Nathan, 2006). Secretion of MMPs allows the recruited neutrophils to cross the bladder epithelial layers and enter the lumen (Abraham and Miao, 2015), as evidenced by their large numbers in the urine of infected mice and humans (Haraoka et al., 1999). Interestingly enough, although not capable of phagocytosis, NK cells seem to also play an important role in the control of the pathogen, as demonstrated by the significantly elevated bacterial loads that accumulate in their absence. We suggest that NK cells help to clear the infection by secreting the pro-inflammatory cytokine TNF-α. This is based on our previous work showing that treatment with the soluble TNF-α receptor Etanercept (Weinblatt et al., 1999) results in the elevation of bacterial loads in infected bladders (Gur et al., 2013).

We further showed that the transient presence of bacteria in the bladder is not sufficient to trigger SDF-1 secretion. A UPEC strain in which the type 1 fimbria operon is locked in the off orientation (Fim OFF) hardly induced immune cell recruitment to the bladder. We demonstrated that the adhesive tip of the type 1 fimbria, FimH, is sufficient to trigger SDF-1 secretion and immune cell recruitment.

Epithelial cells function as an essential barrier, restricting the entrance of microbial pathogens. Their role and importance in sensing threats from the outside and alerting the host's immune system were demonstrated in various pathologies (Marchiando et al., 2010). UPEC uses its type 1 fimbria to adhere to bladder epithelial cells and facilitate infection (Mulvey et al., 2000). FimH, the adhesion portion of type 1 fimbria, is involved in the attachment of UPEC to bladder epithelial cells and was identified as a novel ligand for Toll-like receptor 4 (TLR4) (Mossman et al., 2008). Our observation that increased binding by the Fim ON mutant and, furthermore, the sole presence of rFimH lead to increased SDF-1 secretion suggests that epithelial sensing of the pathogen depends on FimH and that its binding triggers an immense recruitment signal, resulting in rapid accumulation of effector cells at the site of infection.

Although often treatable with oral administration of antibiotics, UTI, the second most common bacterial infection in humans, can cause complications. This is due to recurring infections caused by intracellular bacterial communities (IBCs) that evade immune clearance (Bishop et al., 2007; Miao et al., 2015; Song et al., 2009). Another issue hindering simple, short-term antibiotic treatment is infection with antibiotic-resistant strains of UPEC. This may result in bacteria ascending to the kidneys through the ureter, resulting in a possibly life-threatening infection. The immune system is a potent and effective weapon against UTIs, and our results suggest that increasing recruitment of immune cells to the site of infection can lead to its resolution in an antibiotic-independent manner.

EXPERIMENTAL PROCEDURES

Mice

All experiments were performed using 6- to 8-week-old C57BL/6 female mice. The generation of Ncr1$^{gfp/+}$ mice was described previously (Gazit et al., 2006). All experiments were performed in accordance with the guidelines of the local ethics committee (MD-16-14701-3).

Plasmids and Bacterial Strains

UPEC CFT073, a pyelonephritis isolate (fim+, pap+ hly+ Nal^r) (Mobley et al., 1990), CFT073-OFF (Fim OFF, in which the fim invertible element is locked in the off orientation) (Gunther et al., 2002), and CFT073-ON (Fim ON, in which the fim invertible element is locked in the on orientation) (Gunther et al., 2002) were kind gifts from Professor H. Mobley. All *E. coli* strains were grown in Luria Broth (LB) for 18 hr with aeration (220 rpm) at 37°C. Bacteria electroporated with the GFP expressing pCM18 plasmid (Erm^r; pTRKL2-PCP58-RBSII-gfpmut3*-T3-T4) (Hansen et al., 2001) or the mCherry expressing pKB2690 (Sason et al., 2009, a kind gift from Professor I. Rosenshine) were grown in the presence of erythromycin (300 μg/mL) and ampicillin (100 μg/mL), respectively. Recombinant FimH (rFimH; MyBioSource) was either suspended in sterile PBS or blocked with 6% sterile D-mannose for 30 min and then intraurethrally injected into the bladders of anesthetized mice at 2 μg per mouse.

Quantification and Characterization of Bladder Immune Infiltrate

Lymphocytes were purified from homogenized bladders by centrifugation on Lymphoprep (STEMCELL Technologies). Lymphocytes were characterized using either GFP for NK cells (Gazit et al., 2006) or anti-mouse CD3-allophycocyanin (BioLegend) for T cells. Neutrophils were extracted from urine by centrifugation and stained with anti-mouse Ly6G-allophycocyanin and anti-mouse CD11b-FITC (BioLegend).

