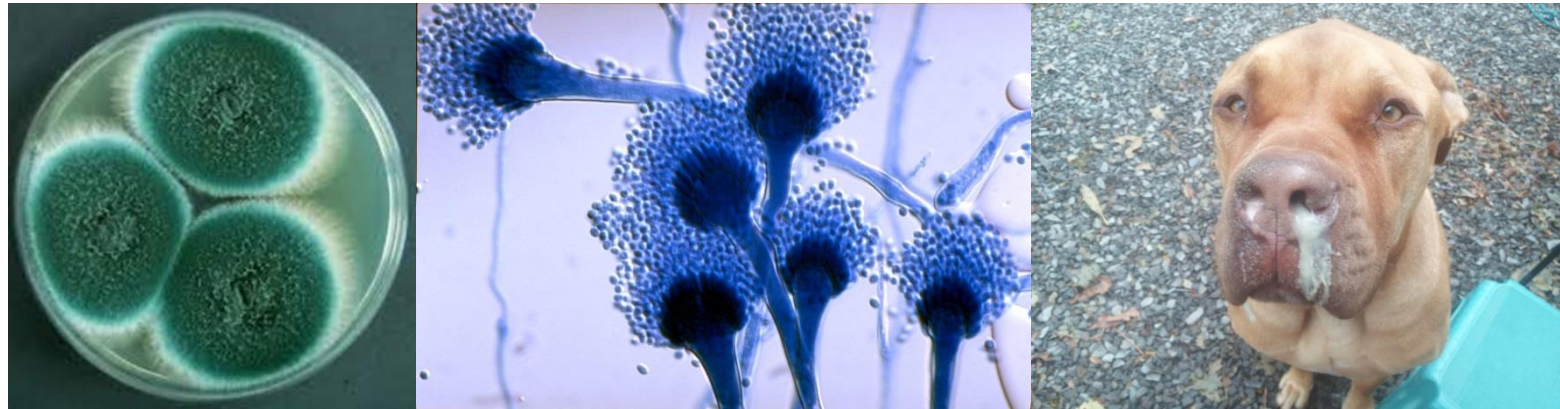




Expression profile analysis reveals that *Aspergillus fumigatus* but not *Aspergillus niger* makes type II epithelial lung cells less immunological alert

*Natalia Escobar, Ivan D. Valdes, **Esther M. Keizer**, Soledad R. Ordonez, Robin A. Ohm, Han A. B. Wösten, Hans de Cock*





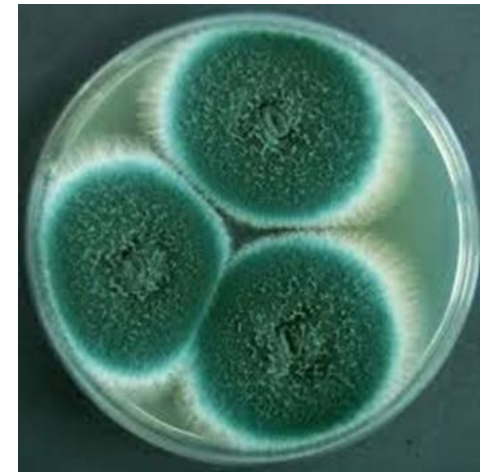
Aspergillus infections

- Very abundant fungi in the world
- Hundreds of different species
- **Majority of infections due to *A. fumigatus***

Why is *Aspergillus fumigatus* so successful as human pathogen?

Is life style in a host different?

