Inflammasome dependent and independent pathways in Coccidioidomycosis

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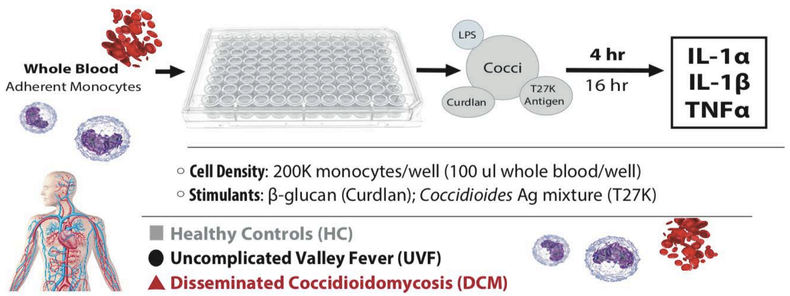
INTRODUCTION

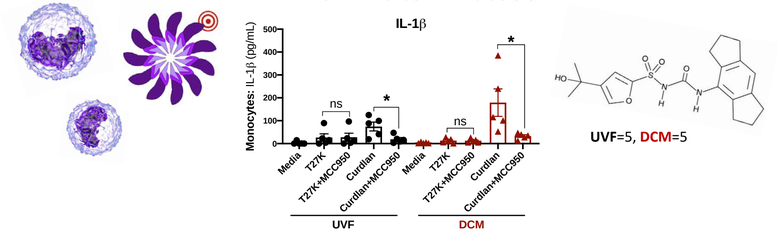
Coccidioidomycosis is an invasive fungal disease presenting symptoms that range from asymptomatic or mild, to isolated pneumonia, to disseminated disease involving CNS, skin, and bone. This variation may be in part due to distinct differences in host innate immune responses. The primary goal of this study was to explore the role of IL-1 cytokine responses using *ex vivo* assays in fresh whole blood or adherent monocytes to measure and compare relevant cytokine responses among affected subjects (disseminated coccidiomycosis - DCM or uncomplicated valley fever - UVF) and healthy controls (HC).

METHODS

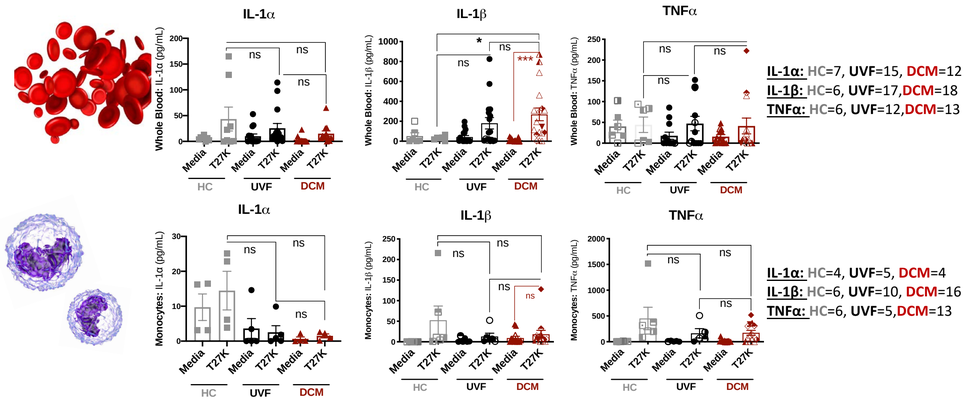


Blood samples from over 70 donors at VFI (34 UVF, 42 DCM) and 8 HC at UCSD were collected with informed consent according to IRB approved protocols. Adherent monocytes (AM) or whole blood (WB) cells were stimulated with either T27K (heat killed *Coccidioides* spherules), Curdlan (β-glucan that activates Dectin-1), or a combination of LPS+ATP (NLRP3 specific stimuli). The NLRP3 specific inhibitor MCC950 (1μM) was also tested to further evaluate NLRP3-inflammasome dependent responses. Plasma from WB assays (4hr post-stimulation) or cellular supernatants from AM (16hr post-stimulation) were collected to determine relative cytokine release between the donor groups. IL-1β, IL-1α, and TNFα levels were measured using ELISA.

