9th ADVANCES AGAINST ASPERGILLOSIS AND MUCORMYCOSIS
Lugano, Switzerland
27 – 29 February 2020
Palazzo dei Congressi Lugano
www.AAAM2020.org

Aspergillus fumigatus – Stenotrophomonas maltophilia co-inoculation model on bronchial epithelial cells: antibiosis effect and inflammatory response according to bacterial strains.

N° 83

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Background

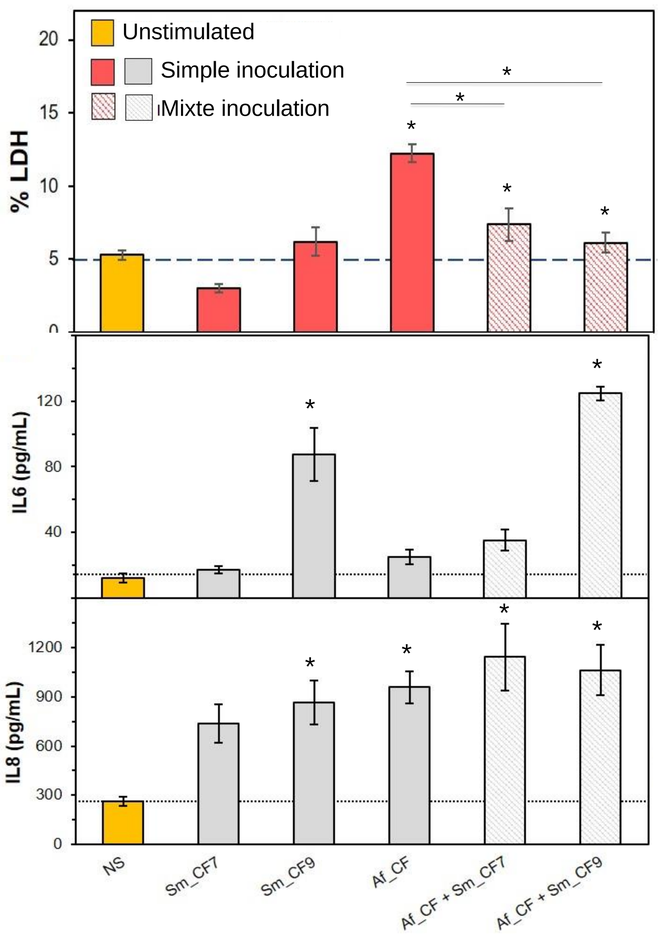
- ***Aspergillus fumigatus*** (*Af*) and ***Stenotrophomonas maltophilia*** (*Sm*) are both opportunistic pathogens which are frequently described and positively associated in the pulmonary microbiota of cystic fibrosis (CF) patients[1].
- We have previously developed an ***in vitro*** **model of mixed *Af-Sm* biofilm** on polystyrene support with clinical reference strains [2,3].
- The aim of the present work was to develop an ***Af-Sm*** **inoculation model on bronchial epithelium cells** (BEAS 2B) **with CF clinical strains**, in order to study the **dynamic of colonization** between both pathogens, as well as the **cell inflammatory responses** to this mixed inoculation.

Material - methods

- Two *Sm* CF strains, **Sm_CF7** and **Sm_CF9** (10^6 bacteria/mL) were used in association with a clinical *Af* strain (10^5 conidia/mL) (**Af_CF**).
- ***Sm* CF** strains presented different fitness, **Sm_CF7 grew slowly** than **Sm_CF9,** with **less plastic adhesion** and **less biofilm formation.**
- We performed:
  - **simultaneous inoculations** with one (***Af*** or ***Sm***) or both pathogens (***Af*/*Sm***) to test **antibiosis effect** on ***Sm*** on ***Af***, 24h
  - **sequential inoculations** to test the **adhesion** (Primo-inoculation (**PI**) at 4 h or 15 h + second inoculation (**SI**) at 4 h) and the **pathogens growth** (**PI** 6 h + **SI** 24 h) after a PI of BEAS 2B cells. 4h 4h / 15h 4h ; 4h 4h / 15h 4h ; 6h 24h / 6h 24h

qPCR, **scanning electron microscopy** (SEM), **cellular survival** (colorimetric LDH assay) and **cell inflammatory response** (IL-6 and IL-8 by ELISA) were used to describe the process.

Results

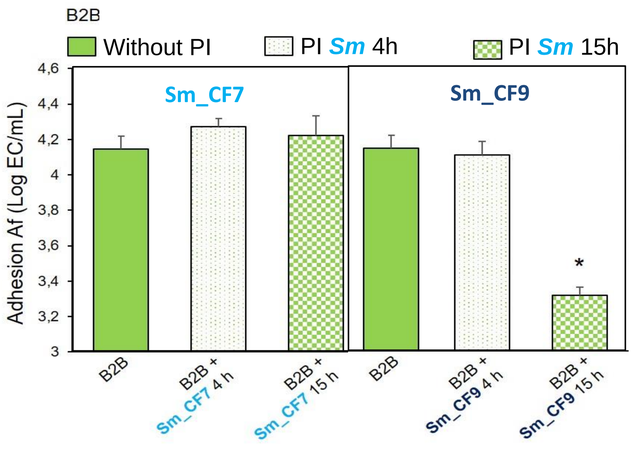


Cellular survival :

