Virtual phagocytosis assays reveal strain-specific differences in the microscopic parameters of the interaction between alveolar macrophages and two A. fumigatus strains

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Biological Background



Experiments and Image Analysis



▷ co-incubation: AM and A. fumigatus ▷ two Af strains: ATCC, CEA10

- ▷ co-incubation for 1 h
- ▷ differential staining



Quantification & Phagocytosis Measures



UPTAKE RATIO VIEWPOINT OF MACROPHAGES



Sporulation Inhalation of airborne conidia

(Adapted from Brakhage *et al.* 2010)

Aspergillus fumigatus is a ubiquitious human-pathogenic fungus. It distributes its small spores (conidia), with a diameter of 2 - 3 µm, via the air. Thus, every human individual inhales several hundred conidia per day. The resident immune cells in the lung - the alveolar macrophages (AM) - phagocytose these conidia and thereby are major players against this pathogen. However, in immunocompromised patients the clearance of the conidia is disturbed leading to severe infections with mortality rates ranging from 30 - 90%. Therefore, it is very important to better understand the host-pathogen interaction of A. fumigatus and AM.





Model & Simulation

Input: binary macrophage images with migration distance



Distribution of cells: using MOI from experiments





Simulation for

optimal parameters

Parameter Estimation Workflow

for all experimental images Experiment Parameter Estimation Screening of parameters φ , α , $\omega \in (0,1)$ for each parameter set (φ , α , ω) Image i



perform simulations with m repetitions



calculate Least-Squares Error (LSE) for probability distibution of adherent and









calculate average Parameter set over all P_i^{opt}

Estimation of Microscopic Parameters

Example: Screening for one image





Results for all experimental images:

▷ higher adherence probability and lower

Resolve Ambiguities of Phagocytosis Measures

Assumption: Two independent experiment with

$$\varphi_p = \frac{N_c^{phag}}{N_c^{phag} + N_c^{adh}} \qquad \text{and} \qquad \varphi'_p = \frac{N_c^{phag'}}{N_c^{phag'} + N_c^{adh'}}$$

$$\Rightarrow N_c^{phag'} = p \cdot N_c^{phag} \qquad \text{and} \qquad N_c^{adh'} = a \cdot N_c^{adh}$$

$$\Rightarrow \varphi'_{p} = \frac{N_{c}^{phag}}{N_{c}^{phag} + \frac{a}{p}N_{c}^{adh}} \quad \text{for} \quad p > 0$$
$$\varphi'_{p} = 0 \quad \text{for} \quad p = 0$$
$$\varphi_{p} = \varphi'_{p} \text{ possible for } p = a > 0$$

 $\varphi_s = \varphi_s$

$\varphi_p \cdot \varphi_u = \varphi_p^{'} \cdot \varphi_u^{'}$



Artificial data set with: $\varphi_s = \varphi_s'$ (for absolute quantifications)







- phagocytotsis probability for ATCC compared to CEA10
- ▷ washing probability similar
- **Outlook:** ATCC ▷ improve estimation of macrophage E CEA10 migration distances with new live
 - cell imaging data ▷ calculate LSE for a combination of probability distribution and absolute cell counts

Outlook:

▷ generate artificial data with probability distribution \triangleright simulations with m \neq 1

▷ simulations of artificial macrophage images

References:

Contact:

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