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Title: Evidence for a replicative intracellular stage of staphylococcus aureus in alveolar macrophages

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Body: Objective. Staphylococcus aureus is particularly difficult to eradicate from respiratory tract. There is accumulating evidence that S.aureus is able to survive within some host cells. Alveolar macrophages are professional phagocytes, responsible for clearing infections by the action of their phagolysosomal machinery. The aim of this study was to define S.aureus interaction with alveolar macrophages. Methods. Newman strain was used to infect MH-S cell line. To assess if S.aureus survives intracellularly and define its intracellular trafficking, we performed quantitative (bacterial plating at different times post-infection) and qualitative analysis (immunofluorescence and confocal microscopy). Results. S.aureus uptake requires host actin polymerisation, microtubule assembly and activation of phosphatidylinositol 3-kinase signalling. Time course experiments showed that the number of intracellular bacteria decreased during the first hours, remaining constant till almost 16.5h and finally increased reaching a plateau at 28.5h. These observations were confirmed by immunostaining. Intracellular bacteria were located inside an acidic subcellular compartment endowed with late endosome features. The containing vacuole shows limited accessibility to lysosomal hydrolases and fluid endocytic markers, suggesting a functional separation from the canonical endocytic pathway. Conclusions. This study shows that S.aureus persists and replicates inside alveolar macrophages by preventing the maturation of the phagolysosome. This mechanism of persistence and replication represents a privileged niche that can potentially offer protection from antibiotics and serve as a vehicle for infection dissemination.