

Receptor expression and epithelial damage response to pathogenic Mucorales species characterized in an alveolar bilayer model

Wurster S, Belic S, Lazariotou M, Waaga-Gasser AM, Dragan M, Springer J, Michel D, Löffler J, Einsele H, Ullmann AJ

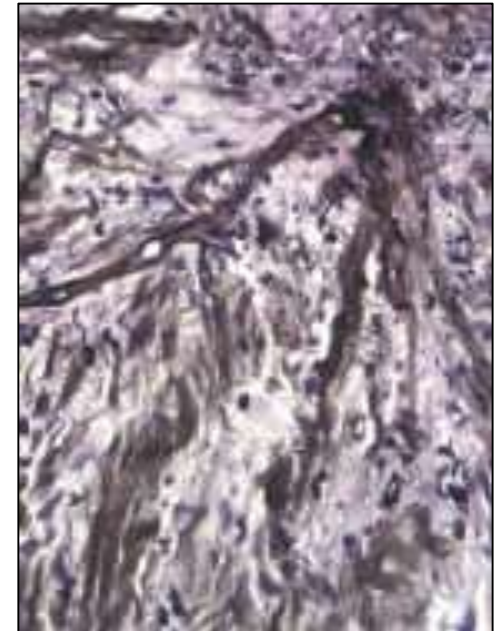


Disclosure of conflicts of interest

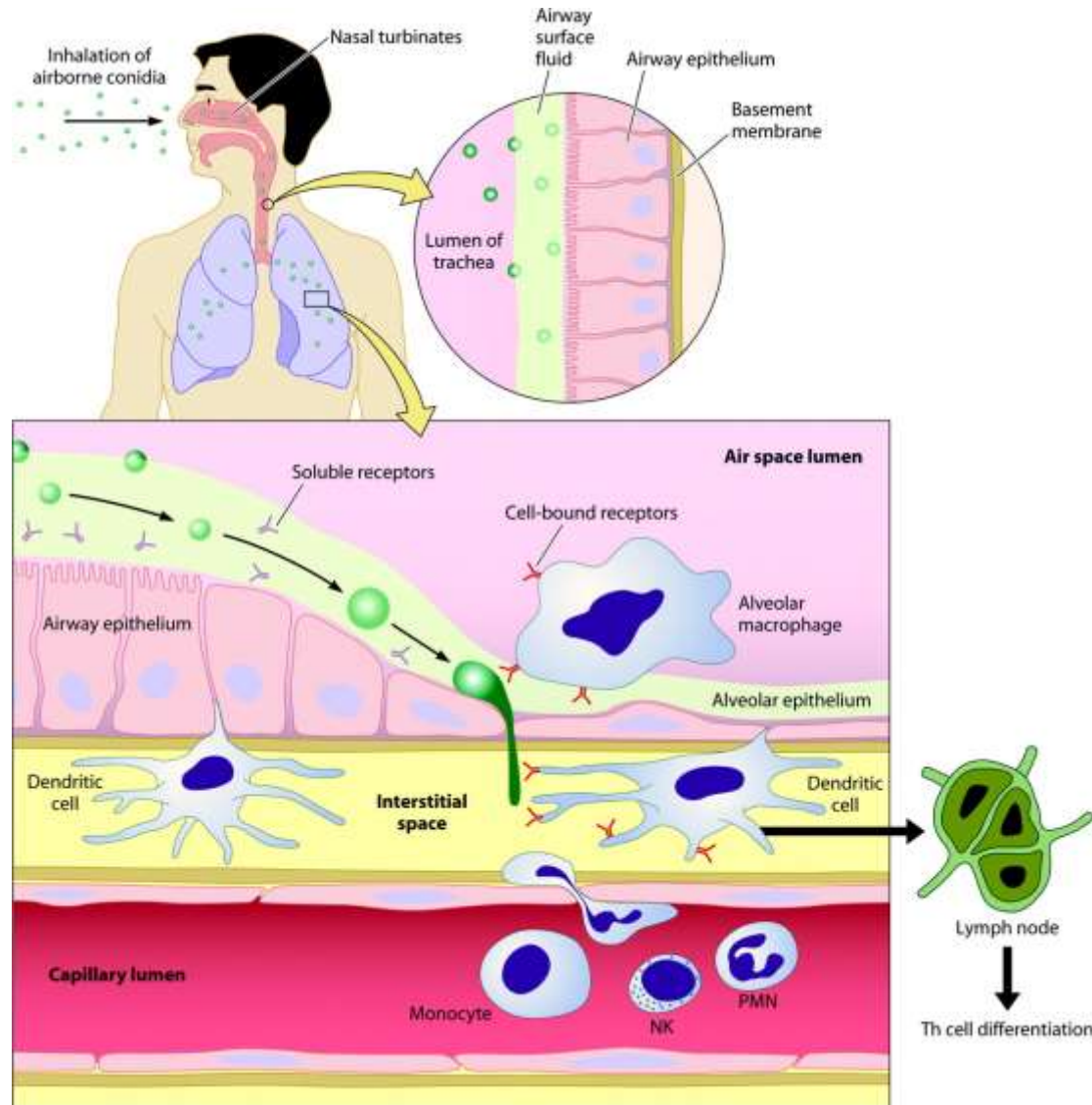
- ▶ SW: none
- ▶ SB: none
- ▶ ML: none
- ▶ AMWG: none
- ▶ MD: none
- ▶ JS: none
- ▶ DM: none
- ▶ JL: received a travel grant from Astellas
- ▶ HE: none related to this project
- ▶ AJU: none related to this project

Mucorales

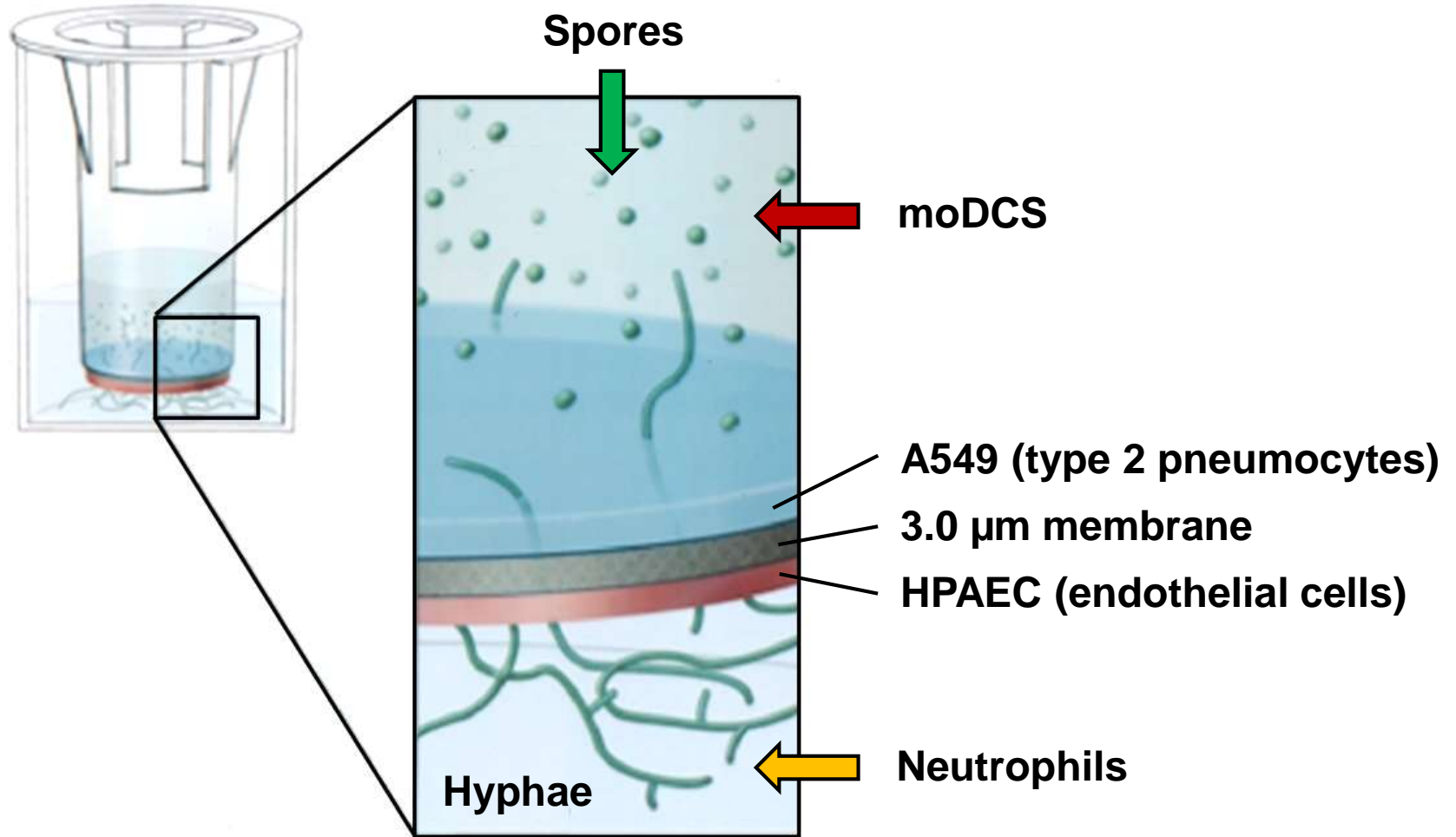
- ▶ Ubiquitous soil organism
- ▶ Aerosolic dispersion of spores
- ▶ Serious threat for immunocompromised patients
- ▶ Increasing incidence of mucormycoses



