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The Innate Immune Response to Uropathogenic
Escherichia coli Involves IL-17A in a Murine Model of
Urinary Tract Infection

Kelsey E. Sivick,* Matthew A. Schaller, † Sara N. Smith,* and Harry L. T. Mobley*
lymphoid tissue cells in response to in vitro restimulation, yet IL-17A seems to be dispensable for the generation of protective immunity. IL-17A is also upregulated in the bladder in response to acute infection, and γδ T cells are a major source of secreted IL-17A in the bladder tissue. IL-17A seems to play a role in regulating the innate immune response to UTI; mice lacking IL-17A exhibit deficient cytokine transcript upregulation and cellular responses during acute UTI, resulting in suboptimal clearance of uropathogenic E. coli.

Materials and Methods

Animals

Mice were maintained in specific pathogen-free conditions, and all experiments were conducted according to protocols approved by University Committee on the Use and Care of Animals at the University of Michigan. C57BL/6 wild type (WT) mice and mice harboring the Tcrdtm1Mom knockout (TCR δ−/−), resulting in deficient γδ TCR expression in all adult lymphoid and epithelial organs (46) were purchased from The Jackson Laboratory (Bar Harbor, ME). Breeding pairs of IL-17A−/− mice were a gift from Yoichiro Iwakura (The University of Tokyo) (47). All experiments were conducted when animals were 6–15 wk old, and C57BL/6 WT mice with birth dates within 1 wk of the IL-17A−/− or TCR δ−/− knockout mice (both in the C57BL/6 background) were used. For manipulation, mice were euthanized by intraperitoneal ketamine (10 mg/kg) and xylazine (5 mg/kg) body weight. When necessary, mice were euthanized using a lethal dose of isoflurane, and appropriate organs were harvested for analysis.

For infections and sensitizations, a 50-μl uropathogenic E. coli suspension in PBS was inoculated transurethrally using a sterile 0.28-mm polyethylene catheter connected to a Harvard Apparatus infusion pump. All infections, sensitizations, and challenges consisted of 5×10^7 CFU per mouse administered through the transurethral route. To determine organism CFU, bladders were harvested from euthanized animals, weighed, and homogenized in PBS with a General Laboratory Homogenizer (Omni International, Kennesaw, GA). Homogenates were plated on Luria-Bertani (LB) media (with GibcoL-glutamine) with 1% sodium pyruvate, 1% L-glutamine. To determine bacterial quantity and distribution, and mucosal changes.

Bladder tissue was harvested at necropsy and fixed in 10% neutral buffered formalin overnight at 4°C. Nonpecific binding sites were blocked with 2.5% BSA in PBS at 37°C for 1 h. Dilutions of purified IL-17A or test samples were made in dilution buffer (0.05% Tween 20, 2% FBS in PBS), and 50 μl was applied to wells at 37°C for 1 h. Biotinylated detection Abs were diluted to 0.25 μg/ml in dilution buffer and applied to wells at 37°C for 45 min. Streptavidin-HRP (Southern Biotechnology Associates, Birmingham, AL) was diluted 1:5000 in dilution buffer, and 100 μl was applied to wells at 37°C for 30 min. E-Phyton ECL kit was used for chemoluminescent readout. All infections, sensitizations, and challenges consisted of 5×10^7 CFU per mL inoculated into 250 mL sterile human urine (pooled from five healthy donors)

Cytokine ELISA

Lysate was cleared by centrifugation (8,000 g, 30 min, 4°C) and sterilized with 0.22-μm filter (Millipore, Bedford, MA). Protein in the lysate was quantified using the BCA Protein Assay Kit (Pierce, Rockford, IL).

