Chapter 7: Summary, conclusion and significance
7.1. Summary

- nHz, but not parasitic stages rings and trophozoites modulated the expression of cytokines, chemokines and surface molecules in THP-1 monocytic cell line. nHz stimulated the production of inflammatory cytokines TNF-α, IL-1β, IL-6. nHz-induced down regulation of surface molecules HLA-DR, CD11b, CD11c and CD54 on nHz phagocytosis in THP-1 cells may be associated with immunosuppression. Additionally, LPS and IFNγ stimulation partially reversed the CD54 expression in nHz-fed THP-1 monocytes.

- nHz induced the expression of M2 like phenotypic and functional characters in adherent human monocytes nHz downregulated the expression of M1 specific surface molecule HLA-DR, cytokine IL-12p70 and NO production in adherent human monocytes, and concomitantly induced expression of M2 associated markers - CD206, secreted IL-10 and arginase 1 activity. nHz treated monocytes expressed M2a and M2b specific CCL17 and CCL1, TNF-α, IL-1β, IL-6 respectively. Moreover, nHz treated monocytes suppressed mitogen stimulated PBMC proliferation.

- The physiologically relevant amounts of Hz i.e. 25-75µg/ml detected during infection possess the ability to induce M2-like phenotypic markers IL-10, CCL1 and CCL17 in monocytes

- The kinetic study of M2 markers in Hz-ingested monocytes suggests that IL-10 production decreased after 48h, even though the levels were still greater than controls. The chemokines CCL1 and CCL17 were still higher in Hz fed monocytes during 48h. The Hz-induced M2-like phenotype may be predominant during early periods after Hz phagocytosis.

- The inhibition of pathways p38 MAPK, NF-κB, PI3k/AKT, STAT3, PPARγ and mTOR using specific pharmacological inhibitors decreased Hz-induced IL-10 production. The inhibition of JNK pathway did not decrease IL-10 at all. The maximum decrease in IL-10 production was seen on p38 MAPK, NF-κB and PI3k/AKT pathway’s inhibition.

- nHz activated PI3K-AKT, NF-κB and p38 pathways and inhibition of these pathways using specific pharmacological inhibitors resulted in downregulation of
M2 like phenotypic and functional characters. The inhibition of PI3K-AKT, NF-κB and p38 pathways, attenuated the expression of IL-10 (M2), CCL17 (M2a), CCL1, IL-6, TNF-α and IL-1β (M2b) in Hz ingested monocytes. Moreover, the arginase activity was reduced and inhibition of above pathways prevented the suppression of mitogen stimulated lymphocyte proliferation, NO and reactive oxygen intermediate production in Hz exposed monocytes.

- Antimalarial drugs, chloroquine and artemisinin partially reversed the expression of M2-like phenotype. The drugs ART and CHQ attenuated the expression of M2-phenotypic markers IL-10 (M2), CCL17 (M2a), CCL1, IL-6, TNF-α and IL-1β (M2b). Moreover, drugs reversed nHz-mediated suppression of mitogen stimulated lymphocyte proliferation in monocytes. The drugs reversed phenotypic markers of M2 phenotype than functional ones i.e. reactive and nitrogen oxidative intermediates.

- The ART and CHQ inhibited NF-κB activation in Hz ingested monocytes. This suggests that ART and CHQ mediated partial reversal of M2-like phenotype in Hz ingested monocytes might be through inhibiting NF-κB pathway.

- Gene profiling by microarray analysis revealed that nHz but not sHz-induced M2 like characters in adherent human monocytes. Further validation suggests that, sHz did not produce M2 specific IL-10 and expressed mannose receptor CD206. Importantly, it did not suppressed PHA- stimulated PBMC proliferation and the cells produced high levels of NO, ROS and exhibited low arginase activity.

The schematic diagram (Fig 7-1) summarizes the Hz-induced alterations in M1 and M2 associated markers, the pathways involved and partial reversal by CHQ and ART in human monocytes. Hz released after schizont rupture is readily ingested by circulating monocytes in blood, activates PI3K-AKT, NF-κB and p38 pathways resulting in increased expression of M2 markers and drives the monocytes towards -M2 (M2a and M2b)-like phenotype. Antimalarial drugs-ART and CHQ partially reverse the process of activation of monocytes towards M2-like phenotype. In conclusion, the findings from this study add a new dimension in our understanding of mechanism/s underlying Hz-induced immunosuppression by demonstrating its ability to drive the monocytes towards M2-like phenotype.
Figure 7-1. Proposed model for Hz induced M2-like monocytes during infection. Hemozoin (Hz) released after schizont rupture is ingested by circulating monocytes in blood, activates PI3K/AKT, NF-κB and p38 pathways; resulting in increased expression of M2 markers IL-10, CD206, arginase activity. Specifically, Hz induces M2a (CCL1) and M2b (CCL17, TNF-α, IL-1β and IL-6) phenotype that regulate TH2 responses. Hz attenuates HLADR expression, nitric oxide (NO) production and reactive oxygen species (ROS) - features of M1 phenotype. Antimalarial drugs- ART and CHQ partially reverse the Hz induced M2 like phenotype.
7.2. Conclusion and significance

*Falciparum* malaria is frequently associated with immunosuppression. Here, we have indicated Hz as the probable component, leading to M2-like monocyte phenotype in human malaria. Parasite may employ this phenotype to exhibit immune evasion. The successful recovery of intracellular parasite infection requires a fast and robust M1/TH1 response, to lower parasitemia, and M2/TH2 immune response, regulating excessive inflammation and tissue injury. sHz, which is widely used as Antimalarials chloroquine and artemisinins, partially reversed the Hz-induced M2 phenotype. Thereby, presenting an attractive option for adjunctive treatment due to immune-modulation as their additional tool along with traditional anti/protozoan nature. Recently, Chloroquine chemoattenuated PfSPZ vaccine was found to be effective in human malaria (293), further signifying the use of immunomodulatory antimalarials in treatment and prevention. In addition, the repurposing of drugs will save a lot of efforts and cost involved in screening and testing new drugs. Even though, resistance against chloroquine and artemisinin is increasing, different derivatives of these drugs endowed with parasite killing and immunomodulatory properties may still be useful. In corroboration with this, artemisinin combination therapy (ACT) is the currently the most effective therapy. Thus, our study provides Hz as the important target responsible for immunosuppressive phenotype and suggests utilizing immunomodulation to design immunopotentiating therapies.