Host and Pathogen Control of Mycobacterial Localisation in Macrophages

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Introduction: For pathogenic mycobacteria to cause disease they must first successfully infect and inhabit host cells of the immune system, most notably macrophages. Within these host cells, mycobacteria have been shown by Transmission Electron Microscopy (TEM) to localise in a variety of environments such as membrane bound compartments and/or the cytosol. The subcellular localisation of mycobacteria defines its relationship with their host cell and ultimately, has implications ranging from how the cell attempts to clear the infection to drug availability in restrictive compartments. Our study focuses on Mycobacterium tuberculosis (Mtb) and Mycobacterium bovis (Mbv), two members of the Mycobacterium tuberculosis complex (MTBC), which are both capable of causing tuberculosis. Previous work has defined the interaction of human macrophages with the human pathogen Mtb, but parallel knowledge of the interaction of Mbv and bovine macrophages is lacking. Understanding of the virulence mechanisms of Mbv is largely based on extrapolation from work in Mtb. However, Mbv and Mtb display contrasting host tropisms and virulence; while Mbv causes disease in cattle (and other animals) but rarely transmits between humans, Mtb rarely causes disease in cattle. Here we investigated if differences in intracellular localisation in human and bovine macrophages can explain contrasting tropisms. For that, human and bovine primary monocyte derived macrophages were infected with Mbv or Mtb and phenotypic differences analysed by TEM and stereological analysis. We seek to understand the intricate role that both host and pathogen play in underlining the ability of pathogenic Mycobacteria spp. to survive, and thrive, in these potentially hostile environments.

Experimental procedures:

Cell culture: Human and bovine peripheral blood monocytes cells (PBMC) are isolated from blood by a Ficoll-Paque™ density gradient. CD14 positive monocytes are then isolated by magnetic separation using CD14 microbeads and LS column (Miltenyi) according to the manufacturer’s instructions. Purified CD14 monocytes are differentiated into macrophages by incubation in RPMI medium supplemented with 10% Foetal bovine serum and with either bovine or human Granulocyte-macrophage colony-stimulating factor (GM-CSF) and harvested after six days. Infection of human and bovine monocyte derived macrophages with mycobacteria: Macrophages were cultured and seeded onto tissue culture treated plastic ware or glass coverslips. Bacterial cultures were grown to mid-exponential phase (OD600 = 0.6 ± 0.2) and prepared as previously described [1]. Mbv and Mtb are incubated with either bovine or human macrophages at a multiplicity of infection (MOI) of 1 and 10 respectively. Cells were washed and fresh media added at 2 h after infection and fixed at 24 h and 2 h after infection.

Ultrastructural Analysis. The protocol for resin embedding was adapted from reference [2]. Samples were fixed by adding warm 2% glutaraldehyde (Electron microscopy sciences) in 200 mM HEPES, pH 7.4, directly to the cell culture medium at a 1:1 volume ratio. After 5 min, the fixative and medium mixture was replaced with 1% glutaraldehyde in 200 mM HEPES buffer, and the samples were incubated overnight at 4°C. Using a modified protocol [3] cells were post-fixed in 2% osmium tetroxide/1.5% potassium ferrocyanide for 1 h on ice, incubated in 1% w/v thiocarbohydrazide for 20 min before a second staining with 2% osmium tetroxide, and then incubated overnight in 1% aqueous uranyl acetate at 4°C. Cells were stained with Walton’s lead aspartate for 30 min at 60°C and
dehydrated through an ethanol series on ice, and embedded in UltraBed resin according to the manufacturer’s instructions (Electron Microscopy Sciences).

Sectioning and image acquisition. Ultrathin sections (~50nm) were cut with a Leica ultramicrotome EM UC7 (Leica Microsystems) using an Oscillating ultrasonic Diamond Knife (DiATOME) at a cutting speed of 0.6mm/sec with frequency in automatic mode and a 6.0 V amplitude. Images were acquired using a Tecnai G2 Spirit BioTwin TEM (FEI Company) with an Orius CCD camera (Gatan Inc.)

Subcellular localization analysis, fractions of membrane bound and cytosolic mycobacteria were determined. At least 15 different infected cells per sample were imaged at ×3,900 magnification by systematic and random sampling. Cross points of the stereological test grid over bacteria were counted with regard to the subcellular localization of bacteria, which was determined from images taken at minimum magnification of ×8,900. Fractions were calculated from total counts per sample. The following criteria were followed for the assessment of subcellular localization: phagosomal bacteria were, at least partially, tightly lined by a phospholipid bilayer, representing the phagosomal membrane; cytosolic bacteria were surrounded by ribosomes, representing the cytoplasm with no indication of the phagosomal membrane; autophagic bacteria were enveloped by double or multiple membrane structures; and lysosomal bacteria resided in loose vesicles containing additional structures [1].

References:

