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Thesis Topic: Characterization and Conformational Studies of High Mobility Group Box (HMGB) Proteins of *Plasmodium falciparum*

Abstract

A combination of computational and molecular analyses is needed to increase our knowledge of transcriptional regulation of *Plasmodium* genes involved in crucial steps of asexual and sexual development. Our work describes the characterization of two *Plasmodium* HMGB factors that appear to exhibit substantial similarity to architectural factors as regards their biological functions, at least when analyzed in vitro for the capacity to interact with distorted DNA, even though their capacities to do so appeared to be quite different. Owing to significant homology between *P. falciparum* and other eukaryotic HMGBs, it can be surmise that Plasmodium HMGBs are also involved in chromatin remodeling. The two proteins (PfHMGB1 and PfHMGB2) were localized in the nucleus at ring stage, but also in the parasite cytosol during the later stages of erythrocytic cycle. We also found both PfHMGB1 and PfHMGB2 are potent inducers of two important mediators of inflammation, TNF-α and iNOS, suggesting that these proteins may have immunemodulatory role in the pathophysiology of *P. falciparum* infection. Recently, HMGB1 has been described as a possible amplification signal in the pathogenesis of malaria (Alleva *et al.*, 2005). Serum from *P. falciparum* malaria patients contained significantly more HMGB1 than did serum from healthy adults (Alleva *et al.*, 2005). Whether parasite's own HMGB, if released in the host's plasma, also contribute to the pathogenicity of the disease in the host, remains to be established. It will be interesting to investigate the presence of parasite's HMGB proteins in the host plasma and to determine if they will contribute to pathogenicity in malaria infection. Furthermore these findings support the need of elucidate the multi-faceted effects of *Plasmodium* parasites and its virulence factors in order to treat or prevent life threatening disease that is caused by this parasite.

The salient features of the work done are summarized below:

- Molecular cloning, expression and purification of PfHMGB1 and PfHMGB2 in *E.coli* expression system.
- Electrophoretic Mobility Shift Assay (EMSA) and Circular Dichroism (CD) spectra analysis showed secondary structure and DNA binding.
- PfHMGB1 and PfHMGB2 are antigenic and showed significant level of humoral and cellular responses.
- IFA and confocal microscopic studies showed that PfHMGB1 and PfHMGB2 were localized in the nucleus at ring stage, but also in the parasite cytosol during the later stages of erythrocytic cycle.
- Significant amounts of proinflammatory cytokines (TNF-α, IL-6, IL-8 and IL-1β) were found to be up-regulated by stimulated macrophage with PfHMGB1 and PfHMGB2. TNF-α was found to be ~10 times higher than the other proinflammatory cytokine.
- *In vivo* studies were also carried out with PfHMGBs and TNF-α mRNA expression, was strongly induced as early as 6 h after exposure of macrophages to PfHMGBs. iNOS mRNA expression was also unregulated with same tendency as TNF-α.
- Neutralization of antigen (PfHMGB1 and PfHMGB2) with anti-PfHMGB1 and anti-PfHMGb2 diminished the up-regulation of TNF-α by 60%.
- PbHMGB1 was cloned, expressed and purified to analyze the cytokine profile in a homologous system.
- *In vivo* studies were also carried out with PbHMGB1 and the TNF-α and iNOS were checked out at transcriptional level. TNF-α mRNA expression, was strongly induced as early as 6 h after exposure of macrophages to PfHMGBs. iNOS mRNA expression was also unregulated with same tendency as TNF-α.
- Neutralization of antigen (PbHMGB1) with anti-PbHMGB1 diminished the up-regulation of TNF-α.
- These results suggest that increase in the inflammatory response during the primary infection is control by various factors and not specifically related to release of HMGBs by the parasite.