Tissue culture

Splenocytes (1.5×10^5 cells/well) or the inner inguinal (lumbar) lymph nodes (5×10^3 cells/well) responsible for draining the pelvic viscera were harvested and made into single-cell suspensions by forcing organs through 40-μm BD Falcon cell strainers. Lymph nodes from the same groups of animals were pooled and plated in replicate wells. Erythrocytes were lysed using 8.02 mg/ml NH4Cl, 0.84 mg/ml NaHCO3, and 0.37 mg/ml EDTA in distilled water. Final cellular suspensions were made in RPMI 1640 medium (with Gibco t-glutamine) with 1% sodium pyruvate, 1% L-glutamine, 1% penicillin/streptomycin, 1% nonessential amino acids, 10% FBS, and 0.001% 50 mM NaOH overnight at 4°C. Non-specific binding sites were blocked with 2.5% BSA in PBS at 37°C for 1 h. Dilutions of purified IL-17A or test samples were made in dilution buffer (0.05% Tween 20, 2% FBS in PBS), and 50 μl was applied to wells at 37°C for 1 h. Biotinylated detection Abs were diluted to 0.25 μg/ml in dilution buffer and applied to wells at 37°C for 45 min. Streptavidin-HRP (Southern Biotechnology Associates, Birmingham, AL) was diluted 1:5000 in dilution buffer, and 100 μl was applied to wells at 37°C for 30 min. E-Phyton ECL kit was used for chemoluminescent readout.

Histology

Bladder tissue was harvested at necropsy and fixed in 10% neutral buffered formalin for 24 h. Tissues were trimmed and processed by standard histological methods and were stained with H&E. Light microscopic histopathological assessment was performed by a board-certified veterinary pathologist blinded to the group assignment of the samples. The presence or absence of inflammation and the predominating type and tissue distribution of inflammatory cells were assessed qualitatively. Additional points assessed were the presence/absence of bacteria, subjective assessment of bacterial quantity and distribution, and mucosal changes.
were acquired using a BD FACSCanto flow cytometer and BD FACSDiva software and analyzed using FlowJo v7.2.4 (Tree Star, Ashland, OR). Mouse anti–CD4-FITC (clone GK1.5), anti–γδ-TCR-FTTC (clone eBioGL3), anti–F4/80-FTTC (clone BM8), anti–IL-17A-PE (clone eBio17B7), anti–CD8a-PE (clone 53-6.7), anti–CD4-PE-Cy7 (clone GK1.5), anti–Ly-6G(Gr-1)-PE-Cy7 (clone RB6-8C5), anti–CD45R(B220)-APC (clone RA3-6B2), anti–MHC class II(I-A/I-E) (clone IA45.2), anti–CD11b-APC-Cy7 (clone M1/70) used for staining and calibration controls were obtained from eBiosciences.

**Statistics**

Graphing and statistical analyses were done using GraphPad Prism 5. Data were represented as mean or median values based on the D’Agostino and Pearson omnibus normality test. Where applicable, the Mann-Whitney U test, paired t test, or Fisher exact test was used to determine statistical significance with two-way ANOVA and 95% confidence intervals.

**Results**

**IL-17A is secreted by spleen and lymph node cells from C57BL/6 mice in response to transurethral infection with uropathogenic E. coli**

To determine whether IL-17A is secreted adaptively in response to uropathogenic E. coli Ags, WT C57BL/6 mice were inoculated via the transurethral route with uropathogenic E. coli strain CFT073 or PBS, according to the outlined schedules (Fig. 1). Cells harvested from the spleen (Fig. 1A) and the inner inguinal lymph nodes (Fig. 1B) were stimulated in vitro with medium alone, α-CD3 mAb, or uropathogenic E. coli strain CFT073 whole-cell lysate and incubated for 72 h before harvesting supernatants for ELISA. As expected, splenocytes treated with α-CD3 mAb had a high expression of IL-17A, regardless of whether they originated from PBS- or CFT073-treated animals; unstimulated cells from both treatment groups did not secrete IL-17A (Fig. 1). However, in response to in vitro stimulation with uropathogenic E. coli whole-cell lysate, only the splenocytes from uropathogenic E. coli-infected mice showed significant secretion of IL-17A compared with unstimulated controls (p = 0.0147; Fig. 1A). Additionally, inner inguinal lymph

**Neutrophil counts**

Urine was collected by massaging the mouse abdomen while holding the urethra over a sterile Eppendorf tube. Urine was mixed 10:1 with Turk’s stain (0.05 mg/ml crystal violet, 3% glacial acetic acid in distilled water), and neutrophils were enumerated using a hemacytometer.

