Interfaces properties of Legionella pneumophila: an issue to explain host-cell association?
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Introduction:

Legionella pneumophila is naturally found in fresh water as well under planctonic bacteria as associated form within protozoa (infection of amoebae) or biofilms. Upon aerosol formation via man-made water systems, L. pneumophila can enter the human lung and cause a severe form of pneumonia, called Legionnaires’ disease. The pathogenesis of Legionnaires’ disease is largely due to the ability of L. pneumophila to invade and grow within macrophages. Unlike its intracellular cycle, first step of L. pneumophila adhesion to the host cell is unexplored. The physicochemical composition of the bacterial cell surface is however an important factor in bacterial pathogenicity. Since it is this surface that first interacts with soluble factors (e.g., the humoral immune system) and the extracellular matrices of the infected host tissues and cells. Many bacteria, in particular gram negative, display a polysaccharide coat, either as a discrete layer of exopolysaccharide (lipopolysaccharide, capsule), or as a loosely attached slime layer. Interfaces properties of bacteria are known to govern the adhesion mechanisms and consequently they may potentially influence the attachment to the host cell during infection, more particularly early step of adhesion.

Phenomena governing bacterial adhesion to the host-cell

Among the exopolysaccharides produced by gram-negative bacteria (lipopolysaccharide (LPS) comprising three distinct sections: lipid A anchored in the outer membrane, oligosaccharide core and O-antigen (O-Ag) polysaccharide. Concerning L. pneumophila, the O-Ag consists of a variable number of repeated saccharide units (n = 18 to 40), identified as levanmannosaccharide, to form a polysaccharide layer that extends into the surrounding media.

Hypothesis:

Lower is the bacterial charge, weaker are the electrostatic repulsions, better is the bacterial adhesion.

Materials & Methods:

1. Bacterial strains and culture condition

<table>
<thead>
<tr>
<th>Clinical strains of Legionella pneumophila</th>
<th>Mab 3/1 positive strains</th>
<th>Mab 3/1 negative strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lene</td>
<td>Bellingham</td>
<td>Oka</td>
</tr>
<tr>
<td>Knoxvile</td>
<td>Camptodrom</td>
<td></td>
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<tr>
<td>Philadelphia</td>
<td>Oxford</td>
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<tr>
<td>Baniodom</td>
<td>Heysham</td>
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</tbody>
</table>

Bacteria were grown sequentially on BCYE 3 days, on BCYE 2 days and finally to early step of stationary phase in 100 ml BYE. Bacteria were pelleted (10 min, 1,000g), washed once with 1 mM KNO3 and resuspended in 1 mM KNO3 at -10°C chilled before electrophoretic measurements.

2. Electrophoretic measurements (Zetaplotrometer IV, CAD Instruments)

Image analysis software allowed recorded images to be processed in real time to calculate the electrophoretic mobilities from the displacement of bacteria subjected to a constant direct-current electric field (300 V/m). Different cycles were performed and 100 mean bacterial trajectory was obtained for each condition. All the experiments were carried out in natural pH (1-6) and repeated thrhoold with different cultures.

Results:

- The LPS nature at the L. pneumophila surface contributes to the charge of the strains
- Among clinical strains, Mab 3/1 positive bacteria are mainly involved in community acquired infection whereas Mab 3/1 negative are rather source of nosocomial cases. From this fact, bacterial charge could explain differences in host cell association and also the infection efficiency.