Factor H binding protein (fHbp) mediates differential complement resistance of a serogroup C Neisseria meningitidis isolate from CSF of a patient with invasive meningococcal disease

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INTRODUCTION

Results of an outbreak of invasive meningococcal disease at University of Southampton in 1997, two Neisseria meningitidis (Nm) group C isolates were retrieved from a person who died (Case) and a person (Carrier) who performed mouth-to-mouth resuscitation on the case without contracting the disease. Isolate were shown to have identical serological (PorA and PorB), MLST and PFGE profiles1-2 and whole genome comparison revealed differences in only eight genes3. Here we expand on previous studies to investigate phenotypic differences which could explain their contrasting clinical outcomes.

RESULTS

Isolates' proteomes show a high degree of homology

Comparative proteomics revealed that the isolates' proteomes are almost identical. Four proteins (prpC, imp, fba, aldA) are upregulated in the Case isolate while one protein (pilC2) is downregulated. Expression levels for prpC, imp, fba and aldA genes were quantified via qRT-PCR and shown to be upregulated in the Case isolate (Table 2).

Table 2. Comparative proteomics results.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Protein ID</th>
<th>ID of native gene expression ( nonsense codon)</th>
<th>Protein product (function)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB01732</td>
<td>clone synthase (fHbp)</td>
<td>29.27</td>
<td>transferrin activity</td>
<td>Uproregulated in the Case isolate</td>
</tr>
<tr>
<td>MB01417</td>
<td>hypothetical integral membrane protein (fHbp)</td>
<td>47.1</td>
<td>hypothetical membrane protein</td>
<td>Uproregulated in the Case isolate</td>
</tr>
<tr>
<td>MB03090</td>
<td>choline-binding protein (Imp)</td>
<td>15.94</td>
<td>choline-binding to human plasma membranes additional function (function)</td>
<td>Uproregulated in the Case isolate</td>
</tr>
<tr>
<td>MB01942</td>
<td>NAD-dependant dehydrogenase (aldA)</td>
<td>20.91</td>
<td>NAD-dependant dehydrogenase (function)</td>
<td>Downregulated in the Case isolate</td>
</tr>
</tbody>
</table>

Factor H binding protein (fHbp) is not expressed by the Carrier

Peptides from Factor H binding protein (fHbp) were not detected in the Carrier isolate in mass spectrometric analysis.

The lack of fHbp was further confirmed by the presence of a one base deletion (AT366) causing a frameshift mutation in the Carrier isolate.

Furthermore Western blot analysis on the isolates detected fHbp in Case but not in the Carrier (Fig. 1).

Figure 1. Western Blot using JAR5 anti-fHbp mAb showed no fHbp expression in the Carrier. Full-length fHbp (variant 1, V1) is expressed by the Case. RmpM was used as loading control.

Abstence of fHbp expression accounts for reduced complement resistance of the Carrier isolate

Increased survival in human serum for the Case compared to the Carrier isolate was observed in a serum survival assay.

To test whether this could be attributed to fHbp, fHbp expression was complemented in the Carrier strain (erm - fHbp-Carrier), resulting in a significant increase in serum survival for this isolate. This indicates an important role played by Hbp in complement resistance (Fig. 2).

Figure 2. Serum survival assay showed an increased survival in serum for the Case compared to the Carrier. Complementation of fHbp expression in the Carrier increases its survival by 31%.

Bexsero induces bactericidal antibodies against both isolates

Bexsero, a vaccine designed to target MenB strains, can be effective in preventing disease caused by other serogroups, if these isolates express one or more of its antigens.

Sera from mice vaccinated with Bexsero or NadA strongly reacted against both isolates in whole-cell ELISA, suggesting that isolates express at least one Bexsero antigen (Fig. 3).

Moreover, bactericidal killing of both isolates was demonstrated in Serum Bactericidal Antibody assays (SBA). Increased titres were observed for the Case compared to the Carrier (killing at reciprocal dilution of 50% with titres of 2048 and 512 respectively), likely due to the presence of anti-fHbp antibodies in Bexsero serum.

Figure 3. Whole-cell ELISA using Bexsero, NadA and NHBa mouse sera on Carrier and Case showed strong reactivity with Bexsero and NadA sera for both isolates.

CONCLUSIONS

- Isolates genomes and proteomes are mostly identical;
- factor H binding protein (fHbp) is not expressed by the Carrier, while it is expressed by the Case isolate;
- fHbp expression by the Case isolate enhanced its survival in human serum. This might have played a role in the virulence of this isolate in the deceased student;
- Re-introduction of fHbp expression in the Carrier isolate increases Carrier's survival in human serum indicating an important role played by fHbp in complement resistance;
- SBA killing of group C Case and Carrier isolates was observed with murine serum raised against Bexsero.

REFERENCES and ACKNOWLEDGEMENTS

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