- **Simple inoculation *: Af_CF*** leads to more cell lysis than ***Sm*.**
- **Co-inoculation** :The cell lysis decreases in both co-inoculation ***Af-Sm*.**

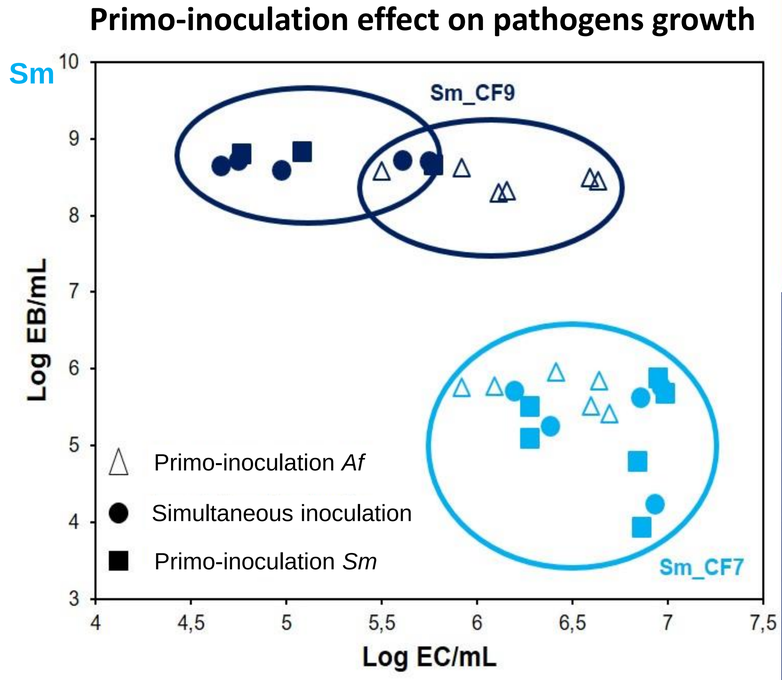
Inflammatory response:

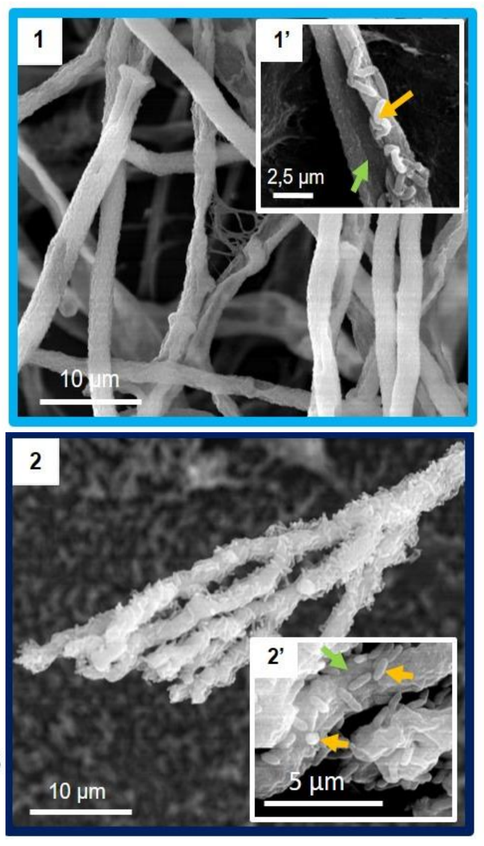
- **IL-6/IL-8** secretion was significantly different according to bacterial strain.
- **No synergism** was observed in co-inoculation ***Af-Sm*** condition.



Adhesion:

- **Af_CF** PI did not change the ***Sm*** adhesion on BEAS 2B.
- Decrease of ***Af*** conidia adhesion on BEAS 2B → ***Sm*** **concentration-dependent.** Indeed, [**Sm_CF7**] at 4h or 15h **did not change** while [**Sm_CF9**] is multiplied by **10** between 4h and 15h.





Antibiosis effect :

- Observed only with **Sm_CF9** when **simultaneous inoculation of *Af-Sm*** and ***Sm*** **PI** (***Af*** inhibition, highly branched hyphae on SEM, Figure 2).

Pathogen growth :

- Whatever the microorganism in PI, no change in terms of concentration for the co-inoculation **Sm_CF7/Af_CF**, ***Af*** and ***Sm*** form one population on the graph (light blue circle).
- Co-inoculation **Sm_CF9/Af_CF** form two populations on the graph (dark blue circle), one with [***Af***] lower concentration (4,5-5,5 log) which includes simultaneous inoculation and PI ***Sm*** condition, and another with [***Af***] higher concentration (6-6,5 log) which only includes PI ***Af*** **(no inhibition** by **Sm_CF9).**
- No antibiosis effect of **Sm_CF9** on young ***Af*** hyphae (15h), but only on **conidia stage.**

Conclusions

- ***Af-Sm*** co-inoculation **decreases** the **cell lysis** compare to simple pathogens inoculation
- **Inflammatory profiles** are **different** according to Sm_CF strains
- We observed two effect:
  - **Decrease** in **conidia adhesion** on BEAS 2B: ***Sm*** concentration-dependent
  - **Inhibition** of **Af_CF** growth by **Sm_CF9**: action on **early Af_CF stage**?

[1] Granchelli *et al.,* J Clin Microbiol, 2018, [2] Melloul *et al.,* PlosOne, 2016; [3] Melloul *et al.,* PlosOne, 2018

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