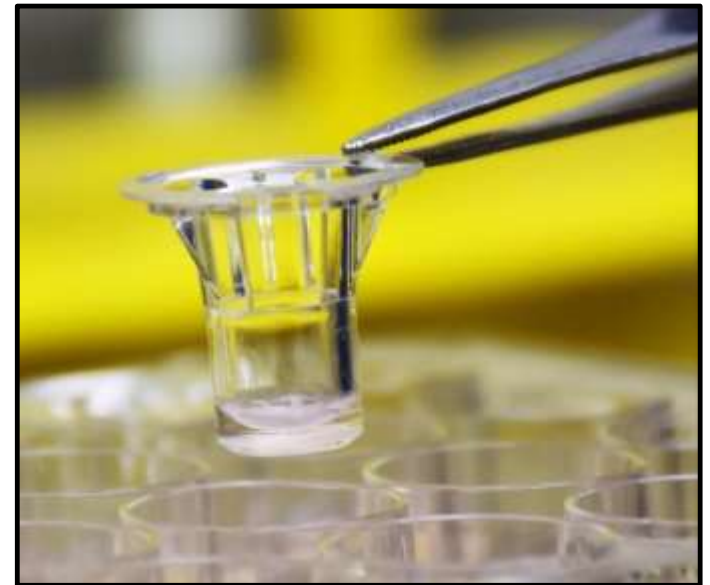
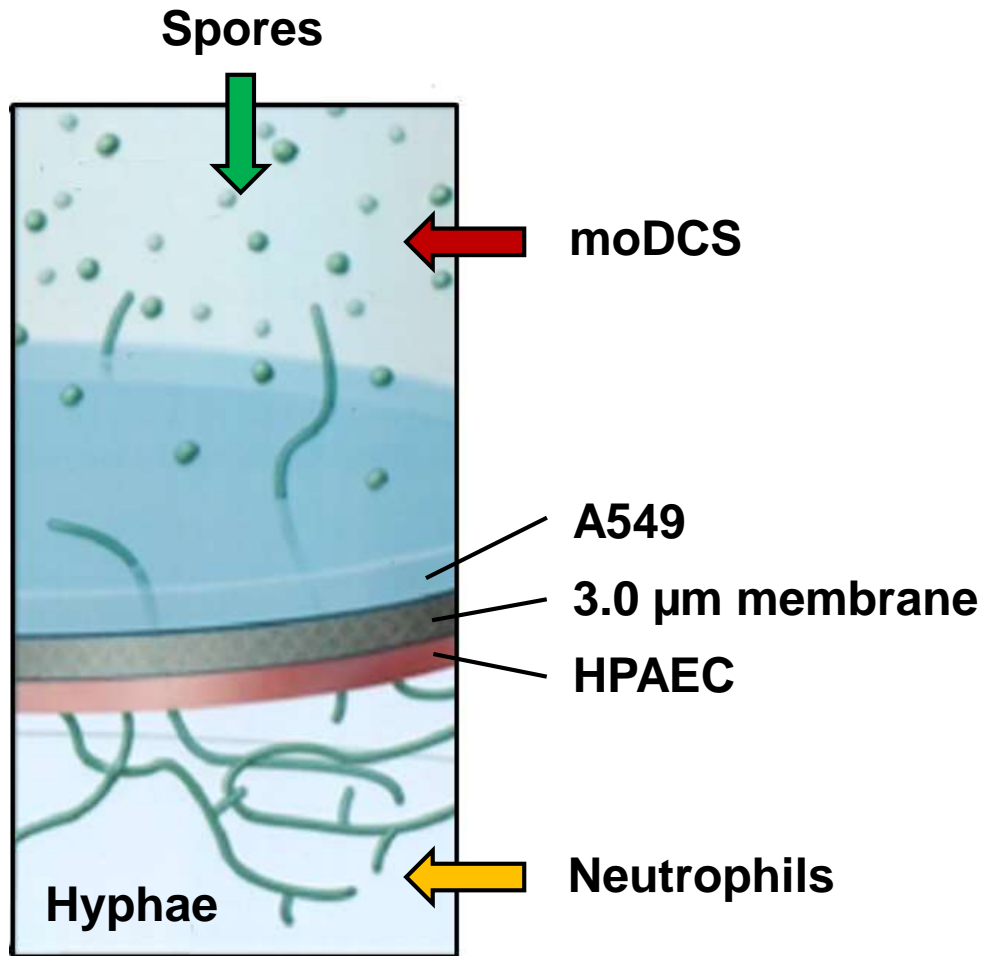
Immune defense against pulmonary mould infections



Transwell-bilayer model of pulmonary mould infections



Transwell-bilayer model of pulmonary mould infections

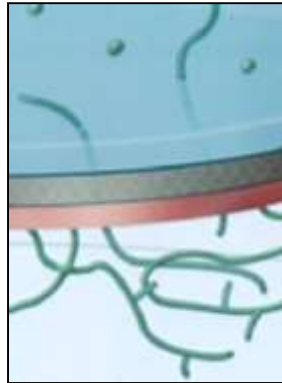


Adaptation of Alveolar Model for Mucorales Research

Study design

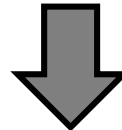
Pathogens

- Medium / uninfected (Med)
- *R. arrhizus* (Rar)
- *R. pusillus* (Rmp)
- *C. bertholletiae* (Cbe)