Compare life style of
A. fumigatus with *A. niger*

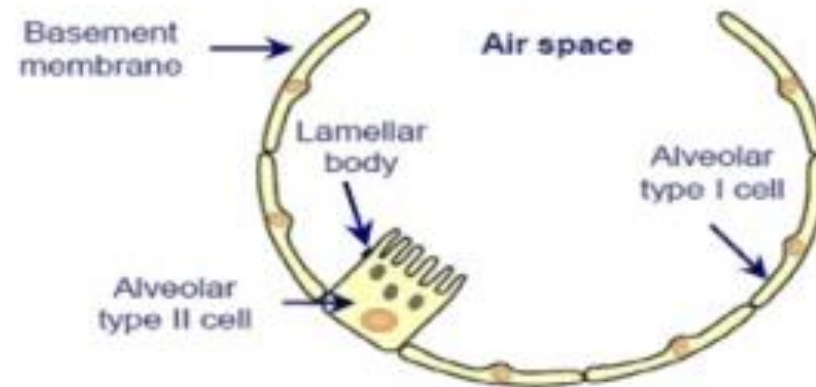




*Why is *Aspergillus fumigatus* so successful as human pathogen?*

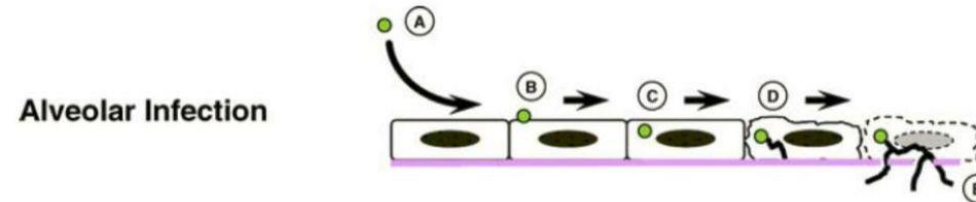
- Invasive and noninvasive infections
- Small conidia can enter more deeply the lung
- First interactions with epithelial lung cells
- Type I (gas exchange)
- Type II cells (surfactant/immunity)

Type II A549 cells as a model system



LIFE STYLE IN A HOST

Adhesion → internalization → germination



transitions from **dormant conidia** into **swollen conidia** to formation of **hyphae**

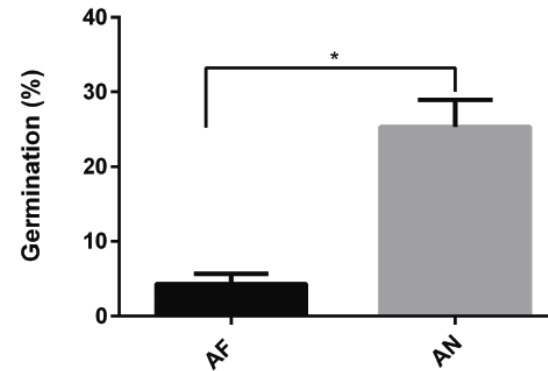
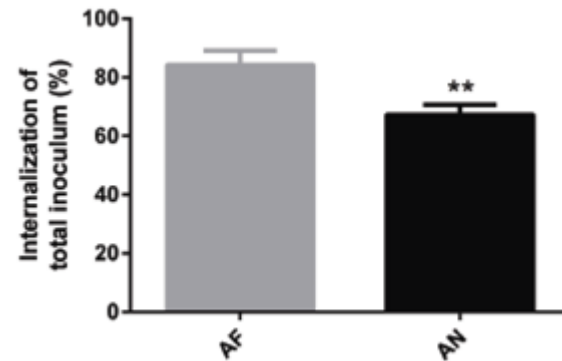
Compare *A. fumigatus* with *A. niger*

Experimental set up

- Red fluorescent conidia with A549 cells for 2 h
- Wash away unbound conidia: **follow only associated conidia !**
- Incubate additional 10 h
- Confocal /CFW staining
- Different morphotypes visible

Comparison *A. fumigatus* and *A. niger*

A. fumigatus is internalized more efficiently and shows delayed germination in A549 cells when compared to *A. niger*



***A. fumigatus* has a different life style → prolonged dormancy**

Escobar N, Ordonez SR, Wösten HAB, Haas P-JA, de Cock H and Haagsman HP (2016) Hide, Keep Quiet, and Keep Low: Properties That Make *Aspergillus fumigatus* a Successful Lung Pathogen. *Front. Microbiol.* 7:438



