Comparing Pro-Inflammatory Responses Produced by Human Bladder Cells Infected with Invasive and Non-invasive strains of *Escherichia coli*

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ABSTRACT

Urinary tract infections (UTIs) are common infections caused by different types of bacteria. *Escherichia coli* (*E. coli*) are the most common uropathogenic bacteria that cause UTIs in women, men, and children. Some strains of uropathogenic *E. coli* are able to invade into the urinary tract tissues that can lead to recurrent UTIs. It is important to understand the mechanisms that enable some strains of uropathogenic *E. coli* to invade into tissues. During an infection process, the innate immune system responds by inducing the production of pro-inflammatory molecules that play an important role in regulating immune responses and inflammation at the site of infection. In the current study, we wanted to determine if there are differences in the production of pro-inflammatory molecules when human bladder cells are infected with an invasive or non-invasive strain of *E. coli.* We used *E. coli* strain K12 as our non-invasive strain and *E. coli* strain CI5 as our invasive strain. The human bladder cell line 5637 was used as the host cell in our experimental setup. We were able to detect more expression of IL-6 and IL-8 in human bladder 5637 cells that were infected with invasive strain of *E. coli.* There was also more IL-6 and IL-8 produced when the 5637 cells were infected with a higher multiplicity of infection (MOI). These findings demonstrate that host cells respond differentially towards invasive and non-invasive strains of *E. coli* by producing different concentrations of pro-inflammatory molecules upon infection.

INTRODUCTION

UTIs occur in men, women and children of all age groups; however women are known to be more susceptible. More that 50% of women will develop a UTI at least once in their life time. Roughly 11 million cases of UTIs are reported in the US each year, with costs estimated to be $5 billion dollars annually (1). Over 80% of UTIs can be attributed to different strains of uropathogenic *Escherichia coli* (UPEC) (2). For a long time, the infectious agents that caused UTIs were considered to be extracellular pathogens. However, recent reports have demonstrated the presence of invasive UPEC that have the ability to invade into urinary tract tissues and cells to form intracellular bacterial communities (IBCs) or quiescent intracellular reservoirs (QIRs) (3). Uropathogens that form IBCs and QIRs are able to hide from the immune system and have been shown to cause recurrent UTIs. While it is vital to study the mechanisms that enable some strains of UPEC to invade into host tissues, it is also important to gain insight into host cell responses.

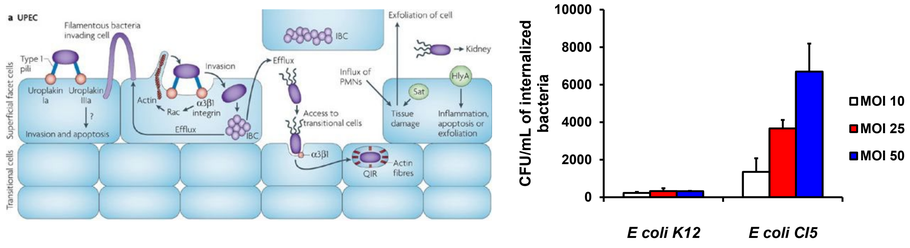


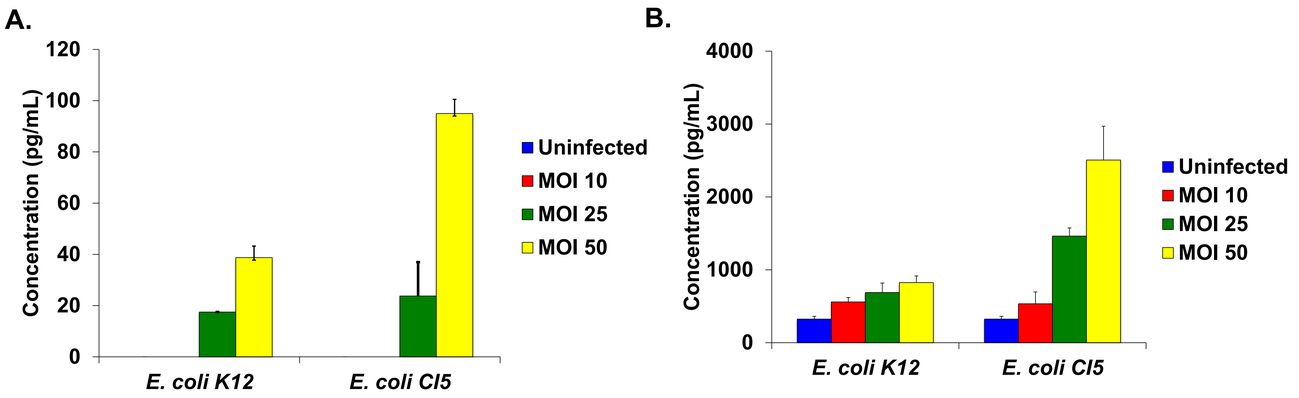
Figure 1: Left: Pathogenic mechanisms mediated by invasive uropathogens to evade the immune system (4). Right: Invasion ability of different strains of *E. coli* in bladder cells.

