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Paper Poster Session
Microbial pathogenesis and virulence

In vivo monitoring of the inflammatory response induced by metalloproteases and other virulence factors secreted by Pseudomonas aeruginosa by using a CFTR-knockout mouse model of cystic fibrosis

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Background: During infection in cystic fibrosis (CF) patients, *Pseudomonas aeruginosa* is capable of releasing several virulence factors in the lung such as pyoverdine, piocianine, different types of enzymes and a series of metalloproteases. These virulence factors have a direct damaging effect on the lung tissue and are responsible of most cases of morbidity and mortality in patients affected by cystic fibrosis. In this study, we used a mouse model transiently expressing the luciferase reporter gene under the control of an interleukina 8 (IL-8) bovine promoter, in which the inflammation, induced by *Pseudomonas aeruginosa* secreted virulence factors, is analyzed detecting and quantifying bioluminiscence (BLI) images of the damaged lung.

Material/methods: The plasmid used to transgenize WT and CFTR-knockout mice carry a gene reporter (luciferase) under the control of an IL-8 promoter of bovine origin. The transgenization was performed by delivering bIL-8-Luc plasmid to lung tissues of mice by intravenous injection. Mice were then injected intraperitoneally with luciferin and subsequently lung was challenge intratracheally with a bacterial inducer and bioluminescence recorded and quantified.
P. aeruginosa culture supernatants were prepared from two different clinical isolates, VR1 and VR2, grown with and without azithromycin, and introduced intratracheally in the transgenized mice. Both clinical strains were characterized as regards a number of virulence factors using standard protocols.

Results: Delivery of the culture supernatant containing metalloprotease and other virulence factors to transgenized WT caused an inflammatory response detectable by a strong bioluminescence signal reaching a maximum peak at 24 hours. The BLI signal was instead significantly reduced when mice were challenged with the culture supernatant from the same strain but grown in the presence of azithromycin, an antibiotic that inhibits protein synthesis and that is used in CF because its anti-inflammatory effect. In this case, the supernatant showed a high reduction of virulence products, similarly to what revealed in the supernatant from the non virulence strain VR2.

When CFTR-KO mice were challenged with the supernatant containing metalloproteases, the BIL signal was stronger and longer-lasting in KO mice in comparison to WT animals despite of the fact that the quantity of immune cells recruited and the cytokine synthesized were not significantly different in both types of animals.

Conclusions: Using this in vivo model, it has been confirmed the pro-inflammatory action of bacterial products released by the most important pathogen in CF and it have been shown that the anti-inflammatory effect of azithromycin is mediated by its capability of inhibiting the synthesis of *Pseudomonas* virulence factors. The use of this animal model will make possible to perform a specific evaluation in CF mice of known and new molecules with a presumptive anti-inflammatory action and to analyze the pro-inflammatory action of bacterial products.