Comparison *A. fumigatus* and *A. niger*

Transcriptome analysis RNAseq

Differential gene expression analysis of fungi and A549 cells

Escobar N, Ordonez SR, Wösten HAB, Haas P-JA, de Cock H and Haagsman HP (2016) Hide, Keep Quiet, and Keep Low: Properties That Make *Aspergillus fumigatus* a Successful Lung Pathogen. *Front. Microbiol.* 7:438



Transcriptome analysis

Changes in fungal gene expression


A. fumigatus (in A549 cells)

- 545 fungal genes up/down

A. niger (in A549 cells)

- 473 fungal genes up/down

Only 53 genes and 4 GO terms are shared between the two fungal species

- Response to hypoxia (up)
- sod3, cat2, and catA (up)  Protection oxidative stress (not up)
- More conidia-associated genes
- More germination-associated genes

	CAGs	GeAGs
<i>A. fumigatus</i>	74	47
<i>A. niger</i>	46	85



Transcriptome analysis

Changes in fungal gene expression

A. fumigatus (in A549 cells)

- 545 fungal genes up/down

A. niger (in A549 cells)

- 473 fungal genes up/down

Only 53 genes and 4 GO terms are shared between the two fungal species

- Re
- SO
- M

Gene expression analysis shows also a marked difference in life style between *A. fumigatus* and *A. niger*

up)

	CAGs	GeAGs
<i>A. fumigatus</i>	74	47
<i>A. niger</i>	46	85



Transcriptome analysis

Changes in A549 gene expression

A549 cells with *A. fumigatus*

- 62 and 47 up- and down-regulated
- GO terms related to immune response **down regulated**
- *CCR and CCR2 chemokine receptor binding*
- *macrophage chemotaxis*
- *cytokine and chemokine mediated signaling pathway*
- *immune system development*
- *down regulation of TF of beta-catenin TCF7L2 (TCF4)*

A549 cells with *A. niger*

- 17 up- and 34 down-regulated
- GO terms were not enriched
- Genes related to immune response **upregulated**
- *TNFAIP6 (tumor necrosis factor alpha induced protein 6 or TSG-6)*



Transcriptome analysis

Changes in A549 gene expression

A549 cells with *A. fumigatus*

A549 cells with *A. niger*

A. fumigatus down regulates immune response in A549 cells

Remarkable difference with other studies which show upregulation eg IL-8

signaling pathway

- *immune system development*
- *down regulation of TF of beta-catenin*
TCF7L2 (TCF4)



Transcriptome analysis

Influence of MOI

This study:

- MOI = 0.1
- 12 hour co-cultivation
 - Removal of unbound conidia by washed after 2

No upregulation
immune response

Previous studies:

- MOI = 1
- 6 hour co-cultivation
 - No washing to remove unbound conidia
- MOI = 10
- 8 hour co-cultivation
 - No washing to remove unbound conidia

Upregulation immune response

Oosthuizen PlosOne 2011, 6(5):e20527

Chen PlosOne 2015, 10(8):e0135720



IL-8 induction at different MOI's

qPCR after 12 hour of co-cultivation with A549 cells
with wash step to remove unbound conidia

	MOI	$\Delta\Delta Ct$	IL-8
<i>A. fumigatus</i>	0.1	1.291	
	1	4.728	UP
<i>A. niger</i>	0.1	1.204	
	1	3.325	UP