Immune cells

- none
- moDCs
- neutrophils
- moDCs and neutrophils



Mucorales DNA

18S-Assay

Cytokine Profiles

HCYTOMAG-Assay,
qPCR

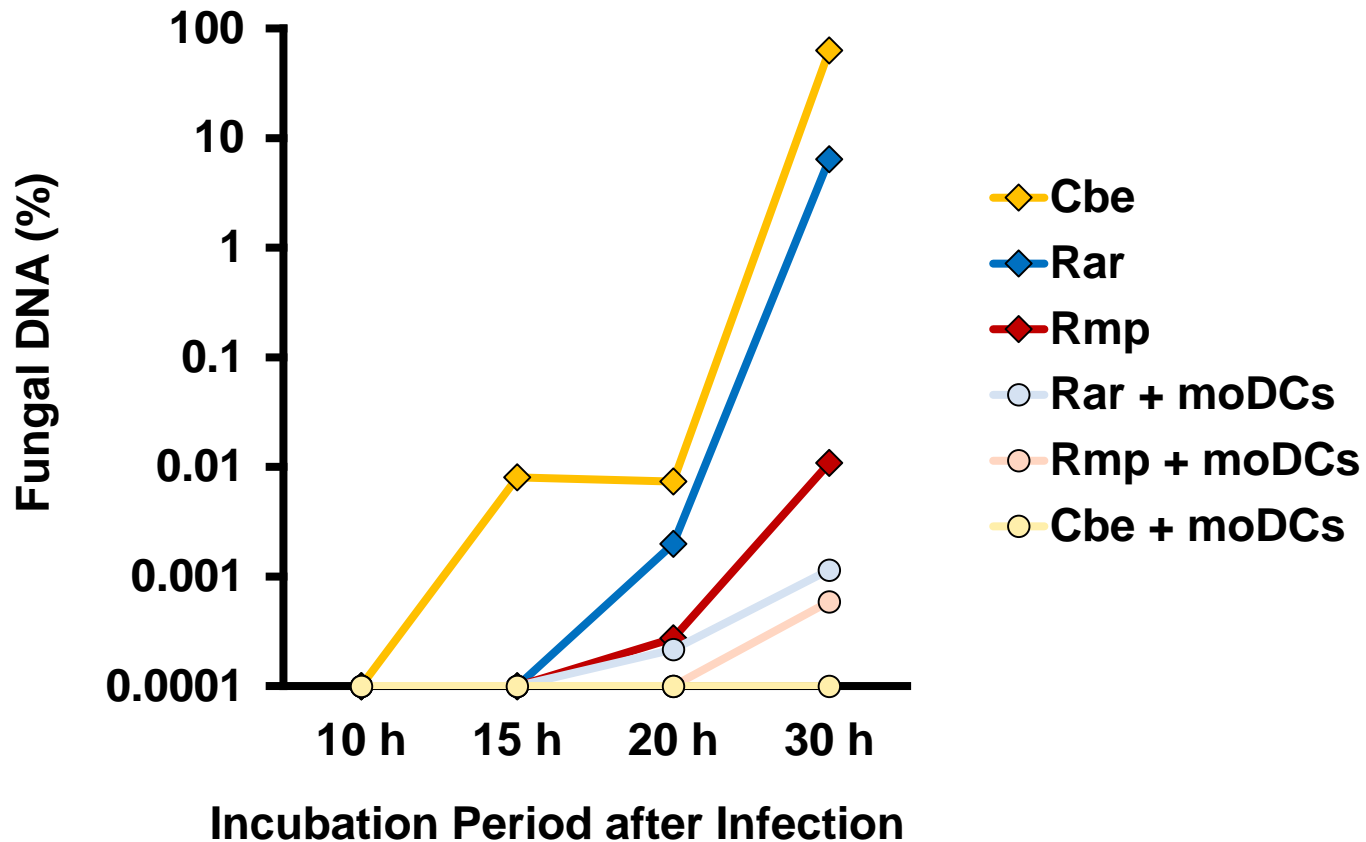
Epithelial Damage

LDH, apoptosis markers,
epithelial cytokines

Receptor Expression

qPCR: TLR2, TLR4,
CLEC6A, CLEC7A, PTX3

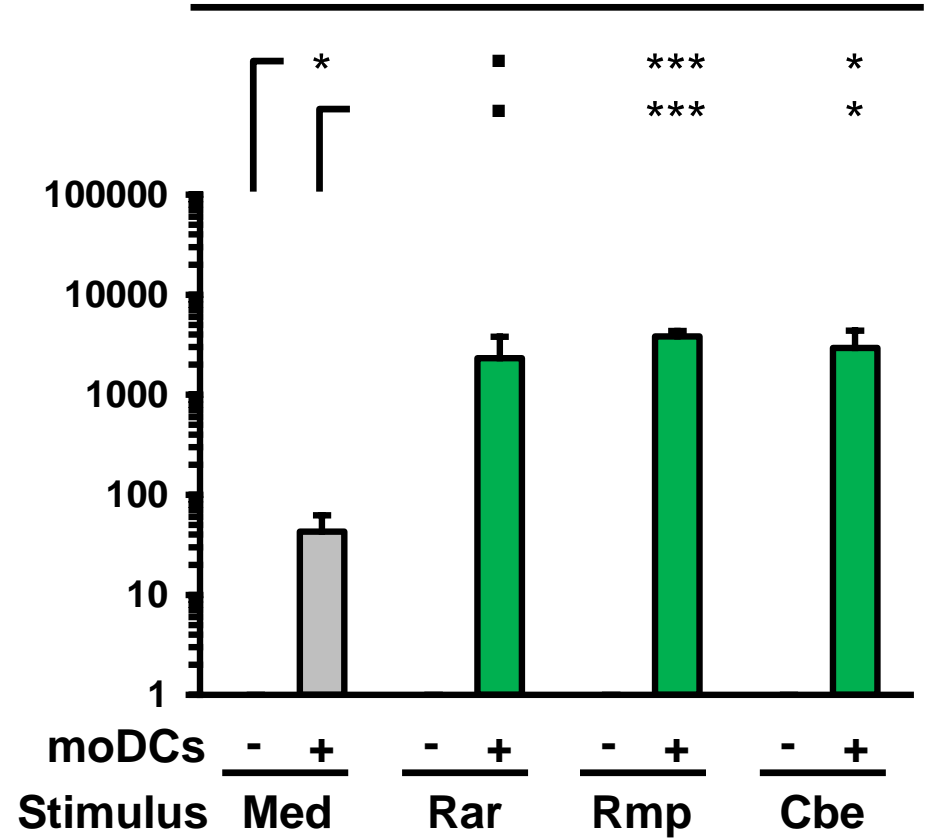
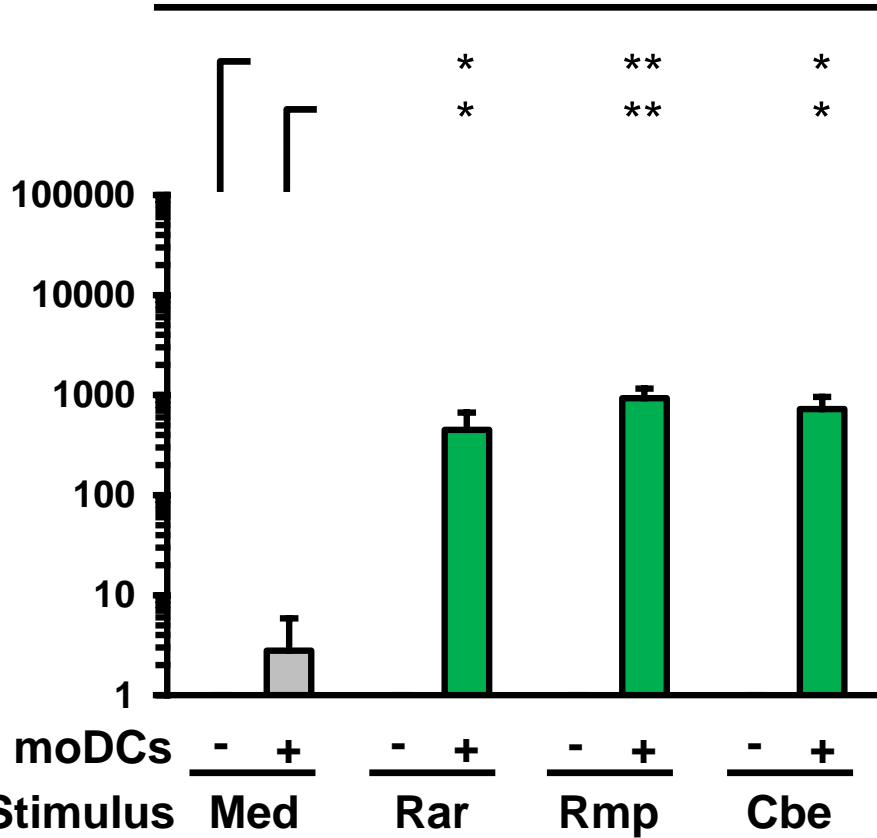
18S Mucorales DNA assay for confirmation of fungal invasion of the lower compartment



Pro-inflammatory response to Mucorales (cytokine concentrations in upper comp. after 30 h)

IL1 β (pg/ml)

TNF α (pg/ml)



Fold Change

< 0.05

< 0.1

< 0.2

< 0.5

0.5 - 2

> 2

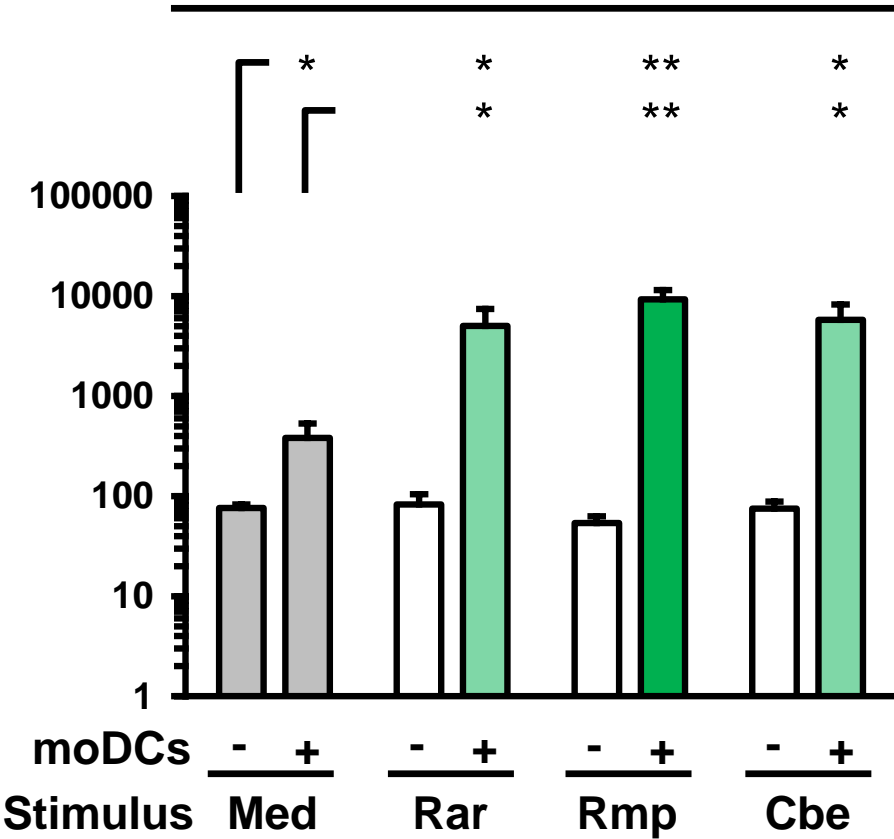
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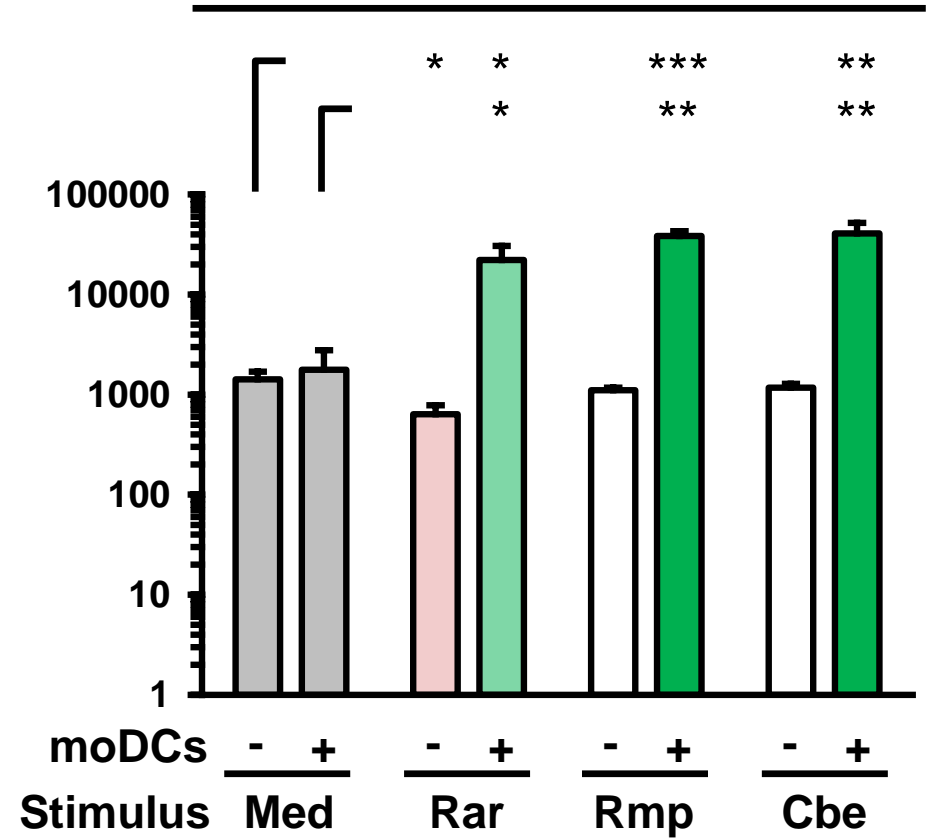
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Pro-inflammatory response to Mucorales (cytokine concentrations in upper comp. after 30 h)

IL6 (pg/ml)



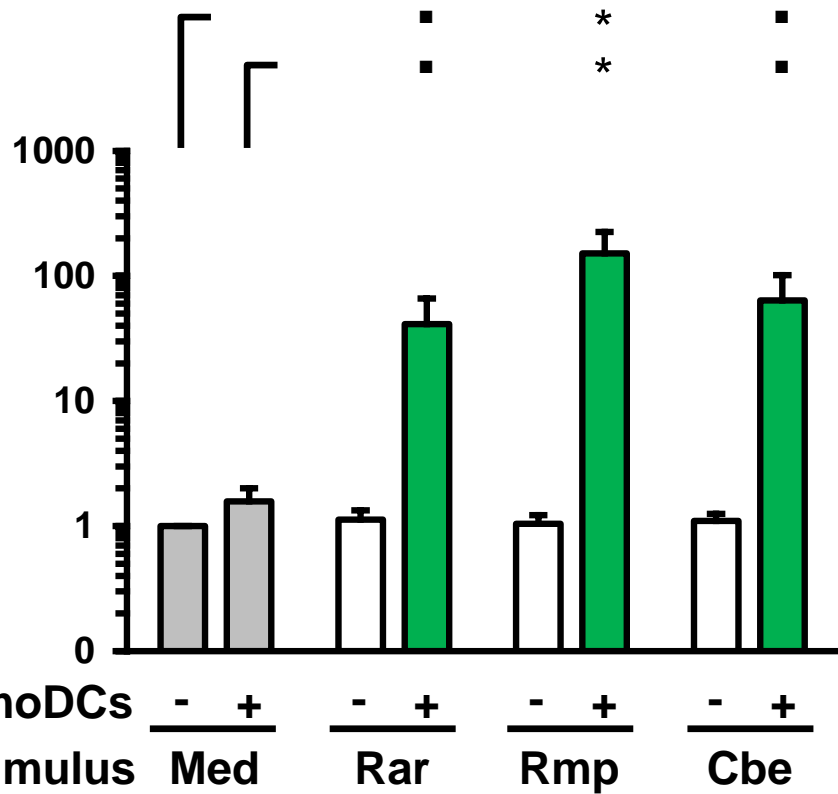
IL8 (pg/ml)