NLRP3 Inhibitor: MCC950



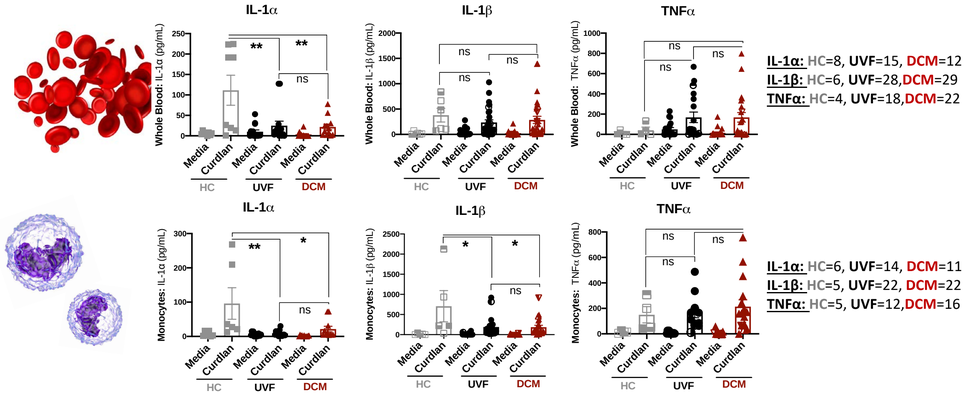
Coccidioides Antigen Mixture: T27K



IL-1α: HC=7, UVF=15, DCM=12
IL-1β: HC=6, UVF=17,DCM=18
TNFα: HC=6, UVF=12,DCM=13

IL-1α: HC=4, UVF=5, DCM=4
IL-1β: HC=6, UVF=10, DCM=16
TNFα: HC=6, UVF=5,DCM=13

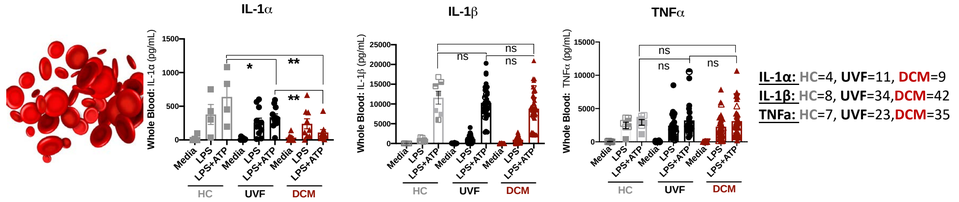
β-glucan: Curdlan



IL-1α: HC=8, UVF=15, DCM=12
IL-1β: HC=6, UVF=28,DCM=29
TNFα: HC=4, UVF=18,DCM=22

IL-1α: HC=6, UVF=14, DCM=11
IL-1β: HC=5, UVF=22, DCM=22
TNFα: HC=5, UVF=12,DCM=16

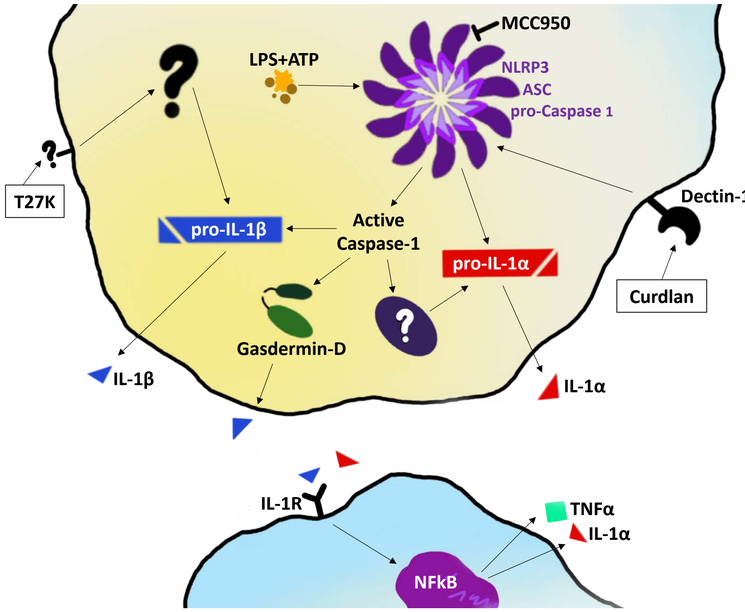
Inflammasome Response



IL-1α: HC=4, UVF=11, DCM=9
IL-1β: HC=8, UVF=34,DCM=42
TNFα: HC=7, UVF=23,DCM=35

RESULTS

T27K elicited a greater IL-1β response in WB from DCM than that of HC but no difference in IL-1α or TNFα, whereas Curdlan stimulated significantly less IL-1α release in WB and AM from both *Coccidioides* affected groups (DCM and UVF) compared to HC. IL-1β and TNFα levels from WB and AM were comparable between all groups with LPS+ATP. However, we observed a significant reduction in IL-1α release in WB from DCM compared to other groups following treatment with LPS+ATP. Inhibition of IL-1β release in AM from affected patients was observed following *in vitro* exposure to MCC950 prior to Curdlan or LPS+ATP but no effect was observed in T27K stimulated samples.



CONCLUSIONS

Host response to *Coccidioides* have apparent NLRP3 inflammasome dependent and independent pathways leading to IL-1α and IL-1β release. Preliminary data suggests that DCM patients may have novel dysregulated NLRP3 inflammasome mediated responses leading to inadequate IL-1α production, however the exact mechanisms driving its release and the cellular sources are still unclear and merits further investigation.