**Cellular staining and flow cytometry**

Bladders isolated from euthanized mice were cut into small pieces with a scalpel. Tissue was digested for 50 min at 37˚C with agitation in 0.5% heat-inactivated FBS, 20 mM HEPES, pH 7. 0.057 Kunitz U/l DNase I (Sigma-Aldrich), and 1 mg/ml collagenase A (Roche) in RPMI 1640 medium, with repeated passage through an 18.5-gauge needle 25 min into the incubation. Erythrocytes were lysed as described above. E-lysed homogenates were filtered through 40-μm cell strainers and washed once with flow cytometry buffer (1% FBS, 0.01% NaN3 in PBS). After enumeration by hemacytometer, cellular suspensions were treated with mouse anti-CD16/CD32 (clone 2.4G2, eBiosciences, San Diego, CA) for 10 min to block Fc receptors. Surface markers were stained for 30 min, and cells were fixed overnight in a 4% formalin solution. For intracellular staining, fixed cells were permeabilized with 1% FBS, 0.1% saponin in PBS and stained for 60 min. Data

**FIGURE 1.** Spleen and inner inguinal lymph node cells from C57BL/6 mice receiving uropathogenic E. coli infection secrete IL-17A. A, Mice were sensitized transurethrally with PBS or uropathogenic E. coli on days 0, 14, and 28 and were sacrificed on day 33. Upon sacrifice, splenocytes from individual animals (n = 10) were stimulated with medium alone, 5 μg/ml α-CD3 mAb, or 25 μg/ml whole-cell lysate from uropathogenic E. coli strain CFT073. Each symbol represents an individual animal, and bars represent the median values. B, Mice were sensitized transurethrally with PBS or uropathogenic E. coli on days 0 and 7 and sacrificed 2 d after the second sensitization (day 9). Pooled inguinal lymph node cells from each group of animals (n = 6) were plated in replicate wells and stimulated as in A. Bars and error represent the mean ± SEM of triplicate ELISA wells in a representative experiment. n.d., not detectable.

**FIGURE 2.** IL-17A is not necessary for the protective immune response to UTI. Cohorts of WT and IL-17A−/− mice (n = 11–12 each) were sensitized by the transurethral route with uropathogenic E. coli strain CFT073 once (1×), twice (2×), or not at all (naive) on the indicated days. Subsequently, all mice were challenged with CFT073 on day 28. Bladder colonization levels 48 h postchallenge for WT and IL-17A−/− mice are shown. The dotted line indicates the 100 CFU/g tissue limit of detection. Each symbol represents an individual animal, and bars represent the median values. N, naive; TU, transurethral infection.
node cells from uropathogenic *E. coli*-infected mice secreted significantly higher amounts of IL-17A in response to in vitro lysate stimulation than lymphoid cells from PBS-treated animals (\(p = 0.0105\); Fig. 1B). These results indicate that treatment with uropathogenic *E. coli* Ags stimulates adaptive secretion of IL-17A from in vivo-sensitized cells of systemic and local lymphoid origins.

