

## SHORT PRESENTATIONS 2

S 8-1

**Fate of the complex formed between nucleolin present on membrane of human cells and LVS surface elongation factor Tu (EF-Tu) during LVS infection**M. Barel<sup>1</sup>, K. Meibom<sup>1</sup>, A. Charbit<sup>1</sup><sup>1</sup>Université Paris V René Descartes, INSERM U570, Paris, France

*Francisella tularensis*, the causative agent of tularemia, is one of the most infectious human bacterial pathogens. Participation of C3, CR3, class A scavenger receptors and mannose receptor in bacterial uptake have been already reported in initial bacterial uptake. However, contribution of an additional, as-yet-unidentified receptor for *F. tularensis* internalization has also been suggested. We previously demonstrated that cell-surface expressed nucleolin is a receptor for *F. tularensis* Live Vaccine Strain (LVS). Nucleolin interacts with bacterial ligand EF-Tu. This interaction allows cell infection (Barel et al., *BMC Microbiol.*, 2008).

**Aim:** Our goal was to determine the fate of nucleolin-EF-Tu interaction after adhesion of bacteria on human cell surface.

**Methods:** Human THP-1 monocyte-like cells were infected for 30 min, washed with gentamycin and further incubated for 24 h. Presence of the complex formed between bacterial EF-Tu and human nucleolin was analyzed at 30 min, 5 h and 24 h, after permeabilizing cells and staining with specific antibodies by fluorescent and confocal microscopy. siRNA were used to abolish nucleolin expression.

**Results:** After 30 min infection by LVS, EF-Tu / nucleolin complex was visible on internalized bacteria, as previously described (Barel et al. 2008). Strikingly, after 5 h, when LVS has started to actively multiply in cytosol, bacteria were still interacting with nucleolin. After 24 h, when cells begin to undergo apoptosis, nucleolin was recovered in the nucleus and EF-Tu was no more able to form complex with it. As these results suggest that intracellular nucleolin may also play a role in LVS infection, we studied the effect of its knocking-down with siRNA targeting nucleolin. Cells were transfected with specific or scrambled siRNAs for 72 h then infected with LVS. CFU number was counted at 24 h. Bacterial infection was decreased by 90 % with siRNA targeting nucleolin.

**Conclusions:** These data suggest that *F. tularensis* hijacks human nucleolin to perform a dual role in infection:

- 1) at the cell surface, nucleolin allows LVS entry through its surface-exposed EF-Tu,
- 2) in the cytosol, where it remains transiently bound to bacteria and may participate to *F. tularensis* intracellular survival.

This is the first report of an interaction between *Francisella* proteins and intracellular proteins of its host. This intracellular interaction may be important for triggering signaling pathways, which may facilitate intracellular infection.