In vivo monitoring of the lung inflammatory response induced by *Pseudomonas aeruginosa*-secreted virulence factors in a mouse model of cystic fibrosis

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BACKGROUND

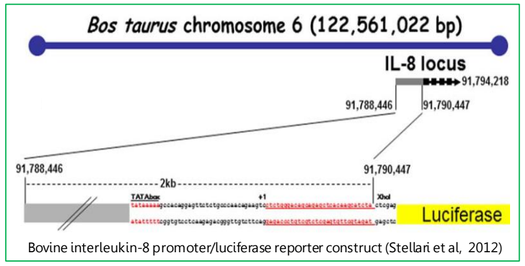
Airway inflammation in cystic fibrosis is frequently associated with bacterial infections, such as those caused by *Pseudomonas aeruginosa*, and contributes significantly to morbidity and mortality in patients. During the early onset of the lung infection *P. aeruginosa* secretes a high number of virulence factors which contribute to tissue damage and inflammation. It has been suggested that azithromycin, used in cystic fibrosis for its anti-inflammatory benefits, could elicit its activity by decreasing the synthesis of *P. aeruginosa* exoproducts.

PURPOSE

We monitored by in vivo imaging the lung inflammation induced by *P. aeruginosa* secreted virulence factors and their modulation by azithromycin (AZM).

METHODS

Culture supernatants were collected from two *P. aeruginosa* clinical strains grown in absence or presence of a sub-MIC concentration (8 µg/ml) of AZM.
Balb/c and C57BL6/J (WT and CFTR-KO) mice were transgenized with a bovine interleukin-8 promoter/luciferase reporter construct, delivered by in vivo JetPEI (Polyplus Transfection) carrier.

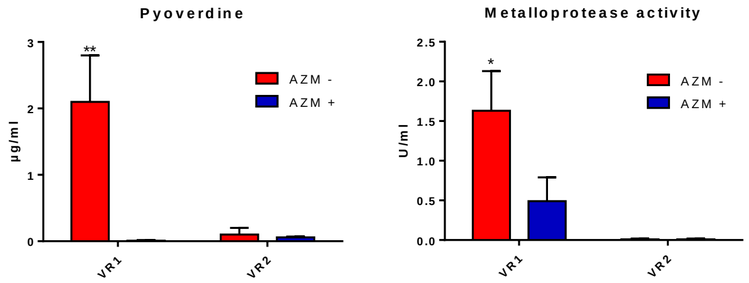


Bovine interleukin-8 promoter/luciferase reporter construct (Stellari et al, 2012)

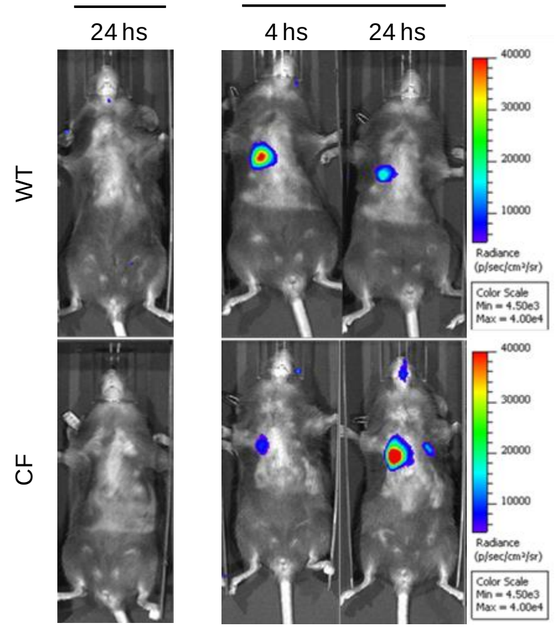
Lung inflammation in mice was induced by non-invasive intratracheal instillation of culture supernatants. After 4 and 24 hrs mice were intraperitoneally injected with luciferin (150 mg/kg) and the bioluminescence emitted from the chest was recorded by IVIS Lumina (Caliper Life Sciences).

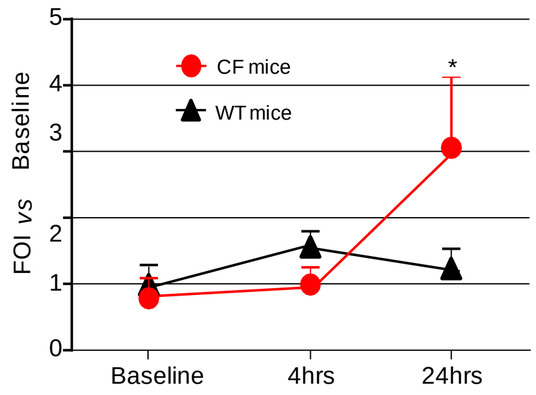
RESULTS

1) VR1 and VR2 strains differ in the release of some virulence factors. In particular, VR1 shows high metalloprotease activity and pyoverdine release, both inhibited after growth in presence of AZM.



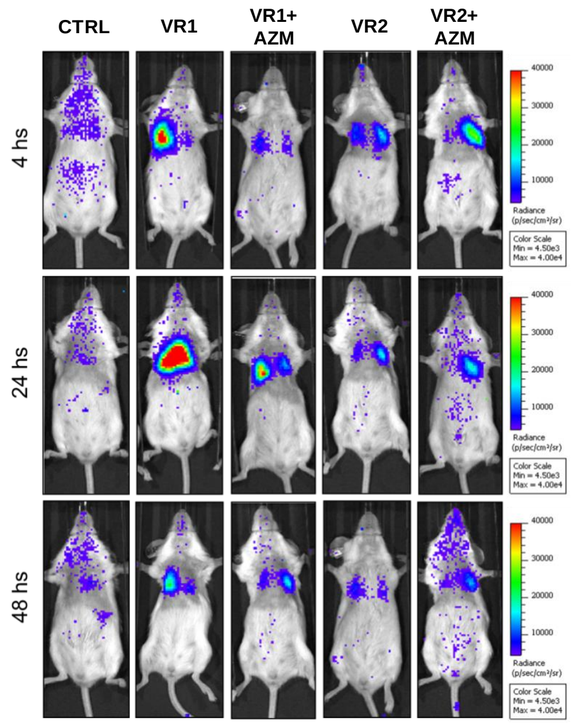
3) After challenge with VR1 culture supernatant bioluminescence (BLI) increased in both WT and CF mice in comparison with untreated controls. At 24 hours the BLI signal was significantly higher in CF than in WT animals.

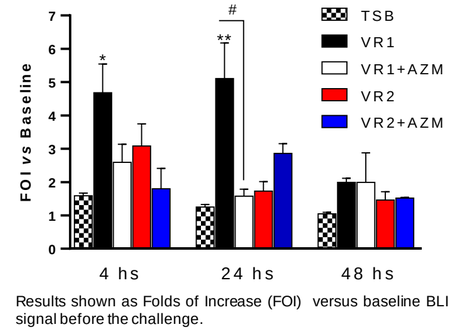




Results shown as Folds of Increase (FOI) versus baseline BLI signal before the challenge.

2) In WT Balb/c mice, VR1 culture supernatant induced a strong bioluminescence emission, significantly higher than those induced by VR1+AZM and VR2.





Results shown as Folds of Increase (FOI) versus baseline BLI signal before the challenge.

CONCLUSIONS

Using this new mouse model of in vivo imaging, it is possible to evaluate the pro-inflammatory action of bacterial products in normal and CF lung, and to study the anti-inflammatory effects of therapeutic molecules.

AKNOWLEDGEMENTS

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ffc — Italian cystic fibrosis research foundation - Onlus