Brain sequelae caused by bacterial meningitis: Interactions between pneumococci and neurons

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Background

Bacterial meningitis: Inflammation of the meninges caused by a bacterial infection of the brain, bacteria reach the brain mainly through bloodstream. *Streptococcus pneumoniae* is the main etiological cause of bacterial meningitis worldwide. Although, bacterial meningitis does not have extremely high mortality(10~30%), but permanent brain damages are a major consequence among meningitis survivors, and neuronal damage is often the reason of such brain sequelae. Previous studies have suggested bacterial toxin, pneumolysin, causes neuronal cell death. Moreover, pilus-I plays important role in bacterial adherence to the cells. However, whether *S. pneumoniae* can physically interact with neurons and cause neuronal damage is still unknown.

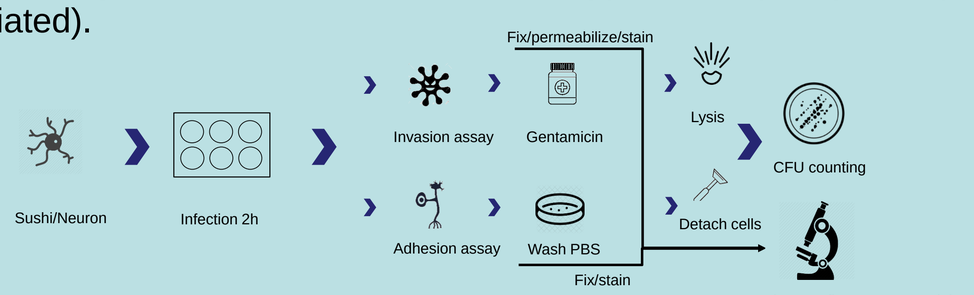
Aim

- To study the capacity of pneumococci to directly interact with neurons.
- To study the molecular mechanism regulating the interaction of pneumococci with neurons.

Material & Methods

1. SH-SY5SY cells: Neuroblastoma cell line (*Human*)
2. Pneumococcal strain

Laboratory strains: piliated serotype 4 TIGR4 wild type and non-piliated TIGR4Δ*rrgA-srtD*. Clinical isolates: serotypes 11A (non-piliated) and 15A (piliated).



Results

1. Validation of neuronal differentiation: SH-SY5Y cells were successfully differentiated to mature neurons after 7 days treatment with Retinoic Acid.

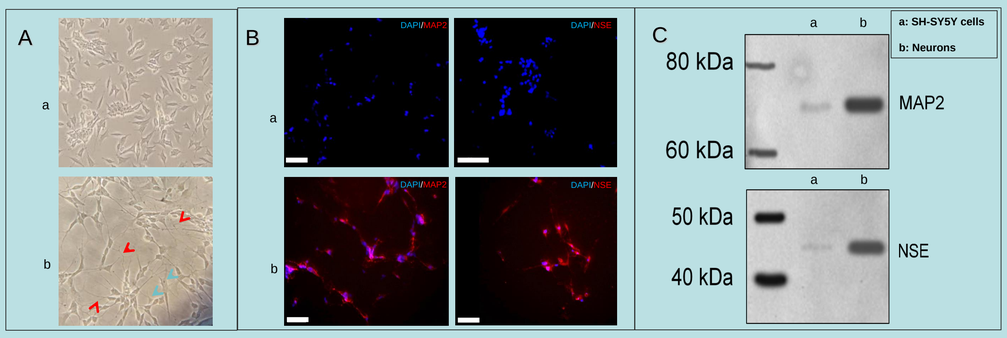
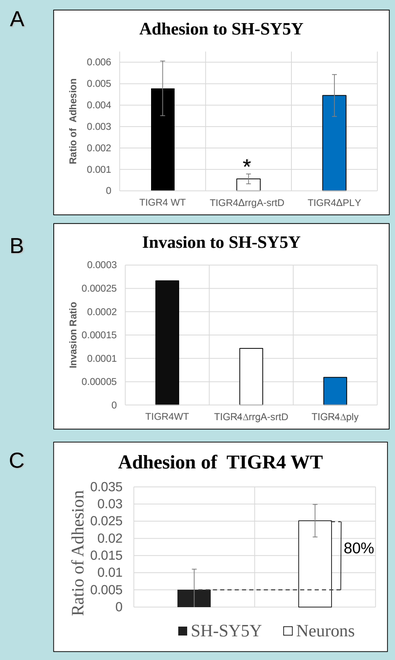