Depletion Experiments

Depleting antibodies were injected intraperitoneally, 12 hr prior to bladder inoculation with UPEC and again 12 hr later. Anti-mouse NK1.1 antibody (clone PK136, BioXCell) at 25 μg per mouse was used to deplete NK cells, anti-mouse CD3 antibody (clone 17A2, BioXCell) at 100 μg per mouse was used to deplete T cells, and anti-mouse Ly6G antibody (clone 1A8, BioXCell) at 100 μg per mouse was used to deplete neutrophils. 1 hr before UPEC inoculation and again right before sacrifice, mice were bled from the tail vein and depletions were validated using flow cytometry.

In Vivo Assays and Determination of Bacterial Loads

Female mice 6–8 weeks of age were anesthetized with ketamine and xylazine before catheter insertion and then infected with 10^8 UPEC in 50 μL sterile PBS. Tissue samples were homogenized using a Fastprep (MP Biomedicals), serially diluted, and spread on MacConkey plates (Novamed). Plates were incubated overnight at 37°C, and colonies were enumerated.

Chemokine Microarray

Bladders were excised and homogenized in PBS with protease inhibitor cocktail (Sigma) and 1% Triton X-100. The amount of extracted proteins was checked both by NanoDrop and a Pierce BCA protein assay kit (Thermo Scientific). The microarray was carried out using Proteome Profiler Mouse chemokine and cytokine array (R&D Systems) according to the manufacturer's instructions. Chemokine expression levels were expressed as an arbitrary unit measured by densitometry, relative to the assay's internal control.

Determination of Tissue Levels of SDF-1 by ELISA

Bladders were excised and homogenized in ice-cold PBS with protease inhibitor cocktail (Sigma). The amount of extracted protein was checked by both NanoDrop and a Pierce BCA protein assay kit (Thermo Scientific). An ELISA was carried out using the mouse CXCL12 DuoSet ELISA kit (R&D Systems) according to manufacturer's instructions.

Blocking of CXCR4/SDF-1

Mice were subcutaneously injected with either 200 μL sterile PBS or 10 mg/kg of the CXCR4 antagonist AMD3100 (Sigma-Aldrich) or received an anti SDF-1 neutralizing antibody (R&D Systems) at 50 μg per mouse 6 HPI.

Bacterial Attachment to Paraffin Tissue Sections

Paraffin-embedded sections of urinary bladders were prepared from 6- to 8-week-old female mice. Sections were blocked in Tris-buffered saline (TBS) supplemented with 15% fetal bovine serum (FBS), 15% BSA and permeabilized with 5% Triton X-100. GFP- and mCherry-expressing bacteria in blocking solution were added to slides and incubated overnight at 4°C. For the relevant slides, 400 mM D-mannose (Sigma-Aldrich) was added to bacteria suspended in the blocking solution. The slides were then washed once with PBS + 0.5% Tween 20, followed by two washes with PBS for 10 min each, and then incubated with Hoechst 33258 diluted 1:5,000 for 20 min at room temperature.

Immunofluorescence Staining

Paraffin-embedded sections of bladders were prepared from uninfected and infected (2 HPI) 6- to 8-week-old C57BL/6J female mice. After antigen retrieval, sections were incubated with anti-mSDF-1 antibody (Abcam) and anti-mUroplakin IIIa (UPKIIIa, Abcam) overnight in 4°C. Sections were then incubated with Cy3-conjugated donkey anti-rabbit antibody and Alexa-Fluor-647-conjugated rat anti-mouse antibody (Jackson ImmunoResearch Laboratories). Sections were then washed and stained with DAPI (Sigma-Aldrich) for 5 min and covered with coverslips. A confocal laser-scanning microscope (Olympus Fluoview FV1000) was used to obtain images.

Statistical Methods

GraphPad Prism software version 6.0 was used for statistical analysis. For statistical significance, Student's t test analysis was used. A statistical test was considered significant (*) when $p < 0.05$. ANOVA was used to identify significant group differences.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.06.034.

AUTHOR CONTRIBUTIONS

B.I. designed and performed experiments, analyzed results, and wrote the paper. T.H., A.G., and C.G. assisted in performing experiments. Z.G. assisted in planning and analysis of neutrophil assays. G.B. provided bacterial strains and helped plan and analyze infection assays. O.M. supervised the project.