**IL-17A is not necessary for the adaptively acquired protective immune response to UTI**

Because IL-17A was secreted in response to uropathogenic *E. coli* Ags (Fig. 1) and the role that IL-17A plays in bacterial vaccination models (56, 57), we wanted to determine whether IL-17A is required for the generation of a protective immune response to UTI. To do this, we used a reinfection model based on one presented by Thumbikat et al. (58). WT and IL-17A \(^{-/-}\) mice were transurethrally sensitized once (1×), twice (2×), or not at all (N) prior to a 48-h challenge with uropathogenic *E. coli* (Fig. 2). Both WT sensitization groups had significantly fewer bacteria in their bladders compared with WT naive mice (\(p = 0.0193\) for 1× and \(p = 0.0016\) for 2× compared with N; Fig. 2), consistent with a previously published study (58). Sensed IL-17A \(^{-/-}\) mice also exhibited a similar pattern of accelerated clearance (\(p = 0.0071\) for 1× and \(p = 0.0062\) for 2× compared with N; Fig. 2). These results suggest that IL-17A is not required for the generation of a protective immune response to UTI.

**IL-17A transcript is upregulated in response to acute bladder infection by uropathogenic *E. coli***

Because there was no overt defect in the generation of protective immunity in IL-17A \(^{-/-}\) mice, we sought to determine whether IL-17A played a role in the innate response to UTI. To do this, we first examined IL-17A transcript dynamics during acute infection. Mice were inoculated transurethrally with uropathogenic *E. coli*, and their bladders were collected for qPCR analysis at several timepoints. As expected, there was a 3.7-fold increase in IL-17A expression at 48 h post-infection (Fig. 3). These results indicate that IL-17A is not necessary for the adaptively acquired protective immune response to UTI.

**IL-17A transcript dynamics in response to acute infection and peak expression levels in WT and γδ TCR \(^{-/-}\) mice.** A, Mice (n = 4–18 per time point) were transurethrally inoculated with uropathogenic *E. coli* strain CFT073, and bladders were collected at specified time points for analysis of IL-17A transcript levels by qPCR. B, WT and γδ TCR \(^{-/-}\) mice (n = 9 each) were inoculated transurethrally with uropathogenic *E. coli* and sacrificed at 48 hpi for analysis of bladder mRNA by qPCR. Each symbol represents an individual animal, and bars represent the median values.

**γδ T cells are a source of IL-17A during acute UTI.** Bladders from mice treated transurethrally with PBS (n = 7) or uropathogenic *E. coli* strain CFT073 (n = 9) were collected at 48 hpi, made into a single-cell suspension, and stained for flow cytometry. Forward-versus side-scatter plots were gated to include all CD4+ and γδ TCR+ cells as determined by backgating. CD4+ and γδ TCR+ gates were then interrogated for IL-17A positivity based on unstained and singly stained bladder control samples. A, Total numbers of CD4+ and γδ TCR+ cells as determined by backgating. CD4+ and γδ TCR+ gates were then interrogated for IL-17A positivity based on unstained and singly stained bladder control samples. B, Flow plots showing CD4+ (left panels) and γδ TCR+ (right panels) cells also staining positive for IL-17A from representative PBS-treated (top panels) and uropathogenic *E. coli* infected (bottom panels) animals. C, The total number of CD4+ and γδ TCR+ cells also staining positive for IL-17A. D, Frequency of γδ TCR+ cells also positive for IL-17A in PBS-treated and uropathogenic *E. coli* infected animals. In A, C, and D, each symbol represents an individual animal, and bars represent the median values.
time points during a 28-d period. Uropathogenic E. coli-infected mice demonstrated a dramatic increase in IL-17A, with median values peaking at 48 h postinfection (hpi) (Fig. 3A), suggesting a role for IL-17A in the innate immune response to UTI.

