Escherichia coli isolated from human polymicrobial bacteriuria are able to suppress in vitro interleukin production

D. Saverino¹, A.M. Schito², A. Mannini³, S. Penco⁴, A.M. Bassi⁴, G. Piatti²*

¹ Department of Experimental Medicine, Section of Human Anatomy, University of Genova, Via De Toni 14, 16132 Genova, Italy
² Department of Specialist and Surgical Sciences, Microbiology and Organ Transplantation, University of Genova, Largo R. Benzi 10,16132 Genova, Italy
³ Department for Territory and Resources Study, University of Genova, Corso Europa 26, 16132 Genova, Italy
⁴ Department of Experimental Medicine, Section of Pathology, University of Genova, Via L.B. Alberti 2, 16132 Genova, Italy
* piatti@unige.it

KEYWORDS: Escherichia coli, urinary tract, polymicrobial infections, microbial synergy, Toll-like receptor, host immunity

Abstract

Urinary tract infections are frequently polymicrobial and mainly due to Escherichia coli. Cystitis and pyelonephritis are usually associated with an inflammatory response. Uropathogenic E. coli possess the capability to evade host defenses, modulating the innate immune response. The aim of this study was to determine if particular E. coli strains correlate with mixed bacteriuria and if the escape from the early host defense and a microbial synergy could be cause of the microbial association. We found significant correlations among polymicrobial urine, absence of pyuria and quinolone/fluoroquinolone susceptible E. coli isolates, and their major capability to suppress the interleukin-8 urothelial production with respect to the resistant strains.

Introduction

Urinary tract infections (UTIs) affects a microbially naive system. They are mainly due to Escherichia coli [1] and, especially when complicated, are often polymicrobial in nature [2-4]. Different strains of E.coli are endowed with different characteristics and Virulence Factors (VFs) [5]. While the laboratory (K-12) strain of E.coli induces proinflammatory signaling in a Toll-like receptor (TLR)-dependent fashion in cultured bladder epithelial cells [6], uropathogenic E.coli (UPEC) strains possess the capability to subvert the early innate defense by not stimulating them and, on the contrary, by suppressing their IL-6 response to K-12 and to the exogenous lipopolysaccharide (LPS), a pathogen-associated molecular pattern [7]. The ability to evade host defenses is broadly distributed among UPEC isolates and is shared by many pathogenic bacterial species [8], among which, some that are involved in mixed colonization and infections.

The aim was to consider the synergistic interaction among different microorganisms as a partial cause of the establishment of human polymicrobial bacteriuria. We analysed monomicrobial and polymicrobial urine cultures positive for E. coli. Isolates had been previously characterized through their biotype, the presence of defined VFs and their susceptibility towards quinolones (Q) and fluoroquinolones (FQ) antibiotics [9]. Here, we report the relationship between UPEC Q/FQ susceptibility and the onset of polymicrobial UTIs, linked to a defect of neutrophil presence, and the peculiar ability of Q/FQ susceptible UPEC to suppress IL-8 and IL-1β secretion by urothelial cells in response to K-12 E. coli.

Materials and methods

Patients, urinary specimens, bacterial culture and characterization of E.coli isolates

188 E. coli isolates were selected from those obtained in 2005 and 2006 from urinary specimens, from internal medicine and surgery wards or from outpatients. A single strain of E. coli was obtained from each patient. E. coli susceptibility to Q/FQ was tested by disk diffusion techniques. Isolates were assigned to four E. coli phylogenetic groups and tested for presence of extraintestinal pathogenic-associated VFs, as reported previously [9]. Pyuria was positive when at least 10 leukocytes were present in 1 ml/urine.

We included in the study urinary specimens positive for E. coli (>1x10⁷ CFU/ml) and where the count of additional microbial species was >1x10⁵ CFU/ml. Finally, we selected bacterial strains according to their susceptibility to Q/FQ.

Cytokines production

IL-8/CXCL8, IL-12/p70 and IL-1β concentration in cell supernatants were measured by ELISA kits (Bender).
Escherichia coli isolated from human polymicrobial bacteriuria are able to suppress in vitro interleukin production

Results
Out of 188 urines, 126 (67%) were monomicrobial, while 62 (33%) were polymicrobial. Polymicrobial cultures were significantly more frequent among the urines containing susceptible rather than resistant strains, i.e. 40 (43%) versus 22 (23%), respectively. \( P = 0.008 \). Leukocytes were present in 88 urines (47%) and absent in 100 (53%). Absence of pyuria was more frequent when specimens yielded Q/FQ susceptible E. coli (63%) than resistant (44%, \( P = 0.013 \)) (data not shown).

