Quantitative impact of cell membrane fluorescence labeling on phagocytosis measurements in confrontation assays.

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Details

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Abstract

Phagocytosis is series of steps where the pathogens and the immune cells interact during an invasion. This starts with the adhesion process between the host and pathogen cells, and is followed by the engulfment of the pathogens. Many analytical methods that are applied to characterize phagocytosis based on imaging the host-pathogen confrontation assays rely on the fluorescence labeling of cells. However, the potential effect of the membrane labeling on the quantitative results of the confrontation assays has not been studied in detail. In this study, we

determine whether the fluorescence labeling processes themselves influence the results of the phagocytosis measurements. Here, alveolar macrophages, which form one of the most important compartments of the innate immune system, were used as an example of host cells, whereas *Aspergillus fumigatus* and *Lichtheimia corymbifera* that cause aspergillosis and mucormycosis, respectively, were studied as examples for pathogens. At first, our study investigated the importance of the sequence of steps of the fixation process when preparing the confrontation assay sample for microscopy studies. Here we showed that applying the fixation agent before the counter-staining causes miscalculations during the determination of the phagocytic measures. Furthermore, we also found that staining the macrophages with various concentrations of DID, as a typical membrane label, in most cases altered the capability of macrophages to phagocytose FITC-stained *A. fumigatus* and *L. corymbifera* spores in comparison with unlabeled macrophages. This effect of the DID staining showed a differential character dependent upon the labeling status and the specific type of pathogen. Moreover, labeling the spores of *A. fumigatus* and *L. corymbifera* with FITC increased the phagocytic measures during confrontation with unlabeled macrophages when compared to label-free spores. Overall, our study confirms that the staining process itself may significantly manipulate the quantitative outcome of the confrontation assay. As a result of our study, we also developed a user-friendly image analysis tool that analyses confrontation assays both with and without fluorescence labeling of the host cells and of the pathogens. Our image analysis algorithm saves experimental work effort and time, provides more precise results when calculating the phagocytic measures, and delivers a convenient analysis tool for the biologists to monitor host-pathogen interactions as they happen without the artifacts that fluorescence labeling imposes on biological interactions.

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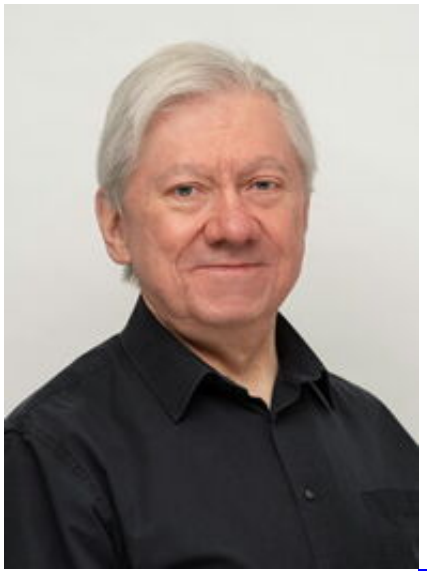

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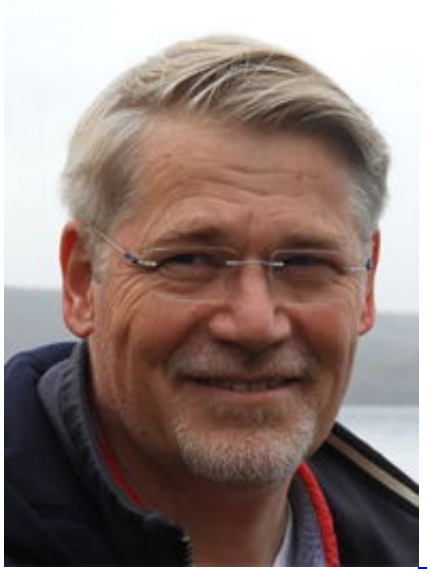

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