SUMMARY

*Mycobacterium tuberculosis* (Mtb) is a facultative, intracellular pathogen which causes tuberculosis (TB). Among diseases with bacterial etiology TB is still the one causing most deaths globally. It is estimated that about one-third of the world’s population develop a clinically silent infection and that TB is responsible for about 1.5 million deaths per year.

Once inhaled, Mtb particles are readily phagocytosed, processed and presented by alveolar macrophages. Subsequently, dendritic cells (DCs) and monocyte-derived macrophages participate in the phagocytic process, playing an essential role in the initiation and maintenance of immune response against Mtb. Thus, during early stages of infection, the control of mycobacterial survival and proliferation is mainly depends on the innate immune response. Among these responses, production of pro-inflammatory cytokines plays a crucial role. Mycobacteria have evolved sophisticated mechanisms to manipulate natural biological processes of host cells to create an environment that is favourable to their survival and proliferation. One of the mechanisms used by mycobacteria to modulate the phagocyte response is their ability to manipulate the secretion of pro-inflammatory cytokines.

Subversion of eukaryotic host responses by bacterial pathogens often requires specialized secretion systems that deliver effector proteins near or directly into host cells. Recently, a novel secretion pathway has been identified in mycobacteria, which has been classified as type VII secretion system. The Mtb genome harbours five gene clusters coding for type VII secretion systems, designated ESX-1 - ESX-5, that export small, highly immunogenic proteins lacking a classical N-terminal signal sequence, belonging to the Esx or WXG-100 family. Among ESX systems the ESX-5 represents the most recently evolved one. The role of the ESX-5 system in the export of selected PPE and PE proteins in *M. marinum*, as well as the involvement of this system in modulation of
cytokine responses by *M. marinum*-infected human macrophages are well established. By the characterization of several Mtb knock-out mutants for ESX-5 components, it has been recently demonstrated that ESX-5 plays a crucial role in host pathogen interaction also in Mtb. Here we have investigated whether the components of Mtb ESX-5 secretion system have an impact on the ability of the bacteria to modulate innate immune response of human professional phagocytes. 

To this aim we focused on three ESX-5 mutants: MtbdeckDsko and MtbΔppe25-pe19, which inactivate the ESX-5 secretion system and show an attenuated phenotype in the SCID mouse model, and Mtbrv1794ko, whose orthologue in *M. marinum* was demonstrated to modulate cytokine secretion by human macrophages. First, we investigated whether the components of Mtb ESX-5 system have impact on human DC maturation and pattern of cytokine production from human macrophages and DCs. To this aim, monocyte derived dendritic cells (MoDCs) and two distinct macrophage subsets with pro-inflammatory (Mϕ1) and anti-inflammatory (Mϕ2) phenotype were stimulated with wild-type Mtb H37Rv (Mtbwt) or the ESX-5 mutant strains. We observed no difference among MoDCs infected with Mtbwt or the ESX-5 mutants both for the maturation markers analysed and the profile of cytokines secreted. Similarly, Mϕ1 and Mϕ2 infected with Mtbwt or the MtbdeckDsko and MtbΔppe25-pe19 strains produced comparable amounts of the cytokines analysed. On the contrary, macrophages infected with Mtbrv1794ko produced significantly higher amounts of IL-1β and IL-18 as compared to Mtbwt or the complemented strain Mtbrv1794ko-C infected cells. This difference was abolished when the macrophages were infected with heat-killed bacteria.

Both IL-1β and IL-18 are produced in inactive “pro-” form and their activation and secretion from macrophages is tightly controlled by a two-step mechanism that involves a family of cytosolic multiprotein complexes known as “inflammasomes”. Activation of inflammasome in
macrophages triggers caspase-1 activation, and, in turn, maturation of IL-1β and IL-18 to their active, secreted forms. Thus, to investigate the possible mechanism(s) responsible of the augmented IL-1β and IL-18 secretion by Mtb*rv*1794ko infected cells, in a second set of experiments, we have tried to dissect the molecular steps involved in inflammasome activation. Analyses of relative mRNA levels by quantitative RT-PCR demonstrated that both Mtbwt and Mtb*rv*1794ko equally activated pro-IL-1β and pro-IL-18 expression in macrophages. These data were confirmed by immunoblot analysis of pro-IL-1β protein levels in cell-lysates. In addition, a FluoChrome Inhibitor of Caspases assay, which was used to detect active caspase-1 inside the cells, indicated higher caspase-1 activation in macrophages infected with Mtb*rv*1794ko compared to Mtbwt infected cells. Altogether these results suggest that the marked IL-1β and IL-18 production by Mtb*rv*1794ko infected macrophages may be correlated with a higher rate of processing rather than a higher production of such cytokines.

Finally, we investigated whether two of the inflammasome triggering mechanisms reported to be involved in Mtb infection (i.e. the efflux of potassium and the release of lysosomal protease cathepsin B) might have a role in the observed augmented IL-1β production/secretion by Mtb*rv*1794ko infected macrophages. Our findings demonstrated that IL-1β and IL-18 levels were drastically reduced when KCl or the inhibitor of cathepsin B were added to the infection medium, suggesting that the release of cathepsin B from the lysosomal compartment or the decrease of intracellular [K+] may contribute to the inflammasome activation during mycobacterial infection.