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Hemozoin and the human monocyte-A brief review of their interactions

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Abstract. In vitro, human monocytes avidly ingest hemozoin (HZ) that modifies a number of monocyte functions. Inhibitory effects: inhibition of: PMA-elicited respiratory burst, ability to killing and repeat phagocytosis, activity of NADPH-oxidase and PKC, expression of ICAM-1, integrin-CD11c, MHC-class-II (IFN-gamma-mediated), differentiation to functional, antigen-presenting dendritic cells. Stimulatory effects: increase in phagocytosis-related respiratory burst and accumulation of lipoperoxidation products; induction of metalloproteinase-9 and pro-inflammatory cytokines and chemokines. Mechanism of action: HZ generates by non-enzymatic catalysis large amounts of lipoperoxidation products, such as monohydroxy derivatives of arachidonic (HETE) and linoleic (HODE) acid, and 4-hydroxynonenal (HNE). Several HZ effects were reproduced by supplementation with plausible concentrations of HETE or HNE, the first most likely via interaction with PPAR-receptors, the second via adduct or crosslinks formation with critical targets.

Key words: Malaria, malaria pigment, hemozoin, monocytes, HETE, 4-hydroxynonenal

Human phagocytic cells avidly ingest hemozoin (HZ) and HZ-containing trophozoites and schizonts. *In vitro*, approx. 9-10 trophozoites/schizonts, or corresponding amounts of HZ were taken up per monocyte. Three hours after start of phagocytosis 79±30% of monocytes were extensively HZ-laden, and approximately 30% of cell volume was occupied by HZ HZ (Schwarzer *et al.*, 2001; Arese and Schwarzer, 1997).

Inhibitory effects of HZ

1. Role of HZ phagocytosis in malaria immunodepression

Altered cellular responses to blood-stage *Plasmodium* antigens, reduced induction of immunity to vaccines, reduced T cell proliferation, and short-lived antibody responses are common observations in malaria. It has been shown by us that induction of MHC class II in response to IFN-gamma stimulation was defective in HZ-laden monocytes (Schwarzer *et al.*, 1998). Abrogation of MHC class II expression was present at protein and mRNA expression level, providing a possible link between HZ loading, suppression of IFN-gamma responsiveness, failure of MHC class II upregulation and disturbances in antigen presentation and immunodepression in malaria (Schwarzer *et al.*, 1998; Scorza *et al.*, 1999). 4-hydroxynonenal (HNE), a potent aldehyde originating from lipoperoxidation of unsaturated fatty acids (Schwarzer *et al.*, 2003), accumulates in membranes and may be causally involved in the effect. Indeed, unpublished experiments (Schwarzer, unpublished) show that low-micromolar HNE inhibited IFN-gamma mediated MHC class II expression and mimicked HZ action. The same studies indicated that HZ-laden monocytes had reduced spontaneous upregulation of CD54 (ICAM-1), an adhesion molecule that contributes considerably to the capacity of monocytes to adhere and stimulate T-cell proliferation (Schwarzer *et al.*, 1998). Thus, our data may contribute to explain defective T-cell response in malaria.

2. Inhibition of differentiation/maturation to DC

Monocytes are a prime source of dendritic cells (DC) *in vivo* and *in vitro*, that play pivotal roles in adaptive immune responses and innate immunity. We have challenged human monocytes before the initial induction/final maturation to mature DC with HZ. Blunted expression of MHC class II and costimulatory molecules indicated that both differentiation and maturation of HZ-loaded monocytes to DC were severely impaired (Skorokhod *et al.*, 2004). These effect were reproduced dose-dependently by HNE supplementation, possibily via stimulation of PPAR-gamma receptor or interaction with CD14/LPS-receptor. Those studies may be significant in malaria immunodepression to explain inhibited response of T and B lymphocytes; reduction in expression of MHC class II; and insufficient antibody production. Recently in confirmatory studies HZ was found to induce failure of DC function in vivo and in vitro in a *P. chabaudi* murine model (Millington *et al.*, 2006). Contrasting results were obtained with highly purified HZ, though, shown to induce DC maturation and activation of murine DC via Toll-like receptor 9 (Coban *et al.*, 2005; Coban *et al.*, 2002).

3. Inhibition of erythropoiesis and thrombopoiesis

Severe malarial anemia, an important cause of mortality, is the result of destruction of parasitized and non-parasitized RBC, and impaired erythropoiesis. Bone-marrow (BM) macrophages produce a variety of

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hematopoietic regulatory or suppressive factors, such as IL-1, TNF, TGF-beta and macrophageinhibitory proteins. Free HZ and HZ-containing trophozoites/schizonts, and HZ-laden macrophages are abundantly present in BM of malaria patients (Arese and Schwarzer, 1997). We have shown that HZ supernatants equivalent to 12.5 trophozoites/progenitor inhibited erythroid growth. Supernatant of delipidized HZ was significantly less effective. Supernatