Borrelia evading the human immune response

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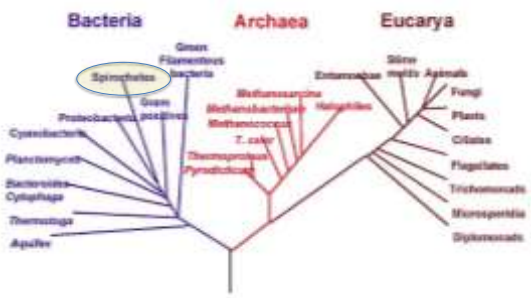
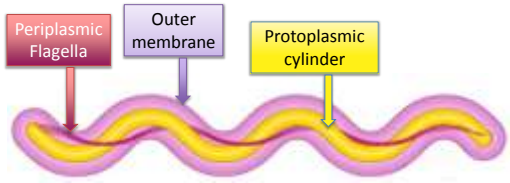
Lecture Outline

- Borrelia
  - A brief introduction to a unique bacterium
- Immune evasion strategies
  - Evading the complement response
  - Adherance to the extracellular matrix
- Research at Huddersfield

Phylogenetic Tree of Life

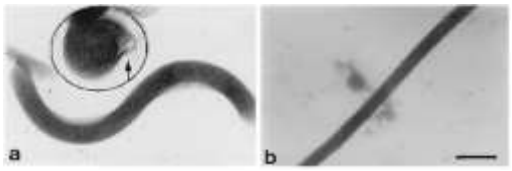


Borrelia cell morphology



Electron Microscopy images

- From Goldstein *et al*, 1996
- The protoplasmic cylinder is rod shaped
- The periplasmic flagella are helical
- Rotation of left-handed periplasmic flagella result in a swimming motion



Wild type Periplasmic flagella deficient mutant

- Interaction between the flagella and the protoplasmic cylinder forms a <u>flat wave structure</u>
- This flat wave beats 5-10 times per second at room temperature in growth media.
- This corkscrew motion is thought to allow greater motility in a viscous environment. (compared with external flagella).
  - (tick gut and human cell cytoplasm)

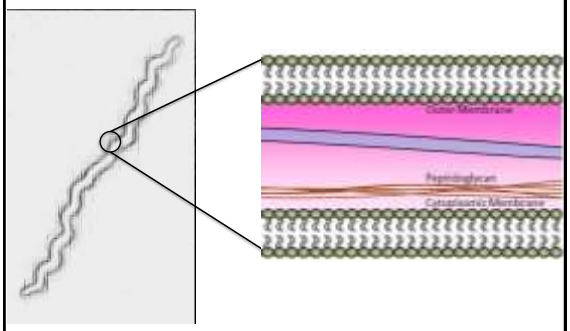
Antigenic variation

- A proofreading 3'-5' exonuclease is present in all other bacteria except *Borrelia*
  - The absence of this enzyme is thought to result in error prone replication of DNA.
  - These genetic changes may lead to new variants of exposed antigens.

The unusual genome of *Borrelia*

- One linear chromosome of 910,000 base pairs
  - with ~850 genes mostly of homology to known proteins
- ~17-21 different linear and circular plasmids
  - ~670 genes, many of which are unique to Borrelia
  - ~167 pseudogenes
- Plasmids comprise approximately 40% of the entire genome
- largest number known for any bacterium

The outer membrane of *Borrelia*



Borrelia outer membrane morphology

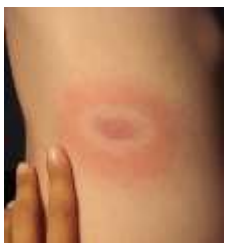
- The basic structure consists of a cytoplasmic membrane surrounded by peptidoglycan and a loosely associated outer membrane (OM).