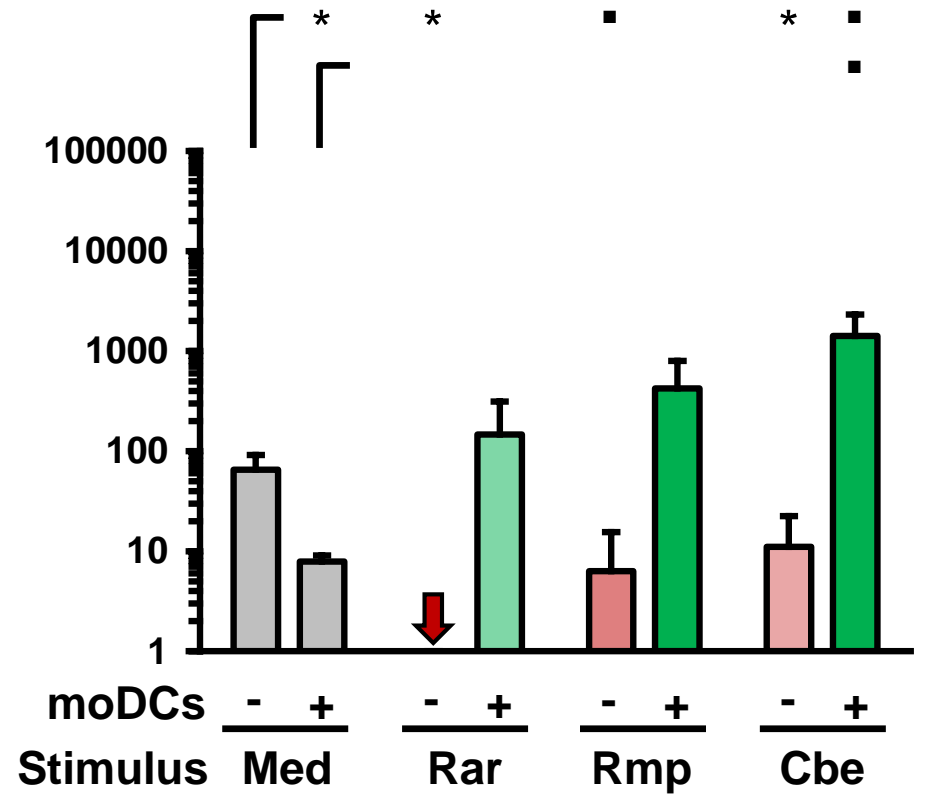
Epithelial Damage Response

Comparison of CCL5 transcription and secretion

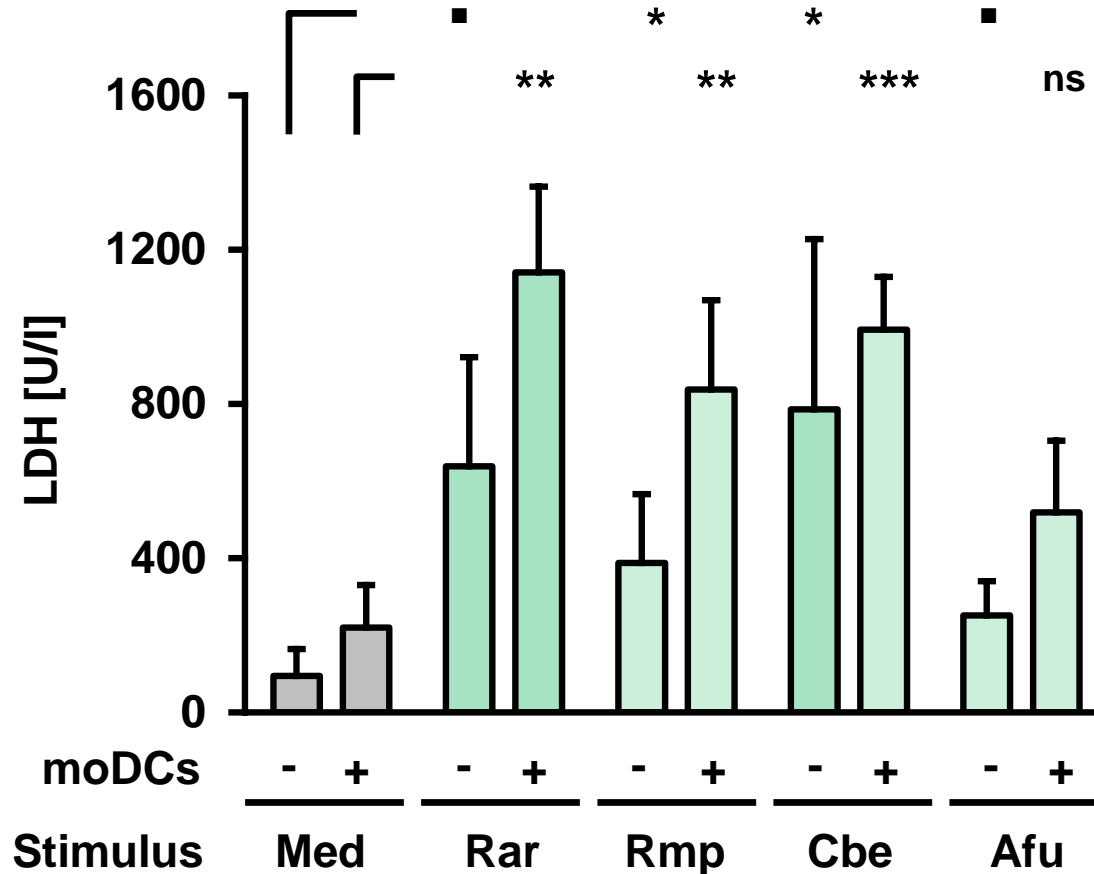
CCL5 mRNA expression



CCL5/RANTES (pg/ml)



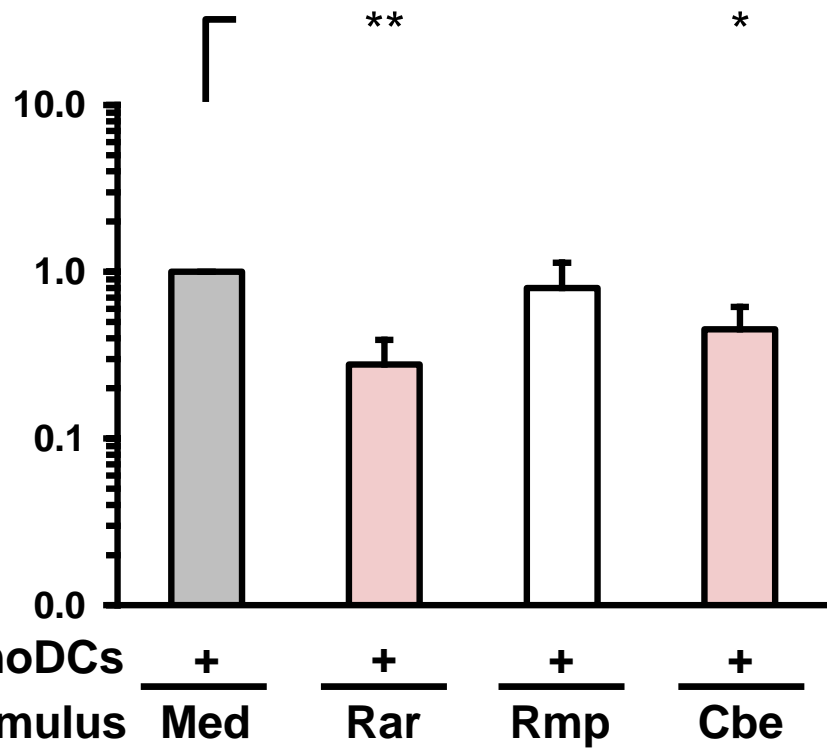
LDH concentrations in culture medium (upper compartment, 30 h)



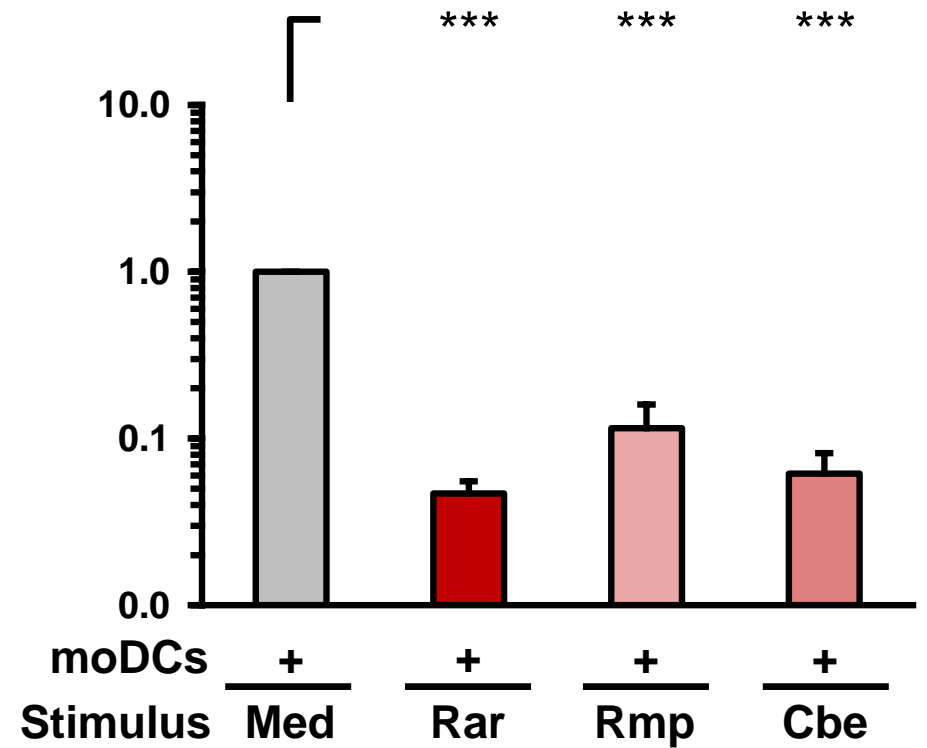
Pattern Recognition Receptor Expression

TLR expression in alveolar model

TLR2 mRNA expression

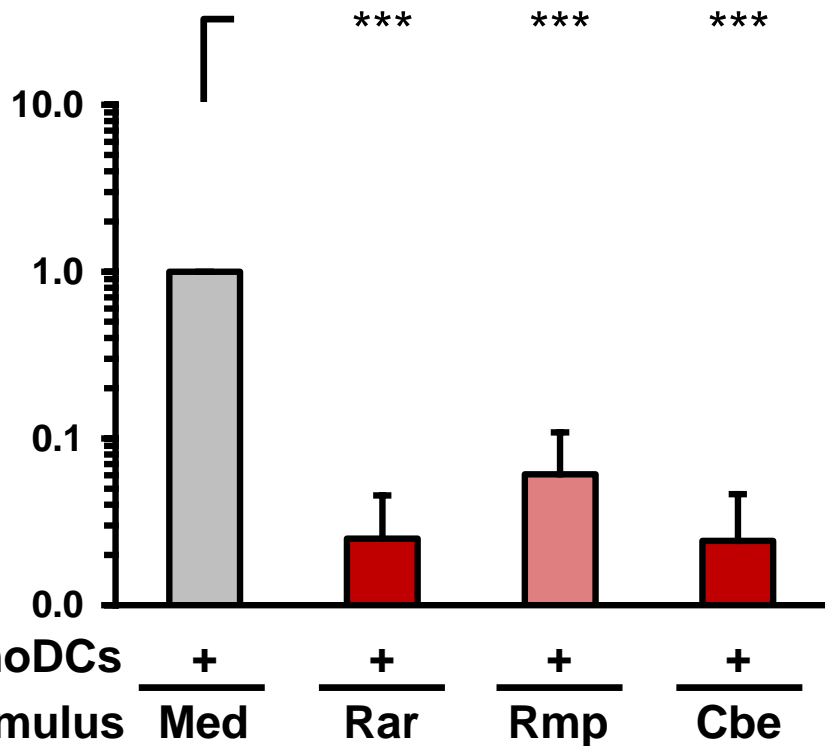


TLR4 mRNA expression

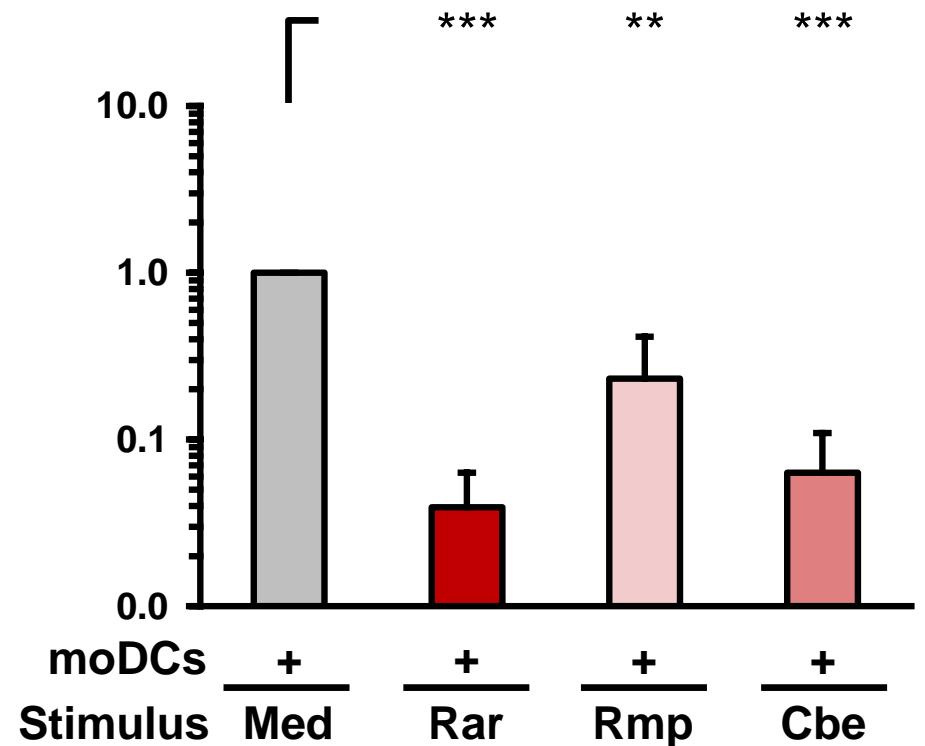


C-type lectin receptor expression in alveolar model

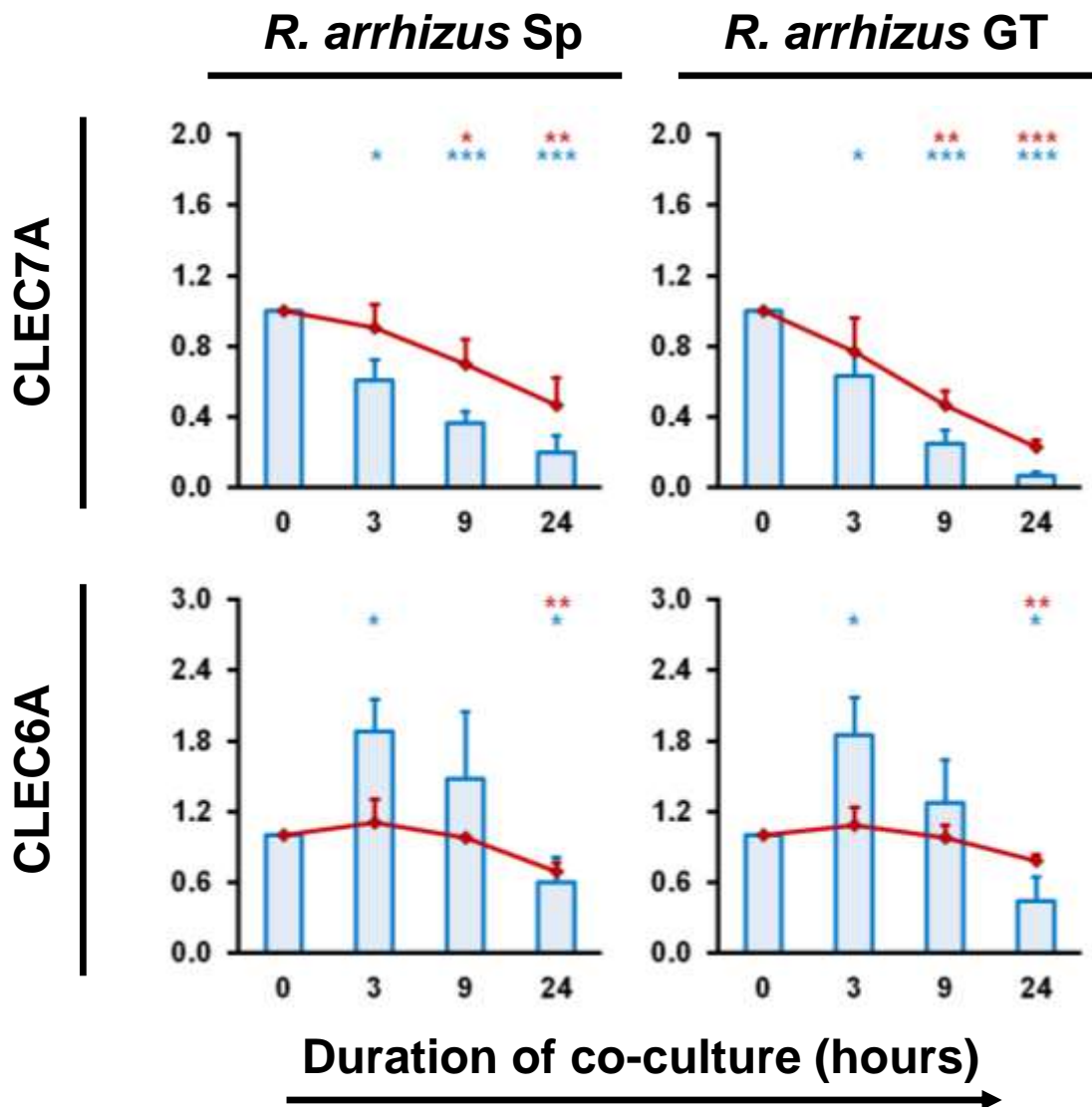
CLEC7A mRNA expression



CLEC6A mRNA expression



Comparison of PRR surface expression (red) and transcriptional activity (blue)



n = 4, Mean fold change + SD, ■ p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001

Summary

- ▶ Successful adaptation of alveolar model with documented invasion of the lower compartment (18S DNA) and strong pro-inflammatory cytokine response
- ▶ Reduced epithelial cytokine secretion and increased LDH concentrations indicate significant epithelial disruption caused by Mucorales
- ▶ Marked differences in epithelial disruption were observed between the studied Mucorales species and *A. fumigatus*
- ▶ Strong transcriptional downregulation of CLEC6A and CLEC7A indicates a potential role of these receptors in Mucorales recognition

Acknowledgement



- ▶ Group members of AG Ullmann and AG Löffler
- ▶ C. O. Morton
- ▶ Institute for Hygiene and Microbiology (IHM) Würzburg
- ▶ Blood donors
- ▶ Interdisciplinary Centre for Clinical Research (IZKF)



Supplementary Slides for Discussion

Outlook

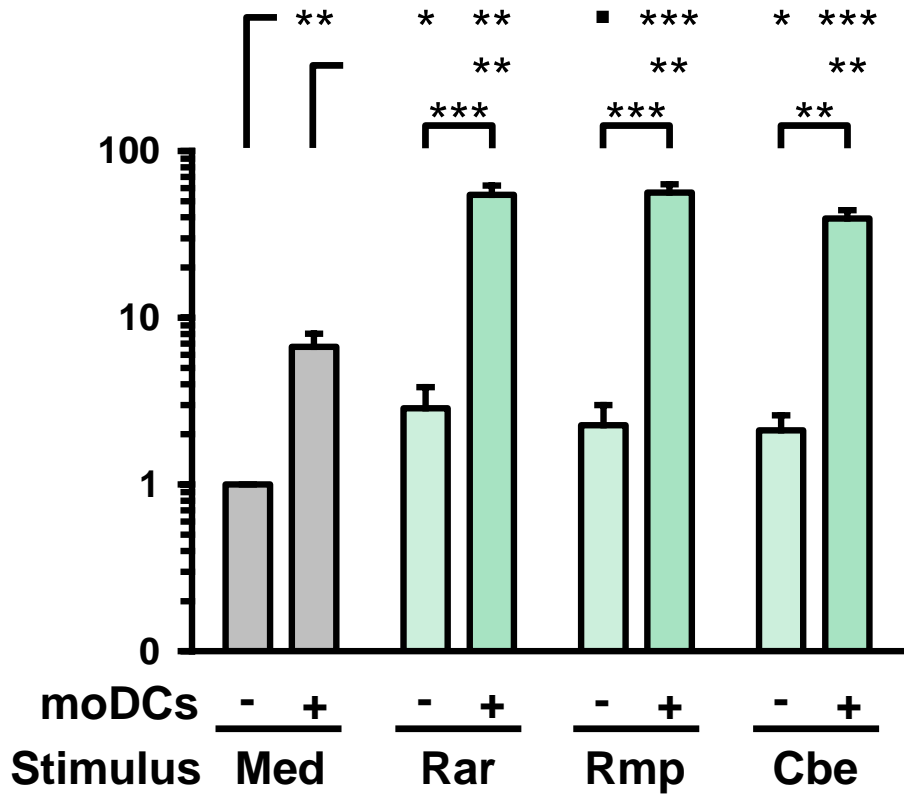
- ▶ Further analyses to study the contribution of neutrophils in the alveolar setting
- ▶ Comparative characterization of epithelial invasion by fluorescence microscopy
- ▶ Functional experiments to characterize the role of CLEC6A/Dectin 2 in the recognition of Mucorales
- ▶ Application of alveolar model for *in vitro* evaluation of novel diagnostic and therapeutic approaches