ACKNOWLEDGMENTS

The authors would like to thank Daphna Borowsky for excellent suggestions and ideas. This study was supported by the European Research Council (ERC) under the European Union's Seventh Framework Programme (FP/2007-2013) ERC grant 320473-BacNK. Further support came from the I-CORE Program of the Planning and Budgeting Committee and the Israel Science Foundation and by the I-Core on Chromatin and RNA in Gene Regulation, the GIF Foundation, the Lewis Family Foundation, an ICRF professorship grant, a Helmholtz Israel grant, a Kamin grant, and the Rosetrees Trust (all to O.M.). O.M is a Crown professor of Molecular Immunology.

Received: January 17, 2017
Revised: May 8, 2017
Accepted: June 12, 2017
Published: July 5, 2017

REFERENCES

Abraham, S.N., and Miao, Y. (2015). The nature of immune responses to urinary tract infections. Nat. Rev. Immunol. *15*, 655–663.

Agace, W.W., Hedges, S.R., Ceska, M., and Svanborg, C. (1993). Interleukin-8 and the neutrophil response to mucosal gram-negative infection. J. Clin. Invest. *92*, 780–785.

Agace, W.W., Patarroyo, M., Svensson, M., Carlemalm, E., and Svanborg, C. (1995). Escherichia coli induces transuroepithelial neutrophil migration by an intercellular adhesion molecule-1-dependent mechanism. Infect. Immun. *63*, 4054–4062.

Ara, T., Tokoyoda, K., Sugiyama, T., Egawa, T., Kawabata, K., and Nagasawa, T. (2003). Long-term hematopoietic stem cells require stromal cell-derived factor-1 for colonizing bone marrow during ontogeny. Immunity *19*, 257–267.

Bishop, B.L., Duncan, M.J., Song, J., Li, G., Zaas, D., and Abraham, S.N. (2007). Cyclic AMP-regulated exocytosis of Escherichia coli from infected bladder epithelial cells. Nat. Med. *13*, 625–630.

Broxmeyer, H.E., Orschell, C.M., Clapp, D.W., Hangoc, G., Cooper, S., Plett, P.A., Liles, W.C., Li, X., Graham-Evans, B., Campbell, T.B., et al. (2005). Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. J. Exp. Med. *201*, 1307–1318.

Choi, H.W., Bowen, S.E., Miao, Y., Chan, C.Y., Miao, E.A., Abrink, M., Moeser, A.J., and Abraham, S.N. (2016). Loss of bladder epithelium induced by cytolytic mast cell granules. Immunity *45*, 1258–1269.

Flores-Mireles, A.L., Walker, J.N., Caparon, M., and Hultgren, S.J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat. Rev. Microbiol. *13*, 269–284.

Foxman, B. (2010). The epidemiology of urinary tract infection. Nat. Rev. Urol. *7*, 653–660.

Gazit, R., Gruda, R., Elboim, M., Arnon, T.I., Katz, G., Achdout, H., Hanna, J., Qimron, U., Landau, G., Greenbaum, E., et al. (2006). Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1. Nat. Immunol. *7*, 517–523.

Gunther, N.W., 4th, Snyder, J.A., Lockatell, V., Blomfield, I., Johnson, D.E., and Mobley, H.L. (2002). Assessment of virulence of uropathogenic Escherichia coli type 1 fimbrial mutants in which the invertible element is phase-locked on or off. Infect. Immun. *70*, 3344–3354.

Gur, C., Coppenhagen-Glazer, S., Rosenberg, S., Yamin, R., Enk, J., Glasner, A., Bar-On, Y., Fleissig, O., Naor, R., Abed, J., et al. (2013). Natural killer cell-mediated host defense against uropathogenic *E. coli* is counteracted by bacterial hemolysinA-dependent killing of NK cells. Cell Host Microbe *14*, 664–674.

Hansen, M.C., Palmer, R.J., Jr., Udsen, C., White, D.C., and Molin, S. (2001). Assessment of GFP fluorescence in cells of Streptococcus gordonii under conditions of low pH and low oxygen concentration. Microbiology *147*, 1383–1391.

Haraoka, M., Hang, L., Frendéus, B., Godaly, G., Burdick, M., Strieter, R., and Svanborg, C. (1999). Neutrophil recruitment and resistance to urinary tract infection. J. Infect. Dis. *180*, 1220–1229.

Hunstad, D.A., Justice, S.S., Hung, C.S., Lauer, S.R., and Hultgren, S.J. (2005). Suppression of bladder epithelial cytokine responses by uropathogenic Escherichia coli. Infect. Immun. *73*, 3999–4006.

Ingersoll, M.A., Kline, K.A., Nielsen, H.V., and Hultgren, S.J. (2008). G-CSF induction early in uropathogenic Escherichia coli infection of the urinary tract modulates host immunity. Cell. Microbiol. *10*, 2568–2578.

