Specific differences in immune activation by bacterial (TLR4) and fungal (Dectin-1) pathogens and their inhibition by small molecules.

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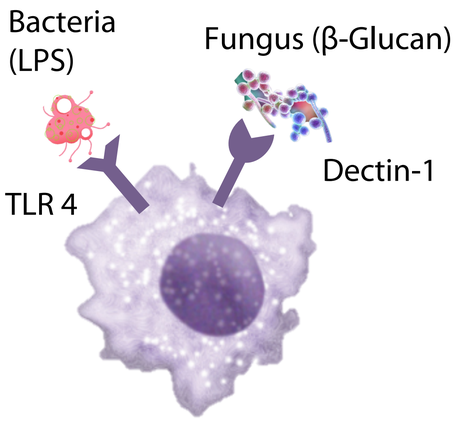
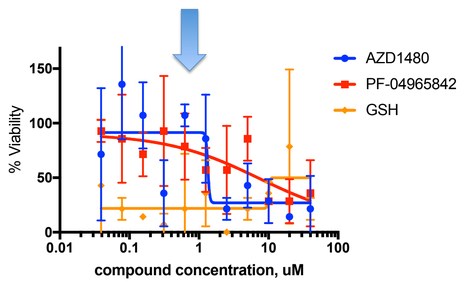
Introduction



The aim of our laboratory this year was to study the cellular work of innate immunity - the first line of body protection from foreign pathogens. The main attention was on the receptors TLR-4 and Dectin-1, which recognize bacterial lipopolysaccharides (LPS) and the glucans of most fungi (Beta-glucan), respectively. We isolated Bone Marrow Derived Macrophages (BMDM) from the mouse bone marrow to study the effect of certain compounds and bacterial/fungal infections on macrophage cells, as well as the macrophage line RAW-264. We used a modern technique qPCR to investigate gene expression.

The main goal of our laboratory was to study the specificity of receptors for the immune response, as well as confirming or refuting the hypothesis about the importance of genes Gclm, NFe2L2, Hmox1, Gclc in the transmission of activation signals from innate immune receptors. The obtained data can be used in the future development of therapeutics against immune diseases.