Comparison of IL8 concentrations in both compartments

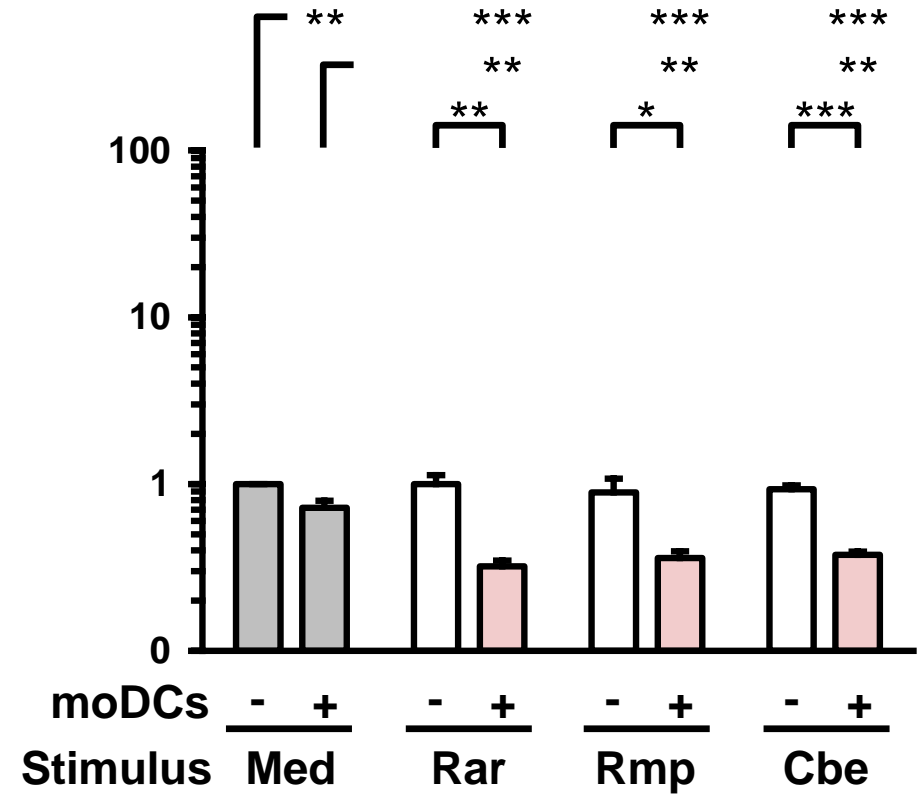
Pathogen	Medium		<i>R. arrhizus</i>		<i>R. pusillus</i>		<i>C. berthol.</i>	
	∅	+	∅	+	∅	+	∅	+
IL8 [pg/ml] Upper compartment	1417 ± 276	1772 ± 1011	636 ± 146	22140 ± 8469	1105 ± 69	38499 ± 4538	1174 ± 107	40823 ± 11348
IL8 [pg/ml] Lower compartment	369 ± 61	674 ± 299	319 ± 70	8658 ± 2577	376 ± 75	14427 ± 3876	414 ± 57	11754 ± 3195
Ratio Lower / Upper comp.	26 %	38 %	50 %	39 %	34 %	37 %	35 %	29%

Epithelial integrity markers

ICAM1 mRNA expression



Cas9 mRNA expression



n = 4, Mean + SD, ■ p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001

Confirmation of PRR expression data in planktonic experiments (moDCs)

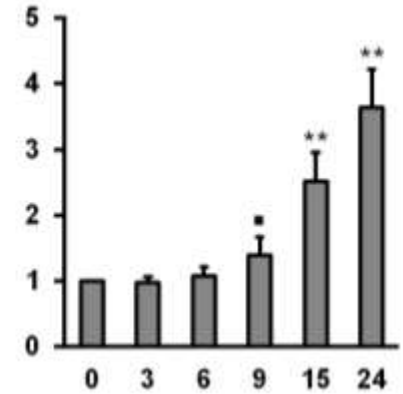
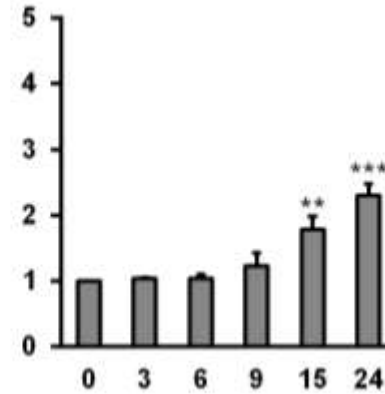
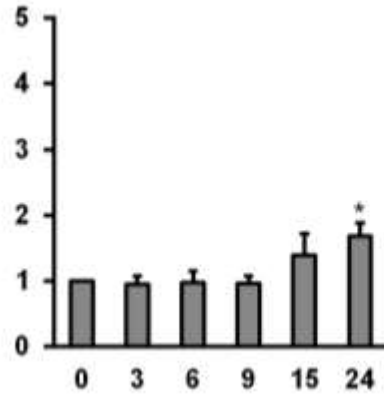
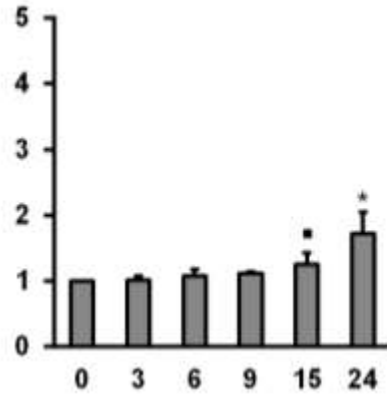
A. fumigatus Sp

A. fumigatus GT

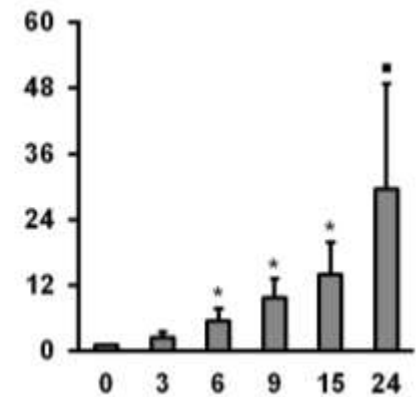
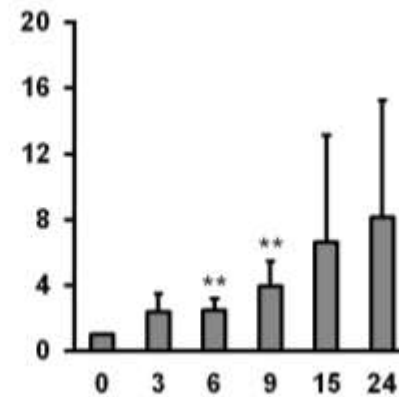
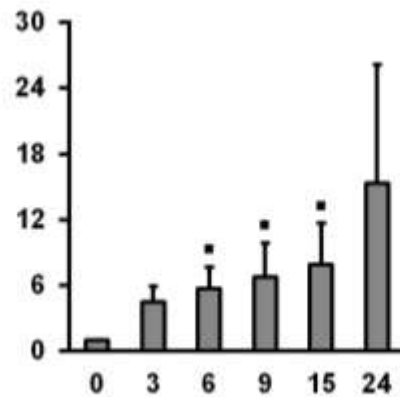
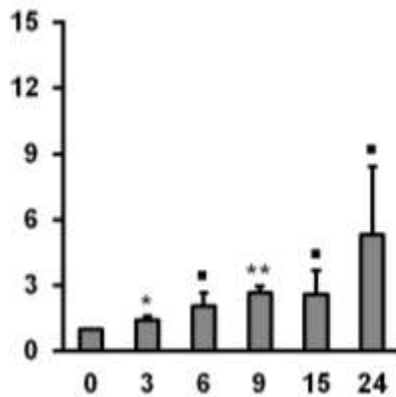
R. arrhizus Sp

R. arrhizus GT

CD80



CD86



Duration of co-culture (hours)

n = 4, Mean fold change of surface expr. + SD, ■ p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001

Confirmation of PRR expression data in planktonic experiments (moDCs)

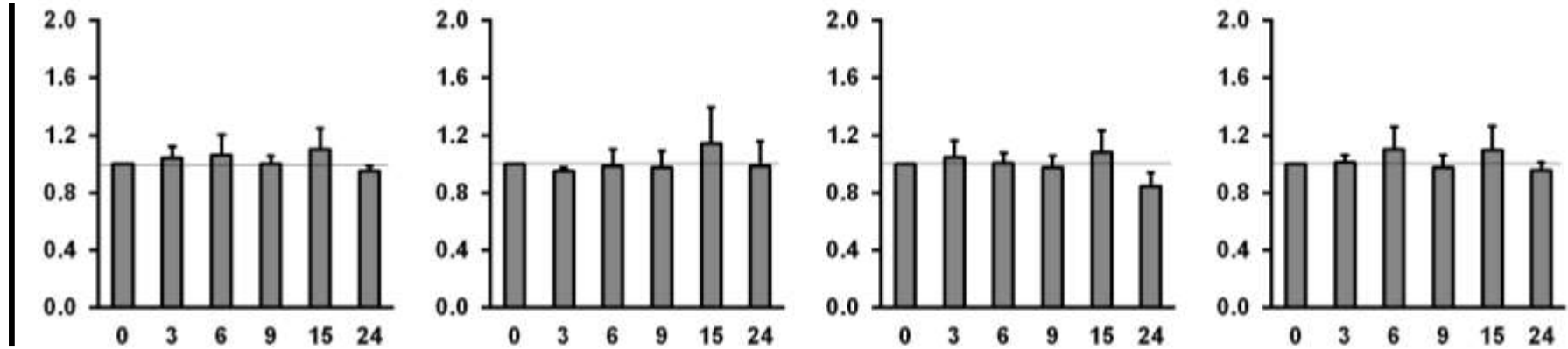
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A. fumigatus GT