Karin, N. (2010). The multiple faces of CXCL12 (SDF-1alpha) in the regulation of immunity during health and disease. J. Leukoc. Biol. *88*, 463–473.

Loughman, J.A., and Hunstad, D.A. (2011). Attenuation of human neutrophil migration and function by uropathogenic bacteria. Microbes Infect. *13*, 555–565.

Marchiando, A.M., Graham, W.V., and Turner, J.R. (2010). Epithelial barriers in homeostasis and disease. Annu. Rev. Pathol. *5*, 119–144.

Miao, Y., Li, G., Zhang, X., Xu, H., and Abraham, S.N. (2015). A TRP channel senses lysosome neutralization by pathogens to trigger their expulsion. Cell *161*, 1306–1319.

Mobley, H.L., Green, D.M., Trifillis, A.L., Johnson, D.E., Chippendale, G.R., Lockatell, C.V., Jones, B.D., and Warren, J.W. (1990). Pyelonephritogenic Escherichia coli and killing of cultured human renal proximal tubular epithelial cells: role of hemolysin in some strains. Infect. Immun. *58*, 1281–1289.

Mora-Bau, G., Platt, A.M., van Rooijen, N., Randolph, G.J., Albert, M.L., and Ingersoll, M.A. (2015). Macrophages subvert adaptive immunity to urinary tract infection. PLoS Pathog. *11*, e1005044.

Mossman, K.L., Mian, M.F., Lauzon, N.M., Gyles, C.L., Lichty, B., Mackenzie, R., Gill, N., and Ashkar, A.A. (2008). Cutting edge: FimH adhesin of type 1 fimbriae is a novel TLR4 ligand. J. Immunol. *181*, 6702–6706.

Mulvey, M.A., Schilling, J.D., Martinez, J.J., and Hultgren, S.J. (2000). Bad bugs and beleaguered bladders: interplay between uropathogenic Escherichia coli and innate host defenses. Proc. Natl. Acad. Sci. USA *97*, 8829–8835.

Nathan, C. (2006). Neutrophils and immunity: challenges and opportunities. Nat. Rev. Immunol. *6*, 173–182.

Okabe, S., Fukuda, S., Kim, Y.J., Niki, M., Pelus, L.M., Ohyashiki, K., Pandolfi, P.P., and Broxmeyer, H.E. (2005). Stromal cell-derived factor-1alpha/CXCL12-induced chemotaxis of T cells involves activation of the RasGAP-associated docking protein p62Dok-1. Blood *105*, 474–480.

Sason, H., Milgrom, M., Weiss, A.M., Melamed-Book, N., Balla, T., Grinstein, S., Backert, S., Rosenshine, I., and Aroeti, B. (2009). Enteropathogenic Escherichia coli subverts phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate upon epithelial cell infection. Mol. Biol. Cell *20*, 544–555.

Schilling, J.D., Mulvey, M.A., and Hultgren, S.J. (2001). Structure and function of Escherichia coli type 1 pili: new insight into the pathogenesis of urinary tract infections. J. Infect. Dis. *183* (*Suppl 1*), S36–S40.

Schiwon, M., Weisheit, C., Franken, L., Gutweiler, S., Dixit, A., Meyer-Schwesinger, C., Pohl, J.M., Maurice, N.J., Thiebes, S., Lorenz, K., et al. (2014).

Crosstalk between sentinel and helper macrophages permits neutrophil migration into infected uroepithelium. Cell *156*, 456–468.

Shahin, R.D., Engberg, I., Hagberg, L., and Svanborg Edén, C. (1987). Neutrophil recruitment and bacterial clearance correlated with LPS responsiveness in local gram-negative infection. J. Immunol. *138*, 3475–3480.

Song, J., Bishop, B.L., Li, G., Grady, R., Stapleton, A., and Abraham, S.N. (2009). TLR4-mediated expulsion of bacteria from infected bladder epithelial cells. Proc. Natl. Acad. Sci. USA *106*, 14966–14971.

Weinblatt, M.E., Kremer, J.M., Bankhurst, A.D., Bulpitt, K.J., Fleischmann, R.M., Fox, R.I., Jackson, C.G., Lange, M., and Burge, D.J. (1999). A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N. Engl. J. Med. *340*, 253–259.

Zec, K., Volke, J., Vijitha, N., Thiebes, S., Gunzer, M., Kurts, C., and Engel, D.R. (2016). Neutrophil migration into the infected uroepithelium is regulated by the crosstalk between resident and helper macrophages. Pathogens *5*, 5.