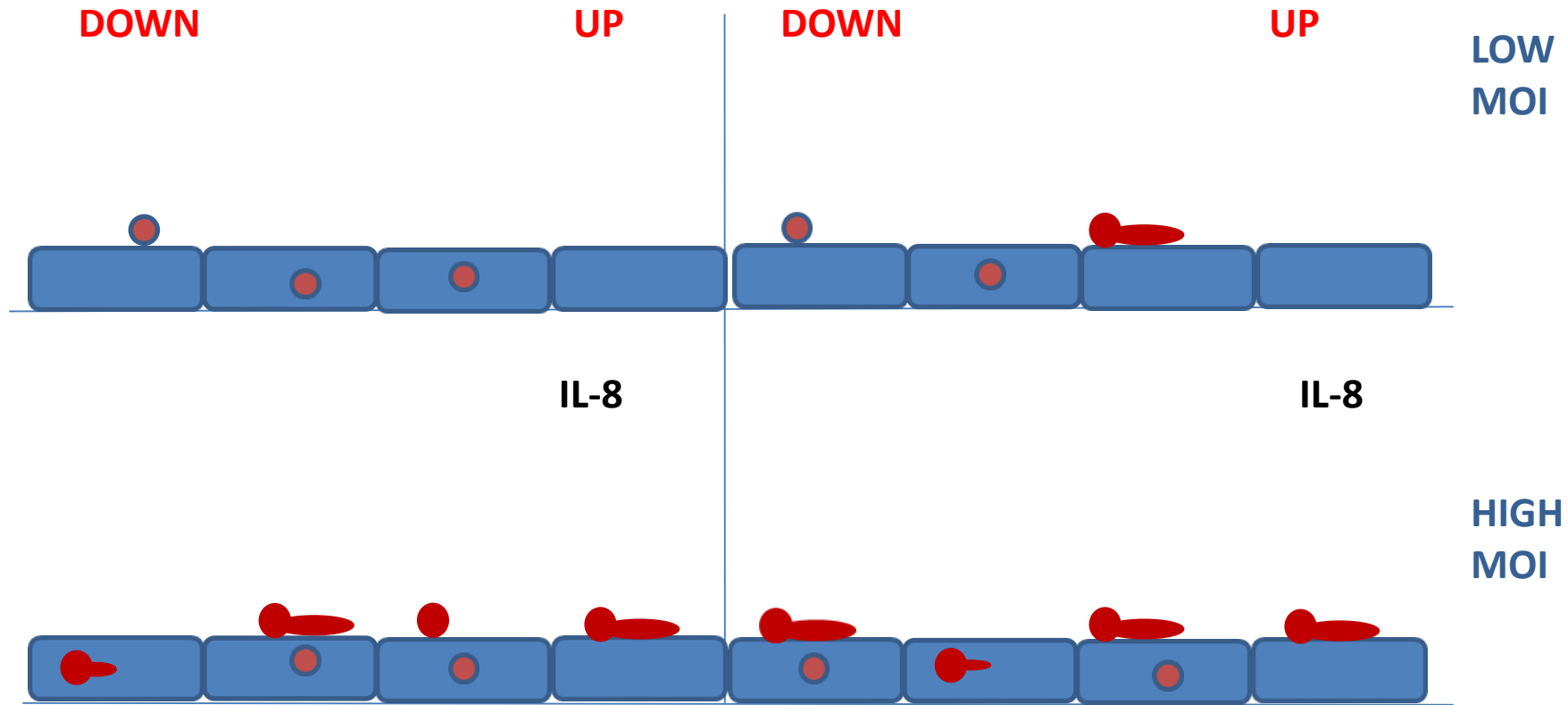


Regulation of immune response

12 h co-incubation of A549 cells with Aspergilli

A. fumigatus

A. niger



Is upregulation of immune response at high MOI regulated via β -glucan receptors?



Conclusions

- Transcriptome analysis confirmed differences in life style between *A. fumigatus* and *A. niger* in A549 lung cells
- *A. fumigatus* has prolonged dormancy and hides in lung cells → higher fungal load in lungs
- *A. fumigatus* (at low MOI) reprograms lung cells to become less immunologically alert
- MOI dependent upregulation of immune response



Acknowledgements



Microbiology & Institute of Biomembranes, Department of Biology, Utrecht University

Department of Infectious Diseases and Immunology, Division Molecular Host Defence,
Utrecht University