<table>
<thead>
<tr>
<th>Urinary specimens</th>
<th>Total (n=188)</th>
<th>No. (%) poly</th>
<th>No. (%) mono</th>
<th>( P )</th>
<th>No. (%) E.coli ( S^\text{a} )</th>
<th>No. (%) E.coli ( R^\text{a} )</th>
<th>No. (%) E.coli ( S^\text{a} )</th>
<th>No. (%) E.coli ( R^\text{a} )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyuria absence</td>
<td>100 (53)</td>
<td>40 (65)</td>
<td>60 (48)</td>
<td>0.03</td>
<td>30 (75)</td>
<td>10 (25)</td>
<td>29 (48)</td>
<td>31 (52)</td>
<td>0.012</td>
</tr>
<tr>
<td>Pyuria presence</td>
<td>88 (47)</td>
<td>22 (33)</td>
<td>66 (52)</td>
<td></td>
<td>10 (45)</td>
<td>12 (55)</td>
<td>26 (39)</td>
<td>40 (61)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Characteristics of urines according to quinolone and fluoroquinolone susceptibility of Escherichia coli isolates. a S, quinolone/fluoroquinolone susceptible; R, quinolone/fluoroquinolone resistant E.coli. b Percentages related only to polymicrobial (n = 40) and to monomicrobial (n = 60) specimens characterized by pyuria absence/presence as indicated.

Tab.1 shows a significant difference (\( P = 0.031 \)) between absence of leukocyte in polymicrobial urines (65%) and the same event in the monomicrobial ones (48%). Out of 40 polymicrobial specimens without pyuria, 30 (75%) yielded Q/FQ susceptible E. coli, while 10 (25%) yielded resistant strains (\( P = 0.012 \)). This finding further underlines the connection among Q/FQ susceptibility, mixed microbial growths and absence of pyuria. In addition, 119 (64%), 128 (69%) and 105 (56%) of the 186 E. coli strains suppressed IL-6, IL-1\( \beta \) and IL-8 production respectively, in 5637 epithelial cells in response to stimulation by K12. When the incidence of cytokine suppression was correlated to Q/FQ E. coli susceptibility, a significant difference (\( P < 0.001 \)) between susceptible and resistant strains (87% versus 26%) emerged for IL-8 suppression (Fig. 1).

Discussion
Polymicrobial infections are investigated with increasing frequency only when they occur on human mucosal surfaces. Poor information is available about mixed infections in microbially naïve mucosa, since this condition is usually considered contamination, chance event or infection due to predisposing factors of the patients.

Here, a correlation among Q/FQ susceptibility E. coli isolates, polymicrobial urines and absence of pyuria was observed. In addition, Q/FQ resistant E. coli presence mostly correlated with peculiarities of a compromised host, known to be frequently affected by polymicrobial UTIs. This finding creates an apparent paradox, but led us to further consider E. coli pathogenicity, related to Q/FQ susceptibility, as a partial cause of both polymicrobial bacteriuria and lack of pyuria. We did not find any relation between mixed bacteriuria or the absence of pyuria and the assessed VFs of Q/FQ susceptible E. coli.

We report that, in a mixed stimulation of 5637 bladder epithelial cells, the ability to suppress their IL-6, IL-1\( \beta \) and IL-8 production in response to laboratory E. coli K-12 was shared by the majority of clinical isolates. Of note, tight correlation between Q/FQ susceptibility of UPEC strains and the suppression of IL-8 production was shown for the first time.

Finally, no correlation between inhibition of cytokines by E. coli strains with polymicrobial urines and

![Figure 1. Urinary E.coli modulate cytokine production in 5637 cells induced by K12 E.coli. Urinary E.coli strains suppressed IL-6, IL-1\( \beta \) and IL-8 production in 5637 epithelial cells in response to K12 E. coli stimulation. Figure displays results from the total cohort of susceptible, S (tot), and resistant strains, R (tot). Asterisk indicates that the amount of cytokines produced was significantly different (\( P < 0.05 \)) when S (tot) strains effect was compared both to K12 alone and to R (tot) effects.](image)
absence of pyuria was observed. This could be due to susceptible strains possessing additional capability, other than cytokine suppression, or that the polymicrobial specimens analyzed represent contamination. In the latter hypothesis, it is unlikely that the finding of polymicrobial urines, absence of pyuria and suppression of IL-8 correlates exclusively with susceptible _E. coli_ isolates.

Acknowledgements

This work is supported by the PRIN’05 program by the Italian Ministry of University and Research (MIUR). We are indebted to Jennifer McDermott for English review.

References