γδ T cells are a significant source of IL-17A during acute UTI in the mouse model

Because TCR δ−/− mice are more susceptible to UTI (22), and γδ TCR+ cell populations are known to express IL-17A in the context of bacterial infection (25, 28–30, 59), we wanted to determine whether TCR δ−/− mice had a deficiency in IL-17A transcript expression upon bladder infection. WT C57BL/6 and TCR δ−/− mice were inoculated transurethrally with PBS or uropathogenic E. coli, and their bladders were analyzed by qPCR at 48 hpi. WT and TCR δ−/− mice exhibited significant upregulation of IL-17A compared with PBS-treated controls (data not shown). However, the median value of IL-17A expression was 3.7-fold higher in the WT mice (1.16 × 10−3 relative to GAPDH for WT compared with 3.1 × 10−4 for TCR δ−/− mice; Fig. 4B). These results demonstrate that mice deficient in the γδ TCR tend to have lower expression of IL-17A at 48 hpi, suggesting that γδ T cells are a source of the IL-17A secreted in response to uropathogenic E. coli bladder colonization.

Because TCR δ−/− mice express less IL-17A in response to experimental UTI, we sought to quantify the level of IL-17A expression by γδ T cells in infected WT animals by flow cytometry. Bladders were isolated from PBS- or uropathogenic E. coli-inoculated WT C57BL/6 mice at 48 hpi and made into single-cell suspensions for staining and flow cytometric analysis. For comparison, the expression of IL-17A by CD4+ cells was also analyzed. Although the number of infiltrating CD4+ cells was an order of magnitude higher than that of γδ TCR+ cells, the increases in both populations were statistically significant (p = 0.0021 and p = 0.0229, respectively; Fig. 4A). Each population was then interrogated for IL-17A positivity, as depicted by representative plots (Fig. 4B). Only γδ cells exhibited statistically significant increases in IL-17A positivity after uropathogenic E. coli infection (p = 0.0225; Fig. 4C). In addition, the median frequency of γδ cells also staining positive for IL-17A was ~5%; in some animals, up to 12% of the γδ cell population expressed IL-17A compared with the PBS group (p = 0.0317; Fig. 4D). These
results indicate that at 48 hpi, γδ T cells are responsible for the upregulated IL-17A transcripts seen in uropathogenic E. coli-injected mouse bladders. Recently, it was shown that responsiveness to IL-23 is important for the expression of IL-17A by γδ T cells in a non–TCR-dependent fashion (60). To determine whether IL-23R was expressed in the bladder and, thus, could be mediating a role in IL-17A expression by γδ T cells in response to UTI, we quantified IL-23R expression in bladder tissue by qPCR. IL-23R transcript was detected in the bladder of C57BL/6 WT mice, regardless of the state of their infection (Fig. 5A), and levels of IL-23R transcript were similar in WT and IL-17A−/− mice (data not shown). These results indicate that IL-23R is present in the bladder tissue of mice and may play a role in the rapid expression of IL-17A in response to UTI.

In addition to IL-23R expression, we wanted to probe the expression of γ and δ variable chains to determine whether a particular subset of γδ T cells is responsible for the secretion of IL-17A in response to UTI. Bladders from C57BL/6 mice that were transurethrally infected with CFT073 for 48 h were harvested and prepared for histological examination (H&E stain, original magnification: A–D, ×40, bar = 500 μm; E–H, ×200, bar = 100 μm). Open arrowhead indicates intraepithelial inflammation; black arrowhead indicates perivascular and interstitial inflammation. B, bacteria; BV, blood vessel; L, lumen; LP, lamina propria; M, muscularis; TE, transitional epithelium; UC, umbrella cell.

**FIGURE 8.** WT and IL-17A−/− mice exhibit similar histological profiles in response to UTI. WT (A, C, E, G) and IL-17A−/− (B, D, F, H) mice were left untreated (A, B, E, F) or were infected with uropathogenic E. coli for 48 h (C, D, G, H). Subsequently, bladders were harvested and prepared for histological examination (H&E stain, original magnification: A–D, ×40, bar = 500 μm; E–H, ×200, bar = 100 μm). Open arrowhead indicates intraepithelial inflammation; black arrowhead indicates perivascular and interstitial inflammation. B, bacteria; BV, blood vessel; L, lumen; LP, lamina propria; M, muscularis; TE, transitional epithelium; UC, umbrella cell.
**IL-17A plays a role in defending the urinary tract from acute uropathogenic E. coli colonization**

