Disruption of membrane microdomains increases resistance to *Mycobacterium marinum*-induced membrane damage and infection

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Mycobacterium marinum is a close relative to *M. tuberculosis*, the etiological agent of the human tuberculosis. Both bacteria have a similar intracellular infectious cycle characterized by the crucial induction of damage to the membrane of the so-called Mycobacterium-Containing Vacuole (MCV) and escape to the cytosol. Strikingly, both bacteria secrete a small membranolytic peptide, EsxA. The MCV compartment shares similarities with endosomes and lysosomes in terms of membrane composition, lipids and proteins. Despite intense study, mechanistic information about these processes is still sparse. Therefore, it is important to strengthen our understanding of the interplay between the host and bacterial components involved. We make use of the model phagocyte *Dictyostelium discoideum* to decipher the role of host membrane microdomain organizers and components during *M. marinum* infection, and during membrane damage induced by the chemical LLOMe.

Microdomains are highly dynamic, liquid ordered membrane domains that are enriched in sterols and sphingolipids. In *D. discoideum*, vacuolins (Vac A, B and C) are microdomain organizers of the flotillin family. We demonstrate that vacuolins and sterols accumulate at the MCV throughout the infection and that vacuolin knockout and/or sterol depletion with MβCD (methyl-β-cyclodextrin) strongly affect the intracellular growth of *M. marinum*. Importantly, absence of sterols and/or vacuolins strongly decrease MCV membrane damage. Most interestingly, absence of vacuolin or MβCD treatment also render the endo-lysosomal compartment more resistant to the lysosomotropic membrane disrupter LLOMe. Our preliminary in vitro results indicate that EsxA partitions into membrane microdomains and we speculate that this is crucial to inflict that MCV-damage, cytosol escape and ensure a successful infection,

Overall, we show that membrane composition and properties might be key in regulating in the sensitivity of the MCV and more generally the endo-lysosomal compartments to damage induced by pathogen toxins and chemical insults.