Part I: Determine compound concentrations to use



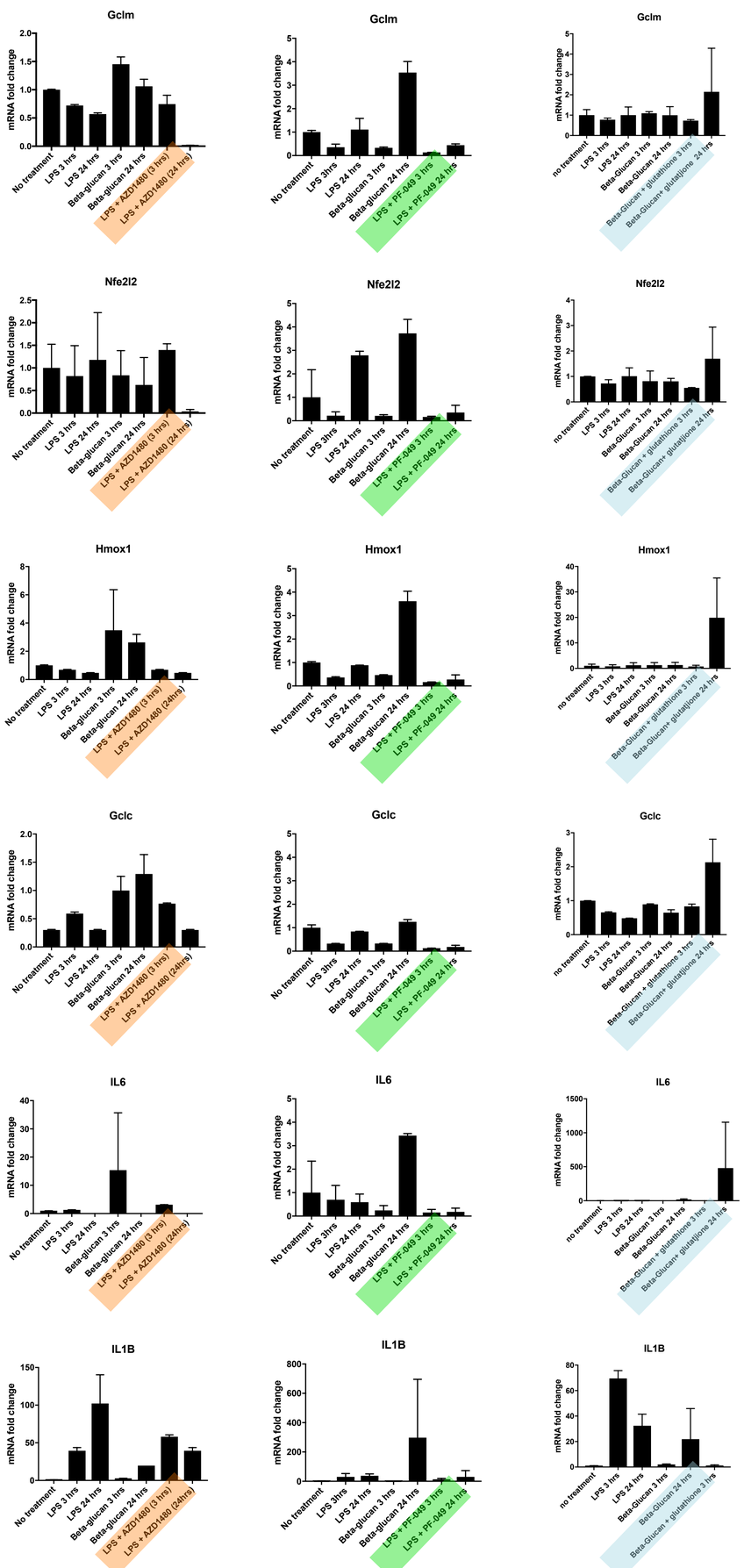
Cell Viability Assay

The purpose of this assay was to measure the viable cells in multi-well plates to estimate non-toxic concentrations of the compounds for subsequent experiments with macrophages, such as qPCR .

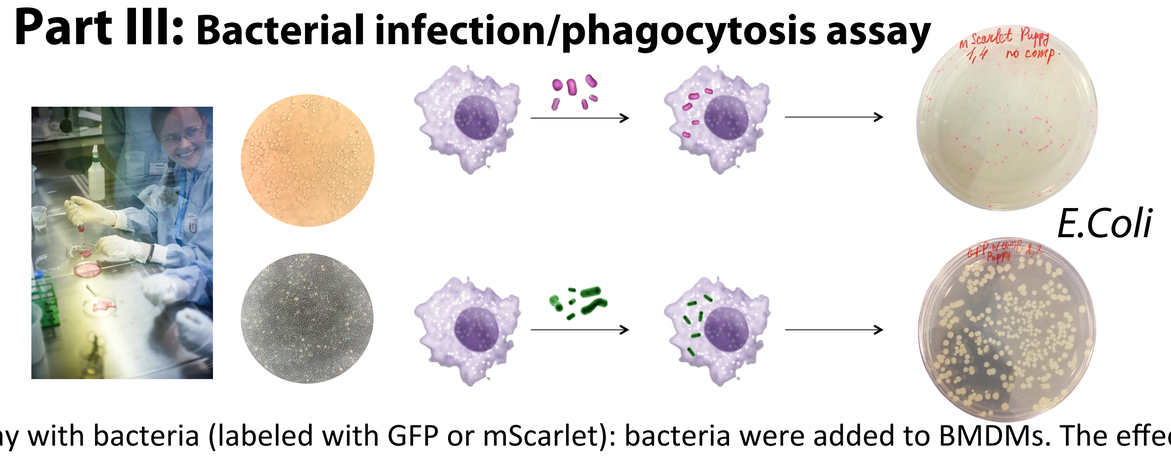
Based on the results, we used 0.5 uM compounds.

Part II:

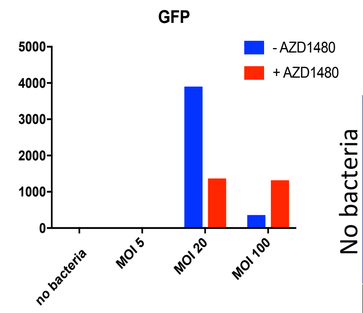
(a) Based on RNASeq, pick most differentially expressed genes.
(b) Measure these genes using qPCR assay in immune cells, with LPS (TLR4) and Beta-glucan (Dectin-1) stimulation.
(c) Compare LPS- and Beta-glucan-driven activation in the presence of compounds