To examine the role of IL-17A in the innate control of UTI, bladder and kidney homogenates from WT and IL-17A−/− mice were cultured at the reported peak (24 hpi) of bacterial colonization in C57BL/6 mice (14). At this time point, IL-17A−/− mice had a 3-fold higher median CFU/g bladder tissue (Fig. 6). By 48 hpi, the peak of IL-17A transcript expression in the bladder (Fig. 3A), this trend increased 10-fold to a 35-fold higher median CFU/g tissue in the bladders of IL-17A−/− mice (Fig. 6). Although these trends were reproducible, we sought to investigate later time points, presuming that the colonization phenotypes resulting from the lack of IL-17A may be exacerbated. Indeed, at 72 and 96 hpi, IL-17A−/− mice had significantly more bacteria colonizing their bladders (p < 0.05; Fig. 6). These results indicate that IL-17A−/− mice are more susceptible to cystitis than are isogenic WT mice.

**IL-17A is necessary for proinflammatory transcript upregulation in response to UTI**

Given that IL-17A mediates inflammatory responses largely by influencing mRNA levels of key cytokines and chemokines posttranscriptionally (39–42), we wanted to determine whether such effects were present in the context of UTI. WT and IL-17A−/− mice were inoculated transurethrally with uropathogenic *E. coli*, and their bladders were collected at 48 hpi for transcript analysis by qPCR. We measured mRNA levels of a panel of chemokines, one antimicrobial effector protein (inducible NO synthase [iNOS]), (Fig. 7A) and cytokines (Fig. 7B) previously shown to be affected by IL-17A expression (63). Strikingly, transcripts for all of the genes investigated were expressed at a significantly lower level in IL-17A−/− mice compared with their WT counterparts (Fig. 7), indicating that animals lacking IL-17A−/− signaling are not able to efficiently upregulate the appropriate mRNA transcripts in infected bladder.

**Infected WT and IL-17A−/− mice exhibit qualitatively similar responses to UTI when examined histologically**

Because IL-17A−/− mice had decreased cytokine and chemokine expression, we wanted to examine the bladder of WT and IL-17A−/− mice histologically to determine whether there were any gross pathological or qualitative differences in inflammation. Longitudinal sections of bladders from WT and IL-17A−/− mice that were uninfected or infected for 48 h (the peak of IL-17A expression) were stained with H&E and visualized microscopically. The sections revealed similar histopathological effects in response to UTI in both backgrounds (Fig. 8). More specifically, bladders from the uninfected mice were histologically within normal limits, without inflammation or other alteration (Fig. 8A, 8B, 8E, 8F). However, bladders from the infected mice had expansion of the lamina propria by edema fluid, accompanied by perivascular and interstitial inflammation (Fig. 8C, 8D, 8G, 8H, black arrowheads). These occurrences ranged from mild to severe in WT and IL-17A−/− mice. Additionally, umbrella cell sloughing was apparent in the infected animals (compare apical surface of the transitional epithelium in Fig. 8E and 8F to that in Fig. 8G and 8H). Inflammatory infiltrates consisted primarily of neutrophils, although sometimes a mixed monocytic and neutrophilic infiltrate was observed (high-power images not shown). Occasional intraepithelial inflammation was also noted in both backgrounds (Fig. 8H, open arrowhead). Small numbers of adherent bacteria were observed in slides from infected animals, whereas larger numbers of adherent and intraluminal bacteria were present in sections from IL-17A−/− mice (Fig. 8H, “B” with arrow), indicative of a decreased ability to eliminate bacteria and consistent with higher bacterial loads in the knockout animals.

