[PS2.33]

The ESX-1 secretion system is the molecular mechanism enabling mycobacterial translocation

D Houben*[1], C Demangel[2], W Bitter[3], R Brosch[2], N van der Wel[1], P Peters[1,4] et al

[1]Netherlands Cancer Institute, Netherlands, [2]Institut Pasteur, France, [3]VU University Medical Center, Netherlands, [4]Delft University of Technology, Netherlands

Mycobacterium tuberculosis is one of the most important human pathogens. It was generally considered that mycobacteria reside inside phagolysosomes within macrophages. The paradigm of exclusive phagosomal localization was recently challenged by the identification of *M. tuberculosis* in the cytosol of host cells. In contrast, *M. bovis* BCG, the vaccine strain, remains phagosomal. RD1, which is present in *M. tuberculosis*, but absent in *M. bovis* BCG, encodes crucial components of the recently discovered ESX-1 or type VII secretion system. This system enables secretion of mycobacterial proteins such as ESAT-6 and CFP-10.

As *M. bovis* BCG is, amongst others, missing the RD1 region, we hypothesized that re-introduction of the RD1 region into *M. bovis* BCG might restore the capacity to translocate. In order to determine if ESAT-6 is involved in the phagosomal escape process we have also tested *M. tuberculosis* H37Rv EsxAΔ84-95 that has a 12 amino acid deletion in the C-terminus of ESAT-6. This mutant is still able to secrete ESAT-6 and CFP-10 efficiently. We used log-phase cultures to infect PMA-activated THP-1 cells (human macrophage cell line). These were subsequently fixed and processed for cryo-immunogold electron microscopy, to determine if and at which rate bacterial translocation occurs.

We show that re-introduction of the ESX-1 secretion system into the attenuated and normally phagolysosomal *M. bovis* BCG vaccine strain is sufficient for translocation and can explain the increased virulence. ESAT-6, and especially the C-terminus of ESAT-6, is involved in translocation.

Here we demonstrated that a proper ESX-1 secretion system is the molecular mechanism that restores the capacity to translocate recombinant *M. bovis* BCG from the host phagolysosome into the cytosol. Within the ESX-1 secretion system, ESAT-6 is involved in translocation. Together with data from other mycobacterial species, ESX-1 is shown to be the key mechanism linking translocation and pathogenicity.

Keywords: Mycobacterium tuberculosis, BCG, Pathogenicity, Cytosol