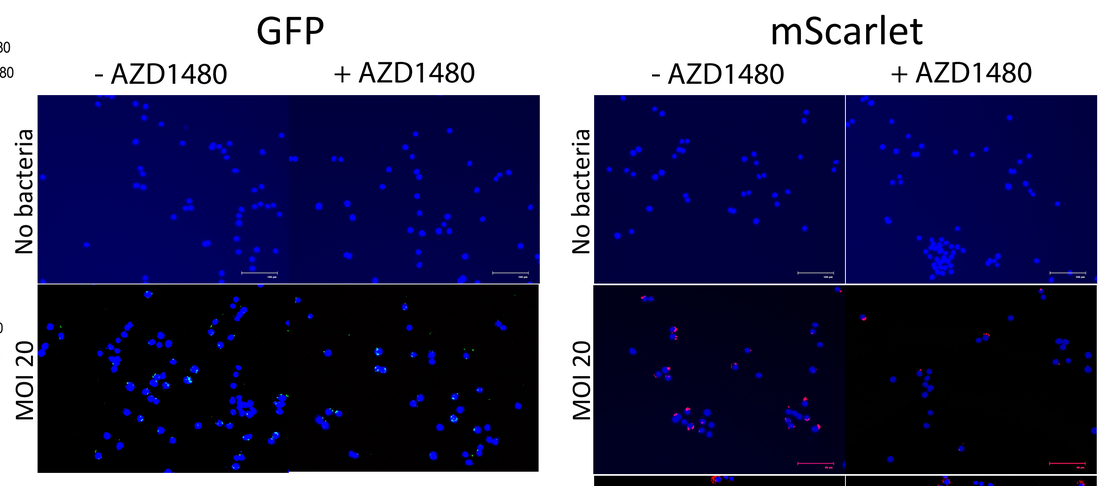


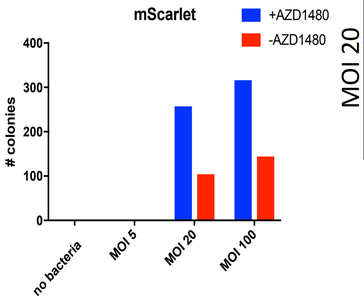
Part III: Bacterial infection/phagocytosis assay

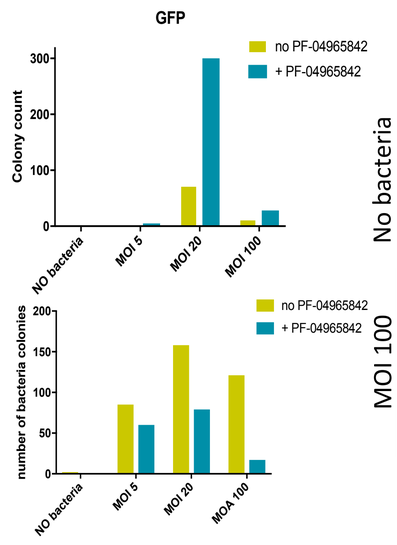


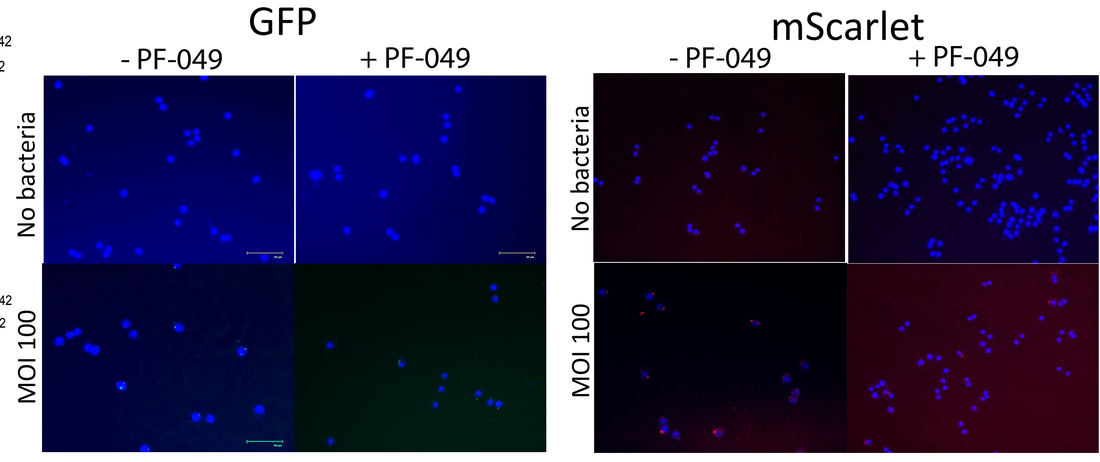
Infection assay with bacteria (labeled with GFP or mScarlet): bacteria were added to BMDMs. The effect of different compounds(AZD 1480, PF-04965842, Glutathione) on bacterial phagocytosis by macrophages was analysed in two ways: by microscopy and by counting bacterial colonies on the agar plates. Macrophage cells were labeled with Hoechst (makes macrophage nucleus blue).