**IL-17A is required for optimal macrophage and neutrophil infiltration in response to UTI**

Neutrophils are the first cell type to migrate to the bladder in the event of UTI, and they are crucial for controlling infection at early time points (10, 19, 64). Because no differences in neutrophil numbers were seen histologically, a more quantitative approach to determine the neutrophil response in WT and IL-17A−/− mice was taken. We counted the number of neutrophils in the urine post-infection using a hemacytometer; the peak of this measurement is 6 hpi in mice and humans (19, 65). At 6 hpi, IL-17A−/− mice had significantly fewer neutrophils in their urine (p = 0.0480; Fig. 9). Additionally, IL-17A−/− mice lacked a population of high-responder mice that was present in the WT cohort; these animals had >1.5 × 10⁶ neutrophils/ml in their urine at 6 hpi (compare 45% of WT animals to 17% of IL-17A−/− animals, p = 0.0262; Fig. 9). Because IL-17A transcript and protein are detectable in the bladder at this early time point (Fig. 3A) (14), these results indicate that IL-17A may be important for very early neutrophil migration to the bladder in response to uropathogenic *E. coli* infection.

To investigate the innate cellular response to UTI in the absence of IL-17A at a later time point (48 hpi), we used flow cytometry to quantify the number of macrophages and neutrophils localized to the bladder tissue in WT and IL-17A−/− mice. Macrophages were determined by interrogating the bladder for F4/80+MHC class II+ cells, whereas neutrophils were defined as Ly-6G+CD11b+MHC class II− cells. Representative plots showing the gating for macrophages and neutrophils in WT and IL-17A−/− bladders are shown (Fig. 10A, 10C). After quantification, the macrophage (p = 0.0145; Fig. 10B) and the neutrophil (p = 0.0031; Fig. 10D) cell populations were significantly lower in IL-17A−/− mice compared with WT mice. These data reveal that IL-17A plays an important role in the recruitment of macrophages and neutrophils to the bladder in response to uropathogenic *E. coli* infection.

**Discussion**

With the exception of asymptomatic bacteriuria (66), the bladder mucosa has been widely accepted as a sterile environment. Mammalian hosts use a number of mechanisms to keep this niche microbe-free, because infection by bacterial and fungal pathogens can lead to serious clinical consequences. In this study, we characterized the role of the cytokine IL-17A during UTI using a murine model. IL-17A was upregulated specifically in response to uropathogenic *E. coli* Ags by secondary lymphoid tissue cells from uropathogenic *E. coli*-infected C57BL/6 mice. IL-17A transcripts were also highly upregulated in the bladders of acutely infected mice; γδ T cells were
Although no role for IL-17A in vaccination-induced UTI protection was observed, we noted a deficiency in bladder neutrophil influx during the very early stages of acute cystitis and higher bacterial burdens in IL-17A−/− mice. Knockout mice also had impaired proinflammatory transcript expression and fewer macrophages and neutrophils infiltrating the bladder tissue. Taken together, these results define IL-17A as an important factor in the innate immune response to uropathogenic E. coli-mediated UTI.

Although the presence of pathogen-specific Abs in the urine and serum of infected humans and experimental animals has been documented for decades (67), the cascade of immunological events that occurs during the generation of adaptive immunity during UTI has not been established. Although cells from uropathogenic E. coli-sensitized mice highly upregulate IL-17A in vitro, IL-17A was not necessary for the generation of vaccine-induced protective immunity (Fig. 2). This result is in contrast to vaccination models for Streptococcus pneumoniae, Bordetella pertussis, and Pseudomonas aeruginosa, in which IL-17A was required for a protective immunity (56, 57, 68). Because the immune system features redundant pathways, it is unclear whether there is a compensatory factor acting in bladder or lymphoid tissue or whether the downstream effects of IL-17A are not necessary for a protective response to UTI.