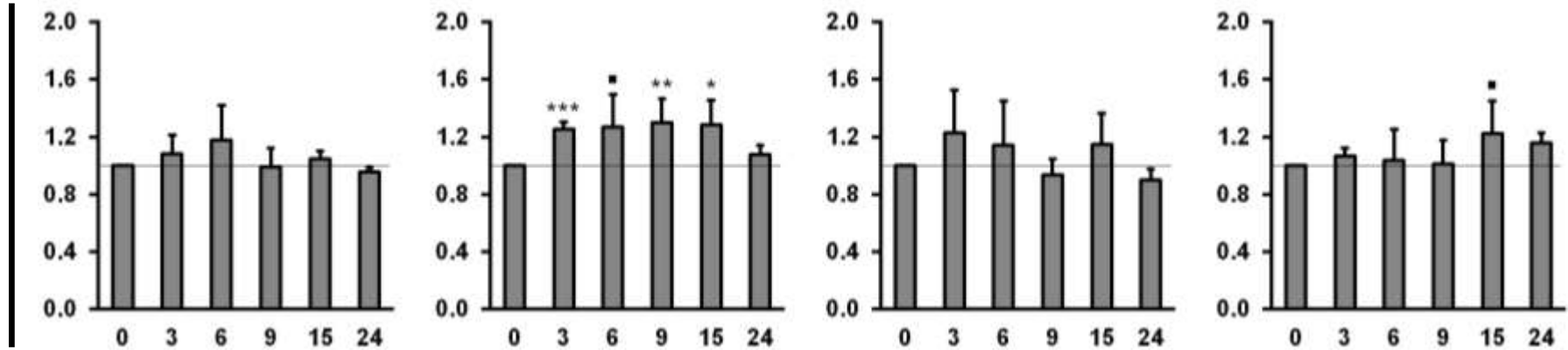
R. arrhizus Sp

R. arrhizus GT

TLR2



TLR4



Duration of co-culture (hours)

n = 4, Mean fold change of surface expr. + SD, ■ p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001

Confirmation of PRR expression data in planktonic experiments (moDCs)

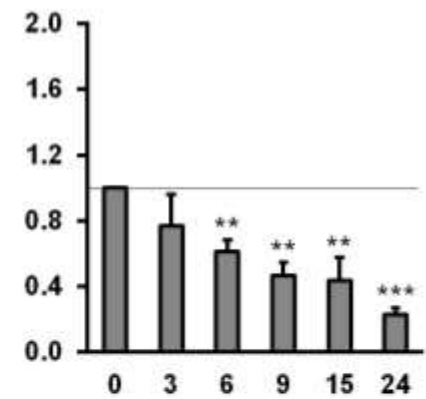
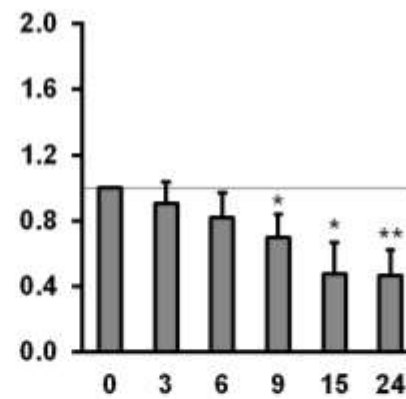
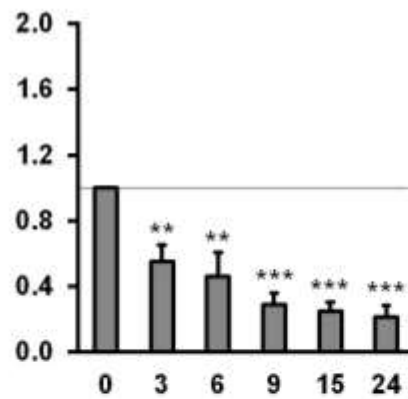
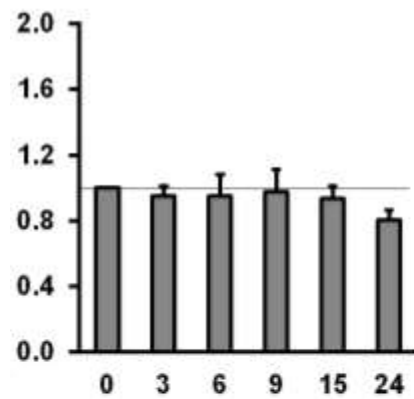
A. fumigatus Sp

A. fumigatus GT

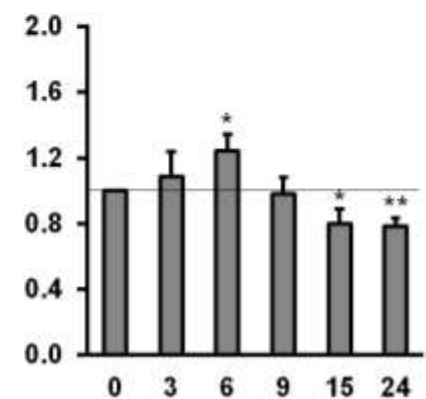
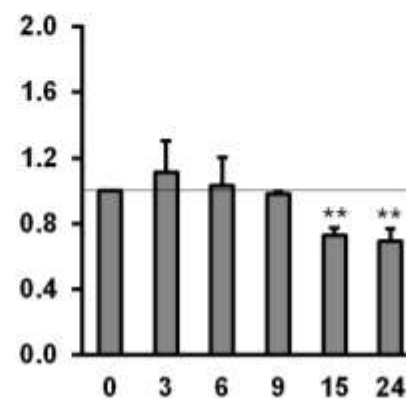
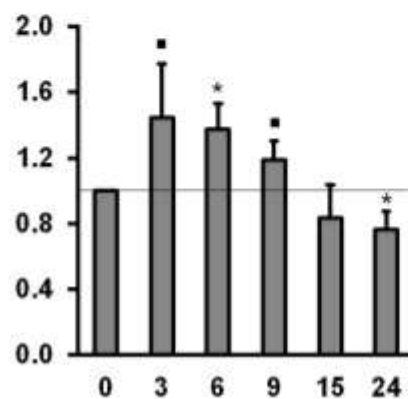
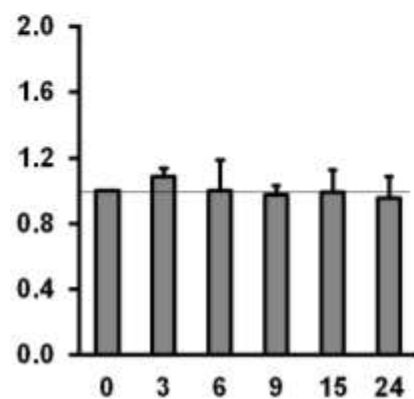
R. arrhizus Sp

R. arrhizus GT

CLEC7A



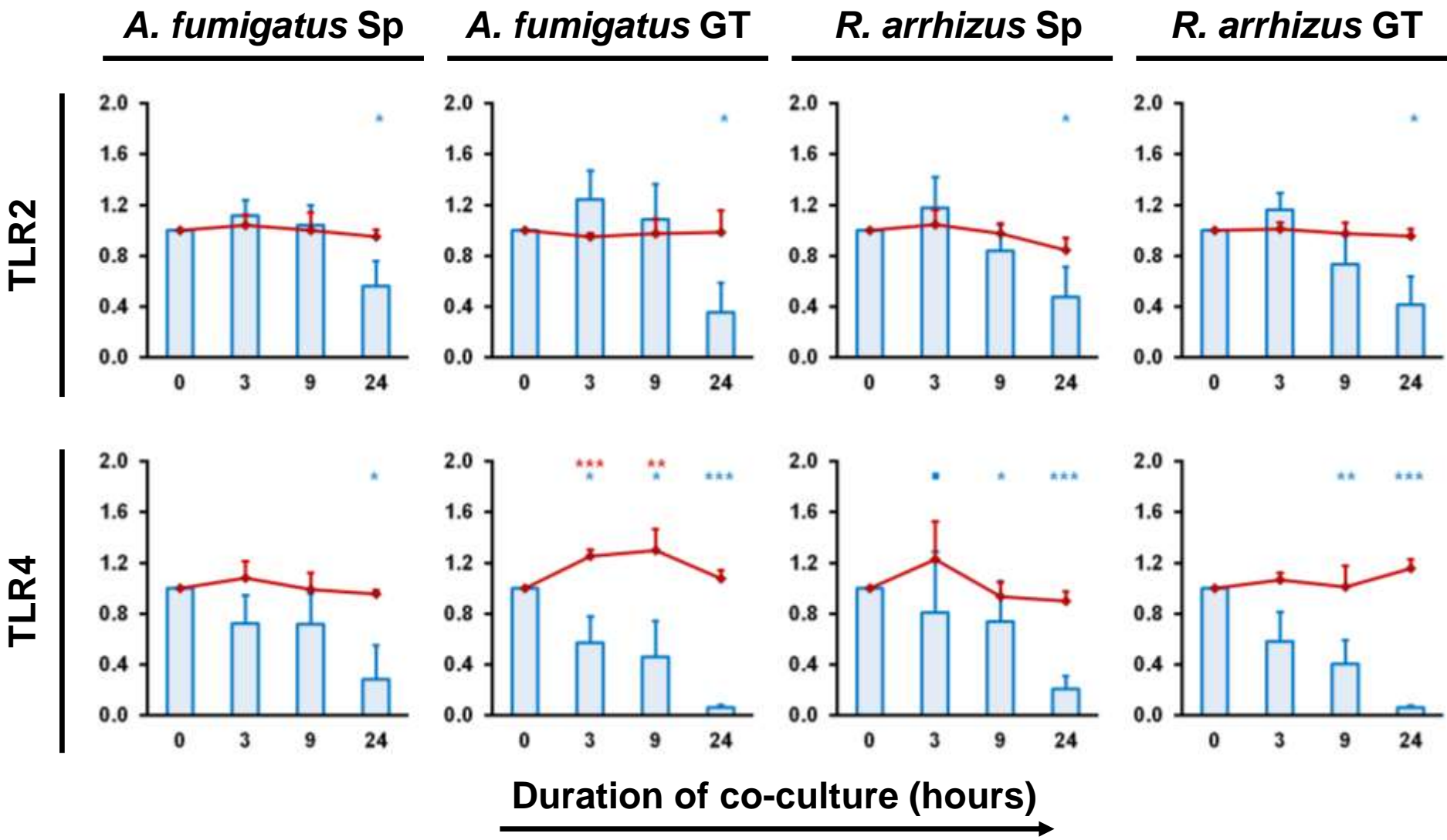
CLEC6A



Duration of co-culture (hours)