  Unique characteristics:
  - Abundance of lipoproteins
  - Glycolipids replace the usual lipopolysaccharide
  - The presence of cholesterol

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

Stage 1 - erythema migrans

- The highly motile spirochetes spread through the dermis, causing a characteristic expanding "bull's eye" rash
  - erythema migrans
- frequently accompanied by headache, joint and muscle pain, and fever.
- High bacterial numbers in the blood stream
  - **Evasion of the complement system**

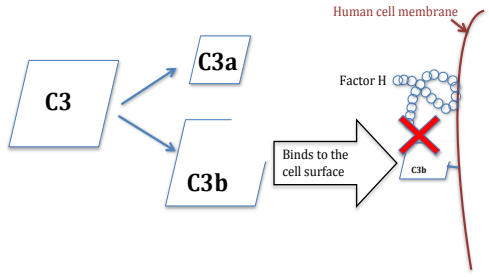


The Complement System

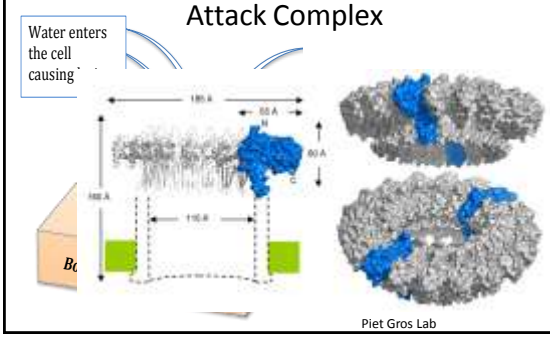
- Part of the innate immune response
- A large number of plasma proteins activated by three initiation pathways.
  - The Classical Pathway
  - The Alternative pathway
  - The Lectin Pathway
- The alternative pathway is continuously activated at a low level
- non-selectively attacks all surfaces in contact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

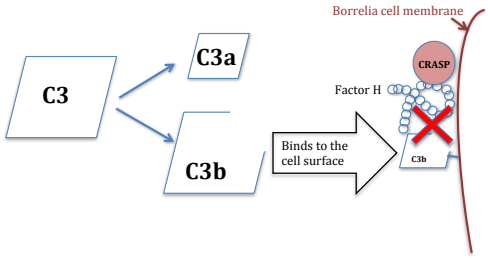
Regulation of Complement

- Various plasma proteins such as factor H, FHL-1, C4b binding protein, and C1 inhibitor regulate the complement system and prevent the system attacking host cells.
- We will focus on Factor H
  - Borrelia has many Factor H binding proteins



C3b causes a cascade of events leading to the formation of the Membrane Attack Complex





Complement Regulator Acquiring Surface Proteins = **CRASPs**

- CRASPs- cell surface proteins
- Bind with high affinity to human factor H (fH) and factor H like-1 (FHL1)
- This gives resistance to complement-mediated killing by inhibiting the formation of the terminal membrane attack complex
- Expression of CRASPs
  - repressed in the tick vector
  - increased in the mammalian host

Numerous CRASPS have been identified

- Numbered CRASPs 1-5
- Structures have been solved for three of them
- CRASP-1 (or BBA68) (Cordes et al 2005)
- CRASP-3 (or OspE) – (Bhattacharjee *et al* 2013)
- CRASP-4 (or ErpC) – (Caesar et al 2013)
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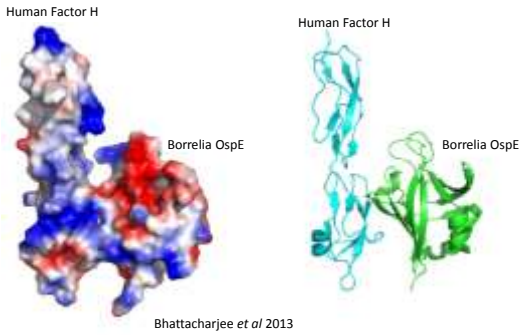
An example - OspE

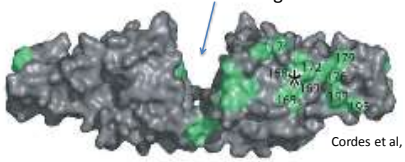


Bhattacharjee *et al* 2013

CRASP-1 Structure

- unusually high levels of non-synonymous substitution onto the surface are green
- Evidence of positive selection
- A "genetic arms race" between Borrelia and host species

This cleft is the Factor H binding site



Cordes et al, 2006

Different Strains

- Complement resistant strains (e.g. *B. afzelii*) survive successfully in body compartments where complement concentration is high,
- Most *B. garinii* strains do not bind fH on their surface and thus are prone to complement-mediated killing (Bhide *et al*, 2009);
  - They ARE able to invade the nervous system where complement concentration is low.