Bladder epithelial cells respond to bacterial infections by producing pro-inflammatory cytokines and chemokines like IL-6 and IL-8 respectively. These molecules mediate appropriate immune responses for the clearance of pathogens. In the current study we wanted to determine if there was a difference in the production of IL-6 and IL-8 in bladder cells infected with invasive (*E. coli* strain CI5) or non-invasive (*E. coli* strain K12) strains of *E. coli.* We hypothesize that the invasive strain of *E. coli* will induce more production of IL-6 and IL-8. We tested our hypothesis by using the 5637 bladder epithelial cell line as the host and evaluated the secretion of IL-6 and IL-8 by performing enzyme linked immunosorbent assays (ELISAs) at different time points and different MOI. The findings from the study will allow us to determine if there are changes in innate immune responses when cells are infected with different strains of *E. coli.*

RESULTS

Invasive *E. coli* CI5 produce more IL-6 than non-invasive *E. coli* K12

Figure 2: 5637 cells were infected with different MOI of bacteria for 6 hr (A) and 24 hrs (B). IL-6 secretion was determined post-infection by an ELISA



Invasive *E. coli* CI5 produce more IL-8 than non-invasive *E. coli* K12

Figure 3: 5637 cells were infected with different MOI of bacteria for 6 hr (A) and 24 hrs (B). IL-8 secretion was determined post-infection by an ELISA

MATERIALS AND METHODS

Maintenance of the 5637 human bladder cell line 5637: The human bladder cell line were grown in RPM 1640 media supplemented with 7.5% fetal bovine serum. The cells were grown at 37 degrees Celsius in a humid environment containing 5% CO_2. The cells were routinely passaged when they reached 80-90% confluency.

Treatment of 5637 cells: 5637 cells were infected with different MOI (10, 25, and 50) of *E. coli* K12 or *E. coli* CI5. The extracellular bacteria were killed by gentamicin treatment 1 hour post-infection, followed by addition of media. The media was collected 6 or 24 hours post-infection, centrifuged and the supernatant obtained was used for ELISA.

ELISA: ELISA were performed by using the appropriate kits (Biolegend) and following the manufacturer's instructions.

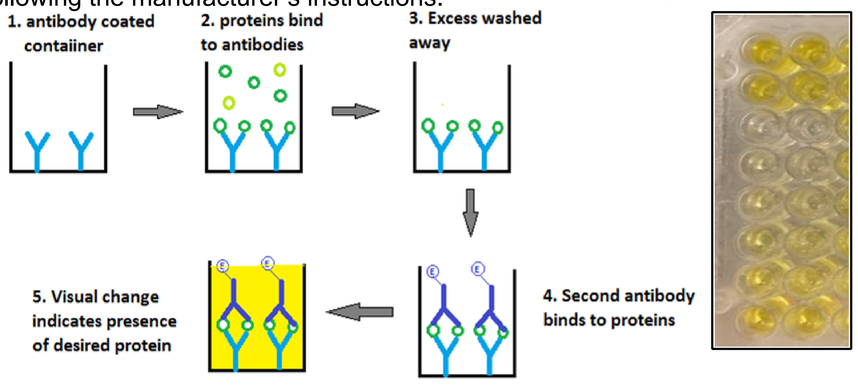


Figure 4: (A) Depiction of the principle of an ELISA. (B) A sample plate is shown after the technique is performed.

DISCUSSION

The results of our experiments indicate that:

- Infection of 5637 cells with the invasive *E. coli* CI5 strain as well as non-invasive *E. coli* K12 strain resulted in induction of IL-6 and IL-8 secretion.
- More IL-6 and IL-8 was produced at 24 hours when compared to 6 hours post-infection
- 5637 cells infected with the invasive *E. coli* CI5 strain produced more pro-inflammatory molecules at all time points tested when compared to 5637 cells

Thus, bladder cells respond differentially towards invasive and non-invasive strains of *E. coli* by producing different concentrations of pro-inflammatory molecules upon infection. These findings will aid us in understanding how host cells mount innate immune responses towards invasive and non-invasive pathogens.

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