Short communication

Influence of mutation in \textit{cj0183} and \textit{cj0588} genes for colonization abilities of \textit{Campylobacter jejuni} in Caco-2 cells using confocal laser scanning microscope

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Abstract

The \textit{cj0183} and \textit{cj0588} genes identified in the \textit{Campylobacter jejuni} NCTC 11168 genome encode proteins with homology to virulence factors found in other bacteria. Previous studies showed that single mutation in the \textit{cj0183} gene does not affect adhesion of \textit{C. jejuni} to the Caco-2 cell line whereas protein encoded by \textit{cj0588} is involved in adherence to the Caco-2 cells. In the presented study differences in invasion index were observed between mutants in both genes and single mutation of \textit{cj0588} in 81116 and 81-176 \textit{C. jejuni} strains. This fact indicates that Cj0183 protein might play some role in invasion of bacteria into host cells.

Key words: \textit{Campylobacter jejuni}, adhesion, invasion, confocal microscopy

Introduction

\textit{Campylobacter jejuni} colonizes the intestinal digestive tract of animals, especially birds, as commensal microbiota. Bacterial transmission to humans, inducing severe gut inflammation, occurs mainly due to the improperly prepared poultry products. Studies demonstrated that adhesion and invasion ability of \textit{C. jejuni} promote the process of colonization. Several cell lines of human and non-human origin have been used to characterize the interaction of \textit{C. jejuni} with host cells (Dasti et al. 2010). Caco-2 cells are most commonly used as an assay which is useful to mimic the behavior of \textit{Campylobacter} in both chicken and human gut (Hanel et al. 2004).

Our previous studies indicate that mutation in the \textit{cj0588} gene influences the adherence abilities of \textit{C. jejuni} to the Caco-2 cell line. This mutation reduces both adhesion and internalization of 81-176 and 81116 \textit{C. jejuni} strains to the epithelial cell line (Sałamaszyńska-Guz and Klimuszko 2008). Interaction studies of the purified Cj0588 protein with

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<th>Strain</th>
<th>Invasion index %</th>
<th>Reference</th>
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<tr>
<td>81-176</td>
<td>10.14 ± 1.02</td>
<td>(Salamaszyńska-Guz and Klimuszko 2008)</td>
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<tr>
<td>81-176-ΔCj0588</td>
<td>9.1 ± 0.09</td>
<td>(Salamaszyńska-Guz and Klimuszko 2008)</td>
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<tr>
<td>81-176-ΔCj0183ΔCj0588</td>
<td>6.04 ± 0.95</td>
<td>this study</td>
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<tr>
<td>81116</td>
<td>17.63 ± 3.1</td>
<td>(Salamaszyńska-Guz and Klimuszko 2008)</td>
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<tr>
<td>81116-ΔCj0588</td>
<td>11.07 ± 1.76</td>
<td>(Salamaszyńska-Guz and Klimuszko 2008)</td>
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<tr>
<td>81116-ΔCj0183ΔCj0588</td>
<td>6.92 ± 1.15</td>
<td>this study</td>
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Fig. 1. A) Comparative analysis of invasion index of the wild type 81-176 strain and mutants 81-176-ΔCj0588, 81-176-ΔCj0588ΔCj0183 and wild type 81116 strain and mutants 81116-ΔCj0588, 81116-ΔCj0183ΔCj0588. Values represent means ± S.E.M. of three independent experiments (P<0.05). B) Representative confocal fluorescence microscopic images of C. jejuni-infected Caco-2 cells 4 h after infection. The microtubules (red) appear as structural skeletons outlining the cells and the FITC-labeled bacteria (green) appear as spots along the microtubules. B1) C. jejuni 81-176, B2) C. jejuni 81-176 ΔCj0588, B3) C. jejuni 81-176 ΔCj0183ΔCj0588.

Materials and Methods

Adhesion and invasion assays for cj0183 and cj0588 mutants were performed as described by Salamaszyńska-Guz and Klimuszko (2008). The C. jejuni cells and the Caco-2 cell tubulin were visualized using mouse monoclonal anti-bovine -tubulin antibodies, Alexa Fluor 546 conjugated goat anti-mouse IgG (Molecular Probes Inc.) and polyclonal anti – Campylobacter jejuni – FITC conjugate (Fitzgerald). Caco-2 cells infected with C. jejuni wild type as well as adequate mutated strains were observed using confocal microscopy (Fig. 1). Confocal microscopy was performed with a confocal laser scanning microscope FV-500 (Olympus Polska Sp. z o.o., Poland).
Results and Discussion

The invasion index, which describes the percentage of bacteria which have infected epithelial cells compared to the number of cells that have adhered, calculated for the mutants in both genes of 81-176-ΔCj0588Cj0183 and 81116-ΔCj0588Cj0183 strains decrease in comparison to mutant in cj0588 gene only (P<0.05) (Fig. 1). According the previous results (Sałamaszyńska-Guz and Klimuszko 2008) single mutation in cj0183 gene was not statistically important for adhesion and internalization of 81-176 and 81116 strains to the epithelial cell line but together with protein encoded by mutated cj0588 gene unexpectedly reduced invasion abilities of examined strains. It may suggest the possible role of Cj0183 protein in colonization of host epithelial cells by C. jejuni which was not so far identified.

Acknowledgements

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References