Figure 1. SH-SY5Y cell differentiation to neurons

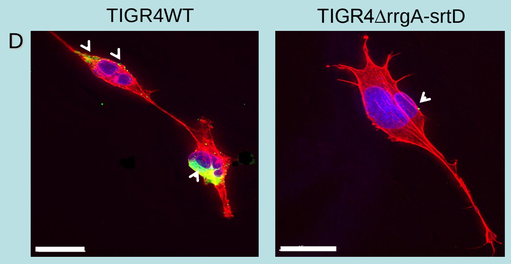
A. Morphological changes of SH-SY5Y cells during differentiation (60 X), Red arrows: neuronal connection upon prolonged axons; Blue arrows: mature neurons. **B.** Expression level of MAP2 and NSE on SH-SY5Y cells and neurons. Cells were stained with MAP2(Left: Red)/NSE(Right: Red) and DAPI(blue). **C.** Expression level of MAP2 and NSE on SH-SY5Y cells and neurons using western blot. Scale bar:25μm

2. *S. pneumoniae* can actively adhere and invade neurons, pilus-I plays important role both in bacterial adhesion and invasion. Pneumolysin is crucial for bacterial invasion.

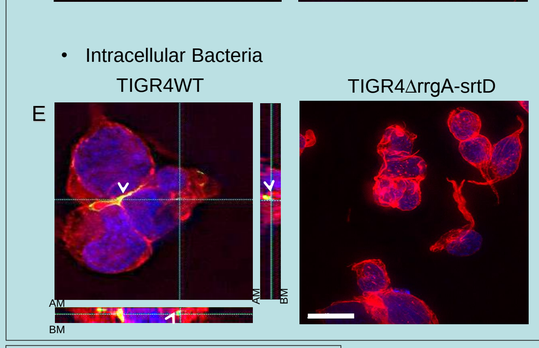
- CFU based bacterial adhesion/ invasion



- Bacterial Adhesion



- Intracellular Bacteria



- TIGR4 WT: wild-type strain
- TIGR4ΔrrgA-srtD: pilus-I knockout strain
- TIGR4ΔPLY: pneumolysin mutant strain

- Ratio of Adhesion=adhered bacteria/total bacteria;
- Ratio of Invasion=invaded bacteria/adhered bacteria.

Figure 2. *S. pneumoniae* bacterial adhesion and invasion to SH-SY5Y cells and neurons

A. Quantification of adhered bacteria on undifferentiated SHSY5Y; **B.** Detected intracellular bacteria in undifferentiated SH-SY5Y cells (* represents $p < 0.05$); **C.** Comparison of bacterial adhesion on undifferentiated SH-SY5Y cells to differentiated neurons; **D.** Immunofluorescence staining of S.pneumoniae adherence to undifferentiated SH-SY5Y cells(Phalloidin). White arrows: adhered bacteria(green: capsule staining). **E.** Immunofluorescence staining of S.pneumoniae invasion on undifferentiated SH-SY5Y cells(Phalloidin). Orthogonal view of detected intracellular bacteria within the cell layer has shown in XZ and XY axis. AM: apical membrane, BM:basolateral membrane. White arrows: intracellular bacteria(green:capsule staining). Scale bar:15μm

3. *S. pneumoniae* co-localize with neuronal protein DBN1 on the plasma membrane through pilus-I and co-localize with MAP2 intracellularly.

- Intracellular TIGR4WT co-localize with MAP2

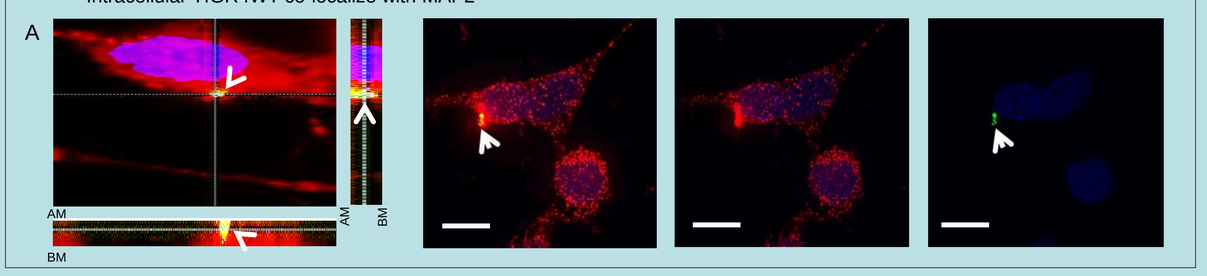
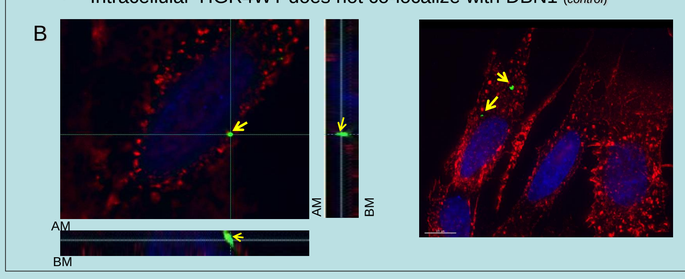