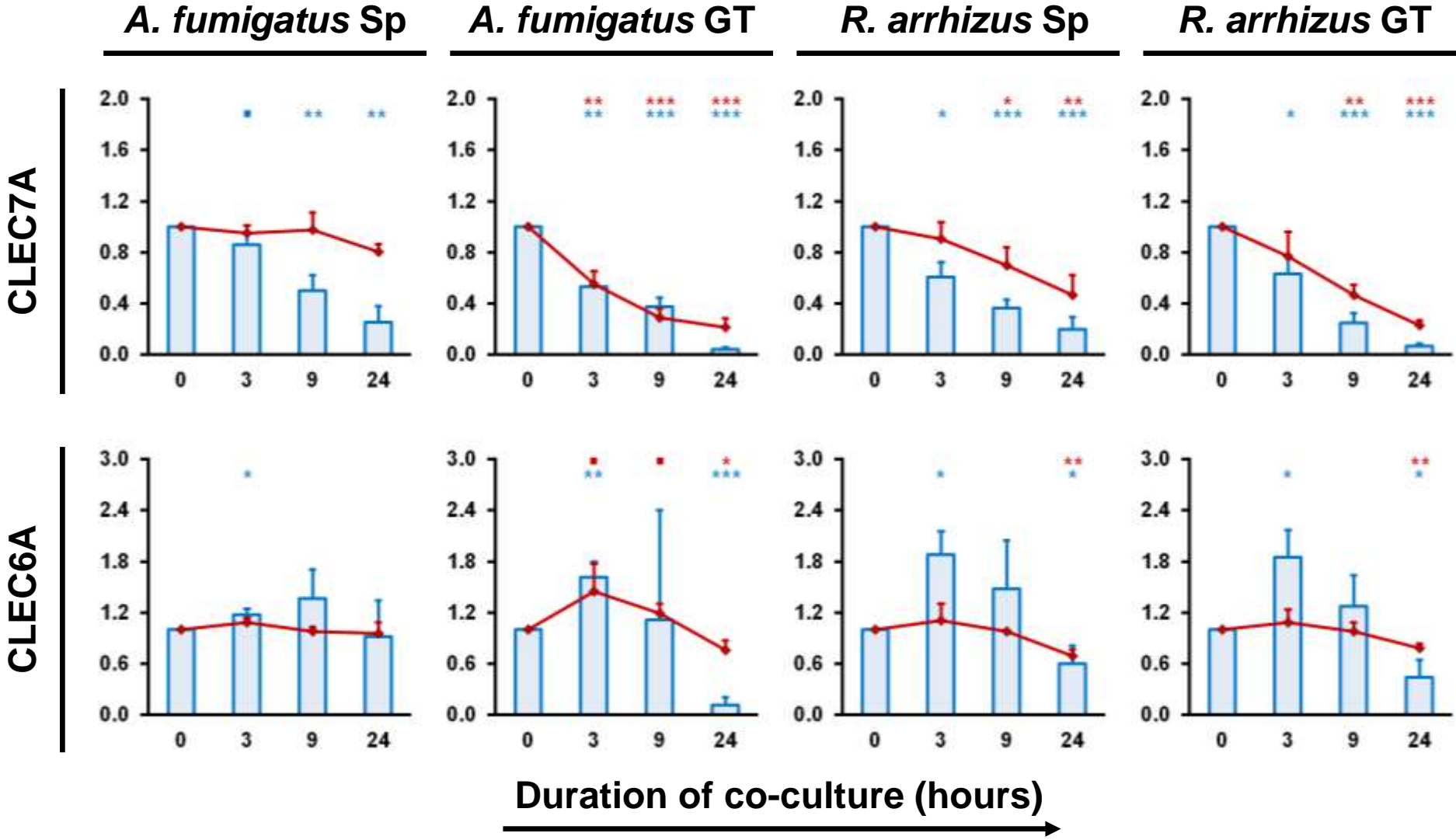
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Comparison of PRR surface expression (red) and transcriptional activity (blue)



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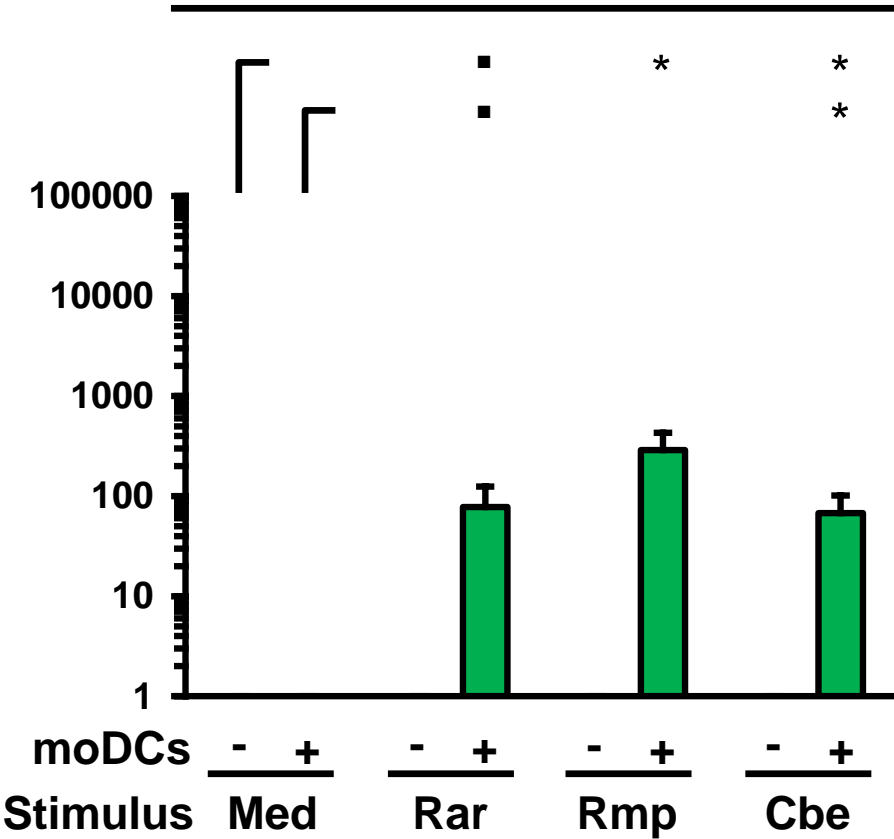
Comparison of PRR surface expression (red) and transcriptional activity (blue)



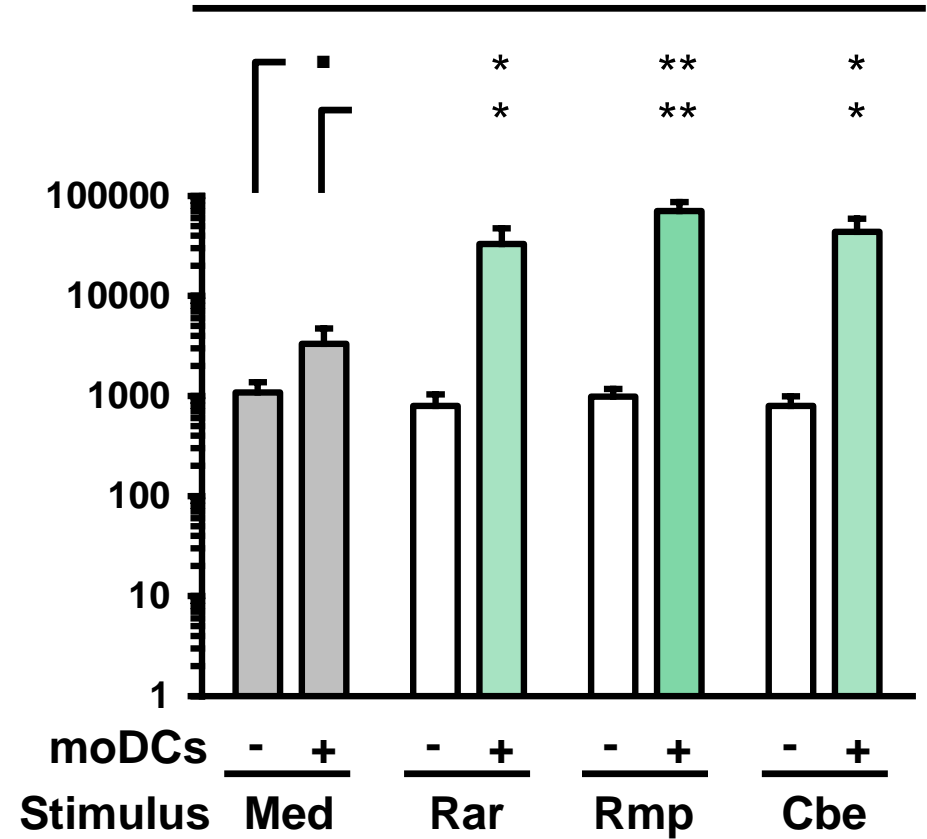
n = 4, Mean fold change + SD, ■ p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001

Pro-inflammatory response to Mucorales (cytokine concentrations in upper comp. after 30 h)

IL12-p70 (pg/ml)

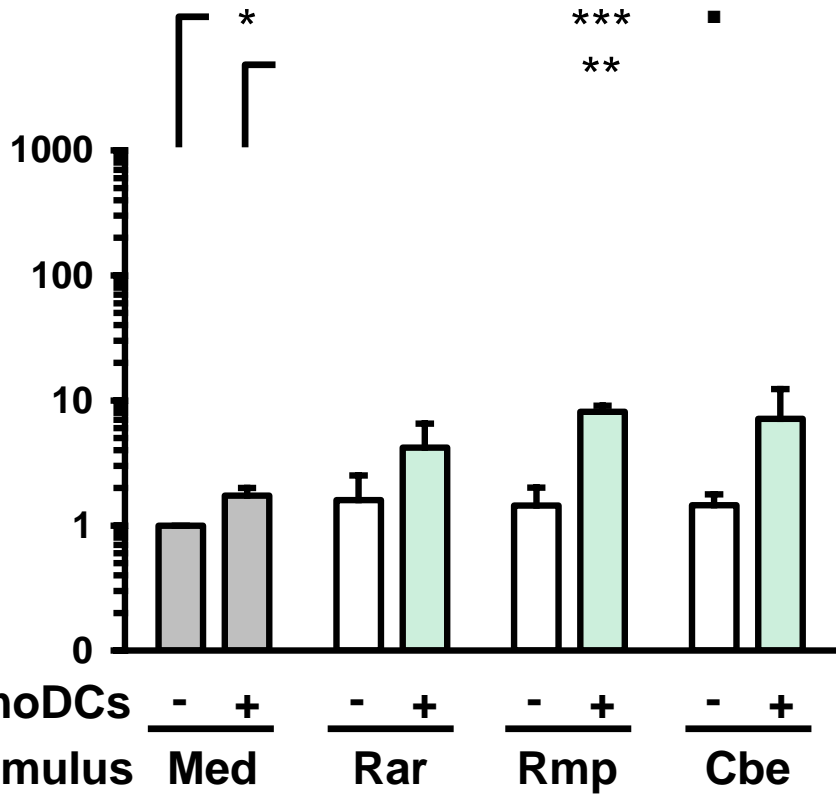


GM-CSF (pg/ml)



Comparison of CCL2 transcription and secretion

CCL2 mRNA expression



CCL2/MCP1 (pg/ml)