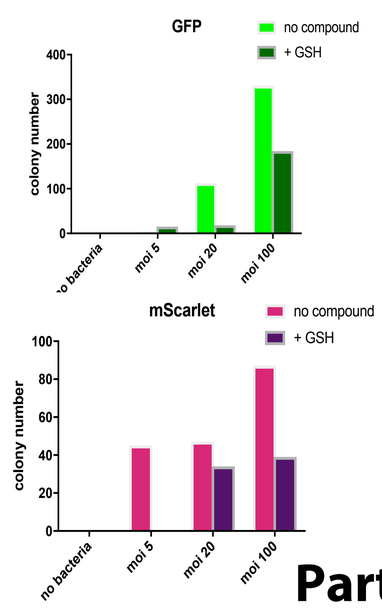


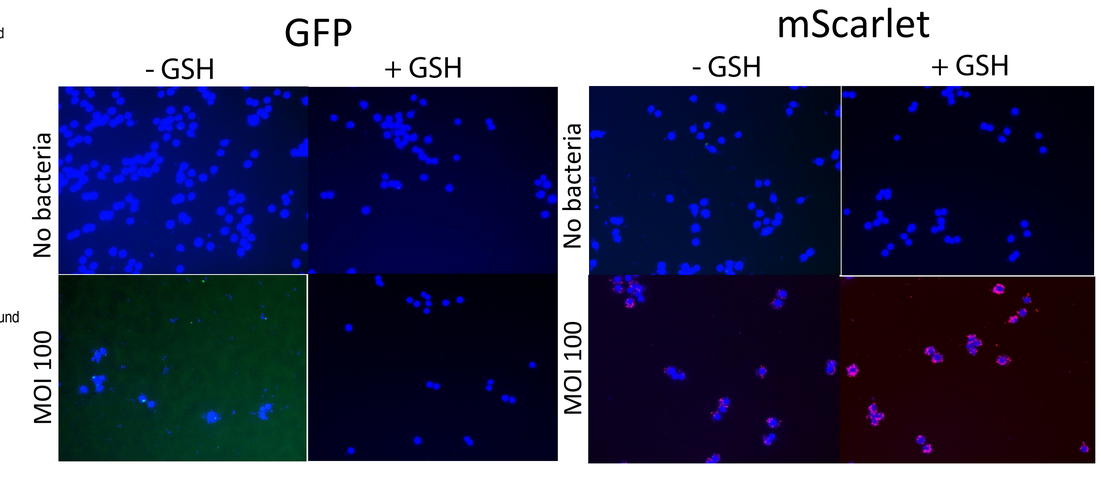




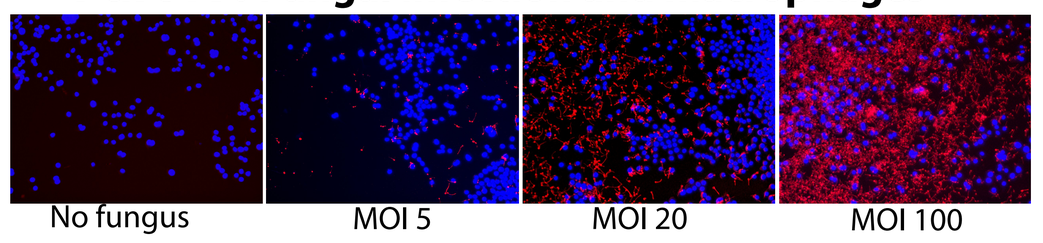








Part IV: Fungal infection and macrophages



Conclusions: Dectin-1 and TLR4 signaling siginificantly differ in macrophages. Compounds inhibiting Jak/Stat pathway affect LPS/TLR4 signaling. Glutathione, an antioxidant agent, promoted immune response and bacterial phagocytosis and bacterial killing.