Figure 3. Co-localization of *S.pneumoniae* with DBN1 on the plasma membrane and with MAP2 intracellularly in neurons.

A. Immunofluorescence staining of co-localization of *S.pneumoniae*(TIGR4WT: wildtype strain) with MAP2. White arrows: intracellular bacteria(green); **B.** No co-localization was found on *S.pneumoniae* (non-piliated TIGR4Δ*rrgA-srtD* strain) with DBN1. Yellow arrows: intracellular bacteria(green); Orthogonal view of detected intracellular bacteria within the cell layer has shown in XZ and XY axis. AM: apical membrane, BM:basolateral membrane. White arrows: intracellular bacteria(green:capsule staining); Scale bar:15μm

- Intracellular TIGR4WT does not co-localize with DBN1 (*control*)



4. *S. pneumoniae* causes increased level of neuronal cell death in the presence of pilus-I and pneumolysin.

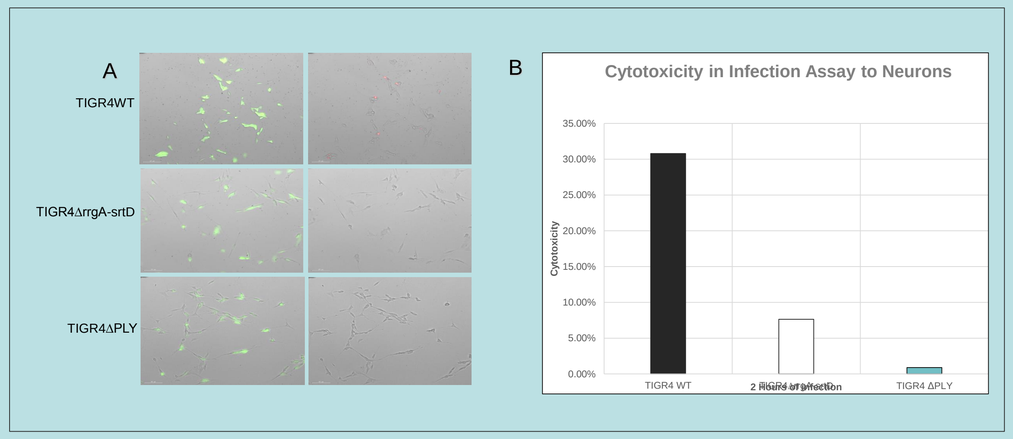


Figure 4. Cell toxicity of neurons during pneumococcal infection

A. Live-dead staining of neurons during bacterial infection(20 X), Left: infection time point zero; Right: infection time point 1 hour. Dead cells stained with Red and live cells stained with Green.

B. Assessment of cell toxicity using LDH assay during 2 hours of bacterial infection.

◆ Take-home messages

Our study shows for the first time in literature that --

S. pneumoniae can directly interact with neurons and ultimately invade neurons; Cell death occurs not only through indirect interaction with neurons such as secreting pneumolysin, but also through direct interaction (adhesion and invasion) with neurons. Possibly, through the interaction with microtubule-associated proteins to induce disruption of cytoskeleton.

- *S. pneumoniae* actively adhere and invade to the neurons, the pneumococcal pilus-I showed to have a important role in this process.
- Pneumolysin does not play as adhesin, but crucial for bacterial invasion.
- MAP2 is important neuronal target for *S. pneumoniae* transport inside neurons.

Future Prospects

- Co-immune precipitation to identify bacterial target protein on the neuronal membrane.
- Blockade of MAP2-bacteria interaction to prevent bacterial interaction with neurons
- Investigate the mechanism of pore-forming toxin pneumolysin in neurons

Funding

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