Abstract

Malaria is an infectious disease affecting populations in tropical and subtropical regions. Of the five *Plasmodium* species, *falciparum* causes the most severe form of human malaria with several complications. It is well established that only the erythrocytic stage of parasites is responsible for malaria symptoms. During schizont rupture, the parasite releases several pyrogens, toxins and malarial pigment—hemozoin (Hz) into the bloodstream along with merozoites. These factors activate immune cells (monocytes, macrophages, dendritic cells etc.) which augment several proinflammatory cytokines, chemokines and other soluble factors. These factors influence the hypothalamus in the brain which results in the fever like conditions and probably influence severe pathophysiology associated with malaria.

During the intraerythrocytic cycle, the parasites digest hemoglobin in the food vacuole, resulting in the production of potentially toxic heme metabolites, which the parasite detoxifies by converting it to an insoluble crystal called malarial pigment or Hz. Hz is released into the blood circulation after completion of the erythrocytic cycle (48h) and is avidly phagocytized by human phagocytic cells such as monocytes, neutrophils and dendritic cells. Hz-containing monocytes are frequently encountered in patients and also related to disease severity. Hz has been reported to alter several functions of monocytes; such as repeated phagocytosis, bactericidal abilities, oxidative burst, MHC Class II expression, antigen presentation and maturation of dendritic cells. The phagocytosis of Hz in monocytes results in stimulation and secretion of pro- and anti-inflammatory cytokines, thereby influencing the immune response.

Monocytes and macrophages are heterogeneous in nature and can mature into pro-inflammatory (M1) or anti-inflammatory M2 (M2a, M2b, M2c) type depending on nature of microenvironmental signals. The response is reflected by an alteration in receptor expression, cytokine production, effector function and chemokine repertoires. IL-10, a well-characterized anti-inflammatory cytokine produced by monocytes plays a crucial role in M2 polarization and is produced through IL10R, STAT3 and NF-κB-mediated pathways. p38-MAPK, PI3K/AKT and NF-κB signaling have been implicated in the IL-10 synthesis and M2 polarization separately; however, their relevance in M2 polarization as a function of IL-10 production is not clearly understood. Monocyte phenotypes have been reported to play an important role in many diseases. The M2 polarization of
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monocytes has been shown to aggravate the clinical symptoms in parasitic and bacterial diseases. Immunomodulators are emerging as a new class of agents to control different infections. Artemisinins have been reported to decrease IL-10, IL-1β, IL-6, TNF-α levels, through p38-MAPK, NF-κB and PI3K/AKT pathways in diseases. Chloroquine (CHQ) has also been demonstrated to act through MAPK and NF-κB pathways and decrease IL-1β, IL-6 and TNF-α in mononuclear phagocytes in different diseases.

Along these lines, we designed our first objective to study the effect of erythrocytic stages and Hz on cytokine, chemokine and surface molecule expression in THP-1 cell line. The phenotypic switching of monocytes to M1/M2 has an indirect role in the activation of TH1 and TH2 response, thereby making it an important topic in immunomodulation and pathology in malaria. While the role of Hz is well established in immunosuppression, it’s involvement in the polarization of monocytes has not been studied. Hence, our second objective was to investigate the role of Hz in the modulation of adherent monocyte phenotype. β-hematin (sHz) is widely used as a structural analog of Hz. However, there are differences in immune responses induced by natural Hz and sHz. Hence, to understand the similarities and differences between their responses in monocytes, we determined the gene profiles of Hz and sHz- fed monocytes and it’s validation to assess the M1/ M2 related phenotypic characters.

Our experiments showed that Hz but not parasitic stages i.e. ring and trophozoites stimulated the production of inflammatory cytokines TNF-α, IL-1β, IL-6 in THP-1 cells. Moreover, only Hz- induced down-regulation of HLA-DR, CD11b, CD11c and CD54 in THP-1 cells. The downregulation of these surface molecules important in antigen presentation, adhesion and cytotoxic killing suggested suppression of immune responses. Since pigment containing monocytes persist for a long time in patients, they may affect immune response against other pathogens. A pro-inflammatory treatment of LPS and IFNγ reversed only the CD54 expression in Hz-fed THP-1 monocytes. Overall, Hz exhibited potent immune-modulatory effects in THP-1 cells in comparison with parasitic stages. In order to get clinically relevant and significant data, all further experiments were carried out with peripheral blood-derived monocytes and focused on unraveling the role of Hz in immunomodulation.

Peripheral blood-derived adherent monocytes exposed to Hz, elevated transcript and secreted level of IL-10, CCL17, CCL1, expression of mannose-binding lectin receptor
(CD206) and arginase activity, which are signatures of M2 phenotype. Hz attenuated HLA-DR expression, nitric oxide (NO) and reactive oxygen species (ROS) production, which are the features of M1 phenotype. This suggested that Hz induces M2-like phenotype and functional properties in human monocytes. Our data also implicated the involvement of p38-MAPK, PI3K/AKT and NF-κB signaling pathways in skewing of Hz-fed monocytes towards M2-like type and suppression of mitogen-stimulated lymphocyte proliferation. Importantly, antimalarial drugs- chloroquine (CHQ) and artemisinin (ART) partially reversed activation of Hz-induced monocytes towards M2-like phenotype. Considering the limitations in the current therapeutic options for malaria, we propose that these drugs may be re-examined for their potential as immunomodulators and candidates for adjunctive treatment in malaria. Further, it would be interesting to find immunomodulators which can reverse M2 phenotype and help in efficient immune response against parasite or aid the current therapy.

Gene profiling by microarray analysis was done for monocytes exposed to Hz, sHz and latex beads. Monocytes fed with inert latex beads as phagocytosis control showed the least number of differentially regulated genes. Furthermore, the differences in sHz and Hz were seen in terms of differential gene expression, functional processes and pathways in human monocytes. The data also revealed that Hz 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. In corroboration with these results, sHz does not inhibit lymphocyte proliferation, and on the contrary, enhanced mitogen-stimulated proliferation. Furthermore, unlike nHz, sHz induces NO and ROS production. sHz is widely used as an adjuvant in different infectious diseases on account of its ability to elevate antigen-specific immune responses. In corroboration with this, the considerable production of inflammatory cytokines IL-6, TNF-α, IL-1β, high chemotactic activity, lymphocyte proliferation, ROS and NO production and absence of IL-10 may advocate the use of sHz as an adjuvant for vaccines. At the same time, the current data also suggest the differential action of sHz in comparison with nHz on monocytes activation and function. Thus, we conclude that use of Hz for immune-related assays might give more relevant information than sHz in monocytes. Considering the fact that most antimalarials act by inhibiting hematin formation, the use of sHz for anti-malarial screening and as an adjuvant is still useful.