- Different *Borrelia* strains infect different hosts.
- The host competence for different strains of *Borrelia* parallels their fH binding ability

  Examples:
  - *B. burgdorferi* binds to human and mouse fH
  - *B. coriaceae* binds to Mouse, Rat and cattle fH, not human
  - *B. bissettiii* binds to Mouse fH, not human
  - *B. valaisiana* binds to human and dog fH

Klaus Kurtenbach

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

Decorin Binding Proteins DbpA and DbpB

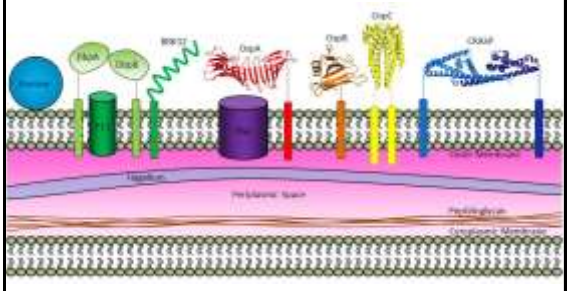
- There is a direct association between *B. burgdorferi* and the proteoglycan **decorin**,
- Decorin is a glycoprotein and binds to collagen

Fibronectin Binding

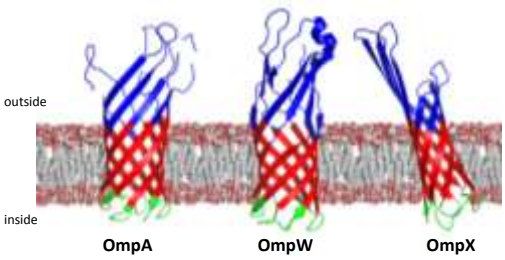
- BBK32 is a surface lipoprotein
- Binds fibronectin
- Binds to glycosaminoglycans (GAGs)
- bbk32 was also shown to be expressed during tick engorgement as well as in the mammalian host.

Invasion of the CNS

- Lyme neuroborreliosis is associated with an inflammation of the central nervous system (CNS)
- neuronal cell damage and loss.
- encephalitis, cranial neuropathy, and meningitis
- B. burgdorferi may cross the blood brain barrier and enter the CNS as a means to circumvent the adaptive immune response of the host.



An introduction to Beta-barrels



These proteins are virulence factors in many other bacteria

- *E. coli* OmpX
  - promotes bacterial adhesion to and entry into mammalian cells, resistance to complement
- *N. meningitidis* OpcA
  - Role in adherence to and invasion of human epithelial and endothelial cells
  - binds vitronectin, which in turn binds integrins (Virji, 1993).
- *E. coli* OmpA - neonatal meningitis
  - Role in penetration of the blood brain barrier (Huang, 1995, Selvaraj, 2007)

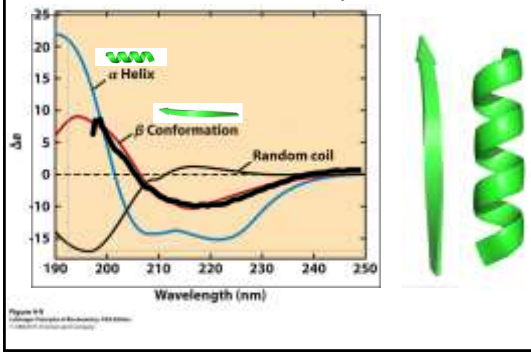
Research at Huddersfield

- Very few beta-barrels are known in Borrelia
  - P66, BamA (BB0795), P13?
- 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**

OmpA as a vaccine target

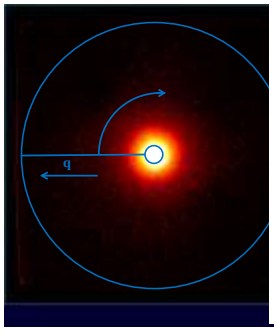
- Exposed outer loops
- High sequence conservation between different Borrelial strains
- OmpA from several other bacterial species have been shown to induce specific humoral and cytotoxic responses in the absence of adjuvant.
-
- OmpA has been proposed in the design of vaccines for numerous other bacteria
  - *Klebsiella pneumoniae, Chlamydia, Neisseria gonorrhoeae, Salmonella*
- Further reading: Jeannin *et al*, 2002

Circular Dichroism Spectroscopy



Small Angle X-ray Scattering

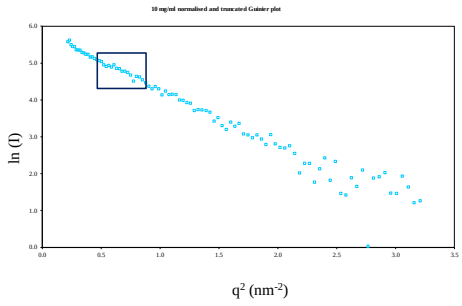
X-ray Scattering



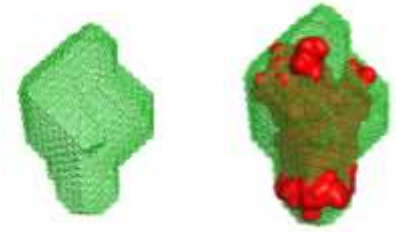
- Low resolution technique
- Protein in solution
  - Molecules free rotating
- Data collection over 15 – 18 hours

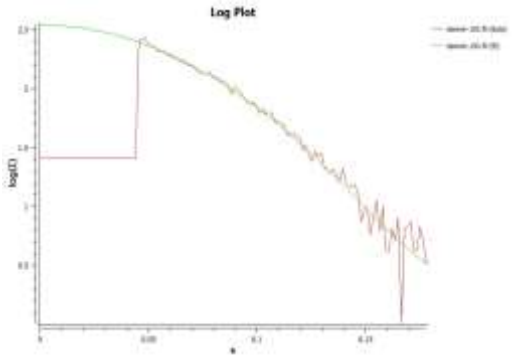
Guinier plot

(q^2 vs ln (I))



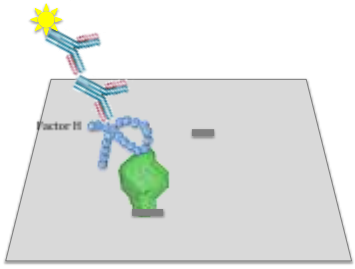
- The data is processed using the ATSAS Package
  - Comprised of many separate programs: PRIMUS, GNOM, DAMMIF, DAMSEL, DAMSUP, DAMAVER, DAMFILT, DAMSTART, DAMMIN, CRYSOL
- Calculates a molecular envelope





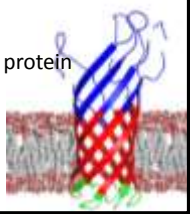
Factor H binding

- Affinity Ligand Binding Immunoblot - ALBI



Conclusion

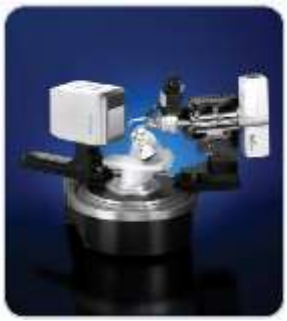
- **Borrelia has numerous adhesins, these bind to a variety of different host proteins**
  - **Allows dissemination to different sites**
- We have identified a family of novel outer membrane proteins in Borrelia
- Potential vaccine target
- Cloned, produced pure recombinant protein
- Basic structural characterisation
  - Circular dichroism
  - Molecular envelope
  - Demonstrated fH binding



Future Work

- X-ray crystal structure- high resolution model
- Clone and express all 4 proteins in this group from *B. burgdorferi, B. garinii* and *B. afzelii.*

Protein X-ray Diffraction



- The University of Huddersfield has recently purchased a Bruker diffractometer.
- This will allow high resolution structure determination

Acknowledgments

University of Huddersfield
- Gemma Brown
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References

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