In addition to secretion in a recall response setting, IL-17A is upregulated in an innate fashion (Fig. 3A). Similarly, airway IL-17A peaked innately in response to intranasal infection with Chlamydia muridarum, another mucosal pathogen, and this was dependent on bacterial replication (26). In experimental UTI, IL-17A upregulation was also dependent on the ability of uropathogenic E. coli to successfully colonize the urinary tract, because a fecal strain that is unable to colonize the bladder efficiently (EFC4) (48) does not induce IL-17A transcript (data not shown). A recent study by Ingersoll et al. (14) surveyed cytokine and chemokine protein in the bladder of mice during a 2-wk experimental UTI. Although most of the cytokines examined returned to near baseline levels 1 wk postinfection, IL-17 remained elevated (higher than in control mice) throughout the experiment (14). Our transcript data agreed with this and the fact that IL-17A is highly upregulated in response to UTI, with peak levels attained only days postinfection.

A number of cell types has been shown to secrete IL-17A (23). Unlike classical γδ T cells, which recognize Ag that is processed and presented in the context of self-MHC molecules, γδ T cells harbor the ability to directly recognize cognate Ag, allowing for the rapid production of effector molecules (61, 69). Therefore, because of the early upregulation of IL-17A in the bladder (Fig. 3A) (14), we reasoned that CD4+ Th17 cells are not the principal source of IL-17A. Despite being in the T cell minority (Fig. 4A), intracellular staining and flow cytometric analyses demonstrated that γδ T cells were a major source of IL-17A during UTI (Fig. 4B–D). Of note, TCR δ−/− mice were still able to generate some IL-17A transcript over background (Fig. 3B), demonstrating that additional cell types

**FIGURE 10.** Macrophage and neutrophil infiltration is deficient in IL-17A−/− mice in response to UTI. WT and IL-17A−/− mice were inoculated with uropathogenic E. coli strain CFT073 via the transurethral route. Mice (n = 14) were sacrificed at 48 hpi, and their bladders were harvested and prepared for analysis of innate cellular infiltrate by flow cytometry. Forward- versus side-scatter plots were gated to include all MHC class II+ F4/80+ and Ly-6G+CD11b+ cells, as determined by back-gating. The Ly-6G+CD11b+ population was further interrogated for MHC class II negatively to enumerate neutrophils. A, Representative flow plots showing MHC class II+F4/80+ cells from WT and IL-17A−/− animals. B, The total number of MHC class II+F4/80+ cells. C, Representative flow plots showing Ly-6G+CD11b+ cells from WT (left panels) and IL-17A−/− animals (right panels). The bottom plots show MHC class II expression by the boxed population in the top plots. D, The total number of Ly-6G+CD11b+MHC class II− cells. Numbers in A and C are the percentage of the parent population; each symbol in B and D represents an individual animal, and bars represent the median values.
In the context of UTI, IL-17A seems to play a role in the optimal restriction of bacterial burden (Fig. 6). Infection models for *Listeria monocytogenes*, disseminated *E. coli*, *Klebsiella pneumoniae*, oral and systemic *Candida albicans*, oral *Toxoplasma gondii*, *C. muridarum*, *Bacillus subtilis*, and others also show that IL-17A signaling is required for acute clearance of the invading organism (25, 26, 29, 31, 32, 34, 70, 71). This collection of data demonstrates the breadth and versatility of IL-17A–mediated pathways in handling various classes of microbes. In contrast, IL-17A was shown to be dispensable for clearance in infection models for systemic *Salmonella enterica* serovar Enteritidis and pulmonary *Mycobacterium bovis* bacille Calmette-Guérin (28, 30, 72).

Collectively, these data demonstrate that IL-17A plays a role in the innate immune response to experimental UTI in a mouse model. Because many of the genes influenced by IL-17A have similar function during UTI in mice and humans (76), we expect that IL-17A also plays a role in controlling the bacterial burdens early during UTI in humans. IL-17A accomplishes such control by enhancing the presence of mRNA transcripts important for the infiltration of neutrophils and other inflammatory mediators. The presence of such cell types is crucial to the defense of the urinary tract from epithelial cell adherence and subsequent invasion by uropathogenic *E. coli*.

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**Disclosures**

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