The Outer Membrane of Borrelia; The Interface Between Them and Us

Richard Bingham
The University of Huddersfield

Lecture Outline

• I will give an overview of the outer membrane of Borrelia
• I will present evidence from various sources showing how Borrelia can:
  – Adhere to extracellular matrix
  – Invade tissues
• I will briefly discuss the biofilm formation
• I will then talk about the research projects at Huddersfield
  • Evasion of the complement immune response
  • SodA
  • Enolase

Phylogenetic Tree of Life

Borrelia outer membrane morphology

• The basic structure consists of a cytoplasmic membrane surrounded by peptidoglycan and a loosely associated outer membrane (OM).

  Unique characteristics:
  – Abundance of lipoproteins
  – Glycolipids (no lipopolysaccharide)
  – The presence of cholesterol

(Ben-Menachem et al., 2003; Schroder et al., 2003; Stubs et al., 2009)
Adhesins

- Adhesins are bacterial cell-surface components that facilitate adhesion or adherence to cells or extracellular matrix

The extracellular matrix

- During persistent infection *Borrelia* is localized to the extracellular matrix
- This provides a protective niche.
- Numerous proteins on the surface of *Borrelia* are involved, binding to various proteins of the extracellular matrix
  - DbpA binds to decorin
  - BBK32 binds to fibronectin

Recent Research on BBK32

- *Borrelia burgdorferi* protein BBK32 binds to soluble fibronectin via the N-terminal 70 kDa region, causing fibronectin to undergo conformational extension Gemma Harris, W. Ma, LM, Maurer, JR. Potts and DF Mosher, J. Biol. Chem. published online June 24, 2014

Fibronectin Binding

- BBK32 is a surface lipoprotein
- Binds fibronectin
- Binds to glycosaminoglycans (GAGs)
- bbk32 was also shown to be expressed during tick engorgement as well as in the mammalian host.
- The mechanism of binding is very similar to FnBPA from *S. aureus*

Decorin Binding Proteins DbpA and DbpB

- There is a direct association between *B. burgdorferi* and the proteoglycan decorin,
- Decorin is a glycoprotein and binds to collagen

The question of Biofilm

- We need to be careful about how we define biofilm.
What is Biofilm?

- Biofilms can be defined as sessile communities of surface-attached cells encased in a self-produced extracellular matrix.
  - Polysaccharides, proteins, and extracellular DNA are the primary matrix components in other bacteria.
- Treatment is challenging due to the resistance to both antimicrobials and host defenses.

Borrelia has Biofilm-like properties

- Borrelia burgdorferi binds to, invades, and colonizes native type I collagen lattices
- Borrelia burgdorferi binds strongly to the extracellular matrix and cells of the connective tissues.
- Bb grew and formed microcolonies

Staphylococcal biofilm life cycle


- Biofilms can be defined as sessile communities of surface-attached cells encased in a self-produced extracellular matrix.

? extracellular matrix?

- There is a lack of evidence to show that Borrelia secretes significant amounts of any polysaccharide, DNA or proteins to form an extracellular matrix.
  - (one paper suggests the polysaccharide alginate, but this has not been repeated and there is a strong possibility of contamination)
- There are many unanswered questions- What genes are involved? Where does the eDNA come from?
- More research is required

Research at Hudderfield

- Lipoproteins can be readily identified by their characteristic signal sequence
- Genome data allows us to identify all potential lipoproteins
- MKKVKSKYLA LGLLFISC

Positive charge
Non-polar
Cysteine amino acid

Yes- There is lots of evidence for this. The Borrelia proteins involved have been identified and we have a reasonable understanding of how this occurs.

So far, no strong evidence for this ( in my opinion ).

LDA Conference 2014 - Richard Bingham
Research at Huddersfield

- Very few beta-barrels are known in Borrelia – P66, BamA (BB0795), P137
- Our aim is to identify and characterise novel membrane spanning beta-barrel proteins in Borrelia
- The major target is the highly conserved OmpA-type membrane-spanning domain

Some *E. coli* Beta-barrels

![Some E. coli Beta-barrels](image)

Pfam family PF01389

![Pfam family PF01389](image)

Topology Prediction

![Topology Prediction](image)

Amino acid sequence alignment

![Amino acid sequence alignment](image)
Literature Search

- BB0405 is expressed in human and tick - see next slide (Brooks et al, 2006)
- BB0405 is surface exposed (Yang et al, 2011)
- BG0407 may bind Factor-H (Bhide et al, 2009)
- BB0405 and BG0407 are homologues of BAPKO_0422
- 87% sequence identity

The Complement System

- Part of the innate immune response
- A large number of plasma proteins activated by three initiation pathways:
  - The Classical Pathway
  - The Alternative pathway
  - The Lectin Pathway
- The alternative pathway is continuously activated at a low level
- Non-selectively attacks all surfaces in contact with host plasma.
  - Such as the outer membrane of an invading bacterial cell.
- This system is regulated by various proteins - (e.g., Factor H)

BAPKO_0422 binds to human Factor H

- Affinity Ligand Binding Immunoblot - ALBI

A cascade of events leads to the formation of the Membrane Attack Complex

General overview of the methods

- Blocking – milk powder 5% made up in TBS – 2 hours
- Factor H wash 73μg/ml (14hr)
- Primary antibody was 1:1000 (1hr)
- Secondary 1:5000 (1hr)
- Washes between each step – 3 washes at 5min each with TBS-Tween

Brooks et al, INFECTION AND IMMUNITY, Jan. 2006, p. 296–304
Negative controls
(done the same day, with identical solutions but **no Factor-H wash**)

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<tr>
<th>Lane</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Human Factor H Positive control</td>
</tr>
<tr>
<td>B</td>
<td>BAPKO_0422</td>
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The absence of a band in lane B shows that the primary and secondary antibodies do not bind to BAPKO_0422.

Loading BSA, SodA or LDAO detergent alone are also blank.

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**Conclusion**

- We have identified four outer membrane proteins in *Borrelia*
- Cloned, produced pure recombinant protein
- Basic structural characterisation
  - Circular dichroism
  - Molecular envelope by SAXS
  - Demonstrated fH binding by ALBI

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