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Poster presentation



# Phagocytosis of non-encapsulated and encapsulated *Streptococcus pneumoniae* by murine microglia is increased after stimulation with Toll-like receptors agonists

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## Objectives

Bacterial phagocytosis by microglia contributes to the resistance of the brain to infections. Microglial cells express Toll-like receptors (TLR) which can be stimulated by pathogen-associated molecular patterns (PAMPs). We hypothesized that PAMPs may stimulate microglia thereby increasing their ability to phagocytose *Streptococcus pneumoniae*.

## Methods

Primary cultures of mouse microglia were exposed to TLR agonists: tripalmitoyl-S-glyceryl-cysteine (Pam3CSK4 at 0.1 μg/ml; TLR2), endotoxin (LPS at 0.01 μg/ml; TLR4) and oligonucleotides containing unmethylated cytosin-guanosin motifs (CpG at 1 μg/ml; TLR9) for 24 h. TLR agonists were used at the lowest concentrations inducing the maximum stimulation of microglia cells in terms of NO release. After stimulation, cultures were challenged with two *S. pneumoniae* strains: the encapsulated D39 or the unencapsulated R6 strains were added at a ratio of 100 bacteria per cell. Phagocytosis was left to proceed for 30 and 90 min at 37°C + 5% $CO_2$. For phagocytosis inhibition studies, 10 μM cytochalasin D (CD) was used. After washing, the microglial cultures were incubated in medium containing gentamicin (200 μg/ml) for 1 h to kill extracellular bacteria. Thereafter, cells were washed and lysed with distilled water. Viable intracellular bacteria were enumerated by quantitative plating of serial 10-fold dilutions. To monitor intracellular survival, microglia cells were allowed to ingest bacteria for 30 min. Then, incubation in medium with gentamicin was performed for 1 h. Thereafter, viable intracellular bacteria were determined at various time points by quantitative plating after cell lysis. Kruskall-Wallis test followed by Dunn's multiple comparisons test was performed to compare phagocytosis between groups ($n \geq 10$); $p < 0.05$ was considered statistically significant.

## Results

Unstimulated microglia ingested bacteria at a low rate. CpG at 1 μg/ml strongly increased the number of phagocytosed bacteria ($p < 0.01$ at 30 and 90 min). The bacterial uptake was enhanced after stimulation with Pam3CSK4 at 0.1 μg/ml ($p < 0.05$ after 90 min) and LPS at 0.01 μg/ml. The phagocytic rates were different for both strains: the uptake of the non-encapsulated R6 strain was approximately 10 times more rapid than the phagocytosis of the encapsulated D39 strain. CD inhibited phagocytosis > 90% in all groups. Intracellular survival assays showed that bacterial killing was similar among unstimulated microglia and cells stimulated with TLR agonists.

## Conclusion

After stimulation with bacterial TLR agonists, phagocytosis of *S. pneumoniae* by microglial cells is increased. The uptake of the non-encapsulated R6 strain is 10 times quicker than the phagocytosis of the encapsulated D39 strain.