Neisseria meningitidis, les secrets de la subversion de l’endothélium vasculaire cérébral

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Human + \textit{N. meningitidis}:
- Commensalism: No disease
- Pathogenesis: Disease
  - Dissemination: Disease
  - No dissemination: No disease

Human + \textit{M. tuberculosis}:
- Pathogenesis: Disease
  - Dissemination: Disease
  - No dissemination: No disease
- Latency: No disease
Neisseria meningitidis, a paradigm of extra cellular bacterial pathogen

Human- nasopharynx + N. meningitidis

Blood

SEPTICEMIA
PURPURA FULMINANS

Cerebrospinal fluid
MENINGITIS

Commensalism

Environment (Influenza virus)

Host factors
+ Deficiency in late complement components
+ Deficiency in maltose binding protein
+ Lack of immunity (young children)

Bacterial factors:
- capsule (+++), Iron adhesines/pili, Opa..
- filamentous phage
Meningococcal infections, a public health burden

- 1 to 1.5 per 100,000 inhabitants,
- Two peaks before 2 years and at 15-25 years of age

- Fulminant septicemia (30% death) even when treated
- Meningitis (3% death)
- Can be responsible for small epidemics in developed countries and large epidemics in Africa (the meningitis belt)

- Two major serogroups in Europe: B (60%) and C (40%).
- A vaccine against C is available
- No vaccine against B serogroup
Structural relation of the capsular serogroup B of *N. meningitidis* to the carbohydrate terminal of the neonatal neural cell adhesion molecule (n-CAM); NeuNac = *N*-acetylneuraminic acid.
LA MÉNINGITE CÉRÉBRO-SPINALE EN AFRIQUE

L. Laparisonnie

ORGANISATION MONDIALE DE LA SANTÉ
GENÈVE
1963
How *N. meningitidis* once in the bloodstream can cross the blood brain barrier?
Subpial vein
Pia mater
Dura mater
Arachnoid
Glia limitans
Subarachnoid space
Brain post-capillary venules and venules (Virchow-Robin Perivascular space)
Ventricle
Choroid plexus
Subarachnoid space
Gray matter
White matter
Sulcus
Brain capillary (gliovascular unit)
Skull
CSF Neuropyle
CSF
Blood-Brain barriers
Blood-CSF barriers
A- Choroid plexus
Secretory epithelial cells
Fenestrated capillary
Stroma
B- Meningeal veins
Subarachnoid space
Glia limitans
Subpial space
Perivascular space
Brain capillary (gliovascular unit)

Blood-brain barriers
A- Brain capillaries
Astrocytic end-foot
Pericyte
Endothelial cell
Microglial cell
Adherent junction
Tight junction belt
Virchow-Robin Perivascular space
5 µm

B- Brain post-capillary venules and venules
NS tight junction
Perivascular macrophage
10-50 µm

Brain capillary inter-endothelial junctional complexes
Luminal face
F-actin
ZO-2,3
ZO-1
Claudin
Occludin
JAM
VE-cadherin
Tight junctions
Adherent junctions
Abluminal face
Catenin
Vinculin
Ficolin
Adherent junctions
Brain

Parenchyma

Blood

Bacteria

Heart

Kidney
117 colonies
24 in the cortex
93 in the white matter

Meninges

Cortex

White matter

24 in the cortex

93 in the white matter
117 colonies
24 in the cortex 93 in the white matter

Meninges

Cortex

White matter

24 in the cortex

93 in the white matter
IMMORTALIZATION OF HUMAN BRAIN ENDOTHELIAL CELLS

Primary culture

hCMEC/D3 cell line

The D3 cell line express junctional proteins and makes tight junctions
Brain

Parenchyma

Blood

Bacteria

Heart

Kidney
Type IV pili (Tfp)
PilD

PilF

PilT

E

Assembly Retraction

Outer membrane

Inner membrane

PilC PilQ

PilW

Assembly

PilM, N, O, P

Stabilization


Maturation

PilE, I, K, W

Assembly

PilQ

Prepilin

Minor pilins

assembly

(PilT) retraction

(PilQ) emergence on the cell surface

Functional Tfp (PilE, PilX, PilV, ComP)

Signaling to endothelial cells by *Neisseria meningitidis*

- ezrin
- CD44
- ICAM-1
- ErbB2
- CD46
- F-actin
- Rho
- Cdc42
- Actin polymerisation
- src
- cortactin

Pili signaling lead to the formation of a « cortical plaque »
Host cell surface reorganization is responsible for mechanical resistance of Nm colonies growing onto the apical surface.
Summary

1. Pili are responsible for the interaction of Nm with endothelial cells and signaling

2. How pilus mediated adhesion is responsible for the crossing of the BBB?
Nm recruit adherens and tight junction proteins

Nm  VE-Cadherin  Actin  Merge

p120-catenin  beta-catenin

ZO-1  ZO-2  claudin-5
Formation of junctions (epithelial cells)

The Par3/Par6/aPKC complex is needed for the recruitment of junctional proteins and then segregation of adherens and tight junctions.
Nm recruits the polarity complex Par3/Par6/PKCζ in a Cdc42 dependent manner
Tagged VE-cadherin is recruited from cell-cell junctions
ErbB2 translocation of AJ proteins - cell-cell junction leakage?

VE-cadherin

Membrane protrusion

Unknown receptor

Actin polymerization (honeycomb)

Translocation of AJ proteins - cell-cell junction leakage?
The polarity complex is required to open the paracellular route

VE-cadherin

PKC η PS

Number and surface of gaps observed in hcmec/D3 monolayer

PKC ζ PS

Nm

Nm

PKCζPS

Nm ΔpilE

0 4 8 12 16 20 24

1.7-3.4 3.4-7.6 7.6-15 15-30 > 30 µm²

Nm

Nm

PKCζPS

Nm ΔpilE
Type IV pilus-mediated signaling induces the opening of the paracellular route

Lucifer Yellow Permeability

Bacterial diffusion through hCMEC/D3 monolayer

Fold increase

Control Nm  ΔpilE  Nm PKCζ PS  Nm PKCη PS  D-mannitol

Fold increase

Nm  Nm +PKCζ PS  ΔpilE
Nm recruit the polarity complex and open the intercellular junctions
Neisseria meningitidis activate the β2-adrenergic receptor (β2AR)
β2-adrenoceptor/βarrestins are sufficient to induce formation of the cortical plaque
β2-adrenoceptors/barrestins pathway open junctions

Nm transmigration
5h after infection
Nm recruit the polarity complex and open the intercellular junctions.
Conclusions

1. *N. meningitidis* franchit la BHE grâce à ses pili qui permettent adhésion et signalling par deux récepteurs différents

2. Le passage de la BHE est due à l’ouverture des jonctions intercellulaires suite à un recrutement au siège de l’interaction bactérie-cellule des protéines de jonction intercellulaire

3. Le récepteur membranaire induisant la signalisation cellulaire est le récepteur beta2 adrenergique
Applications

1. Utilisation des composants du pilus interagissant avec le récepteur pour ouvrir la BHE

2. Utilisation de ces mêmes épitopes pour une application vaccinale contre *N.meningitis* de sérogroupe B