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Paper Poster Session
Host-pathogen interactions

Role of transcriptional factor EB (TFEB) in the pathogenesis of Acinetobacter baumannii

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Background: Given the lack of treatment options against *Acinetobacter baumannii* infections, it is prioritary the development of novel and effective treatments. To this end, we propose to study an intracellular host cell factor as a potential target to develop inhibitors to block the entry and persistence of *A. baumannii* in the host. Endosome/lysosome and autophagosome/lysosome systems play an important role in the bacterial intracellular trafficking. These systems are regulated, among others, by TFEB. The role of TFEB in the entry of *A. baumannii* in the host is unknown. The aim of this study is to determine the involvement of TFEB in the entry and persistence of *A. baumannii* in the host.

Material/methods: Human lung epithelial cells (A549) were infected with ATCC17978 strain during 0.5, 2 and 6 h to analyze the TFEB expression by Western Blot (WB) and immunofluorescence assay. TFEB was then down- or overexpressed by transfecting A549 cells with small interference RNA (siRNA) or with the plasmid pEGFP-N1-TFEB, respectively. These deficient and overexpressing TFEB cells were subsequently infected with ATCC17978 strain to study bacterial adherence and invasion. Moreover, lysosome biogenesis and autophagy activation were studied using lysotracker and determining LC3BII expression, respectively. Additionally, A549 cells were pretreated with three autophagosome inhibitors (Bafilomycin, Pepstatin and Wortmannin) and infected with ATCC17978 strain to study bacterial adherence and invasion to host cells. Finally, the role of TFEB in cell death caused by ATCC17978 strain using a cell viability assay was analyzed.

Results: We demonstrated that infection with ATCC17978 strain increases progressively and significantly the expression of TFEB reaching an increment of 180% at 6 h compared to control non-infected cells. Bacterial invasion in A549 cells was reduced in TFEB deficient cells by 64%, and was increased in TFEB overexpressing cells by 150% compared to the control non-infected cells; while bacterial adhesion in both cells didn´t show any difference. The lysosome biogenesis and autophagy activation by increasing LC3BII expression was observed in infected cells. In addition, bacterial invasion in A549 cells was reduced when these cells were pretreated with autophagosome inhibitors. Interestingly, the treatment of A549 cells with TFEB siRNA and autophagosome inhibitors reduced more significantly the bacterial invasion into these cells in comparison with A549 cells without

treatment. Finally, the viability of A549 cells infected with *A. baumannii* decreases significantly in TFEB overexpressing cells compared to the control A549 cells.

Conclusions: The results of this study help to clarify the role of endosome/lysosome and autophagosome/lysosome systems TFEB-dependent in the pathogenesis of *A. baumannii*. Exploitation of this network "node" could potentially represent a novel therapeutic approach to treat *A. baumannii* infections by modulating autophagy/lysosome function, and may boost the design of a novel class of antimicrobial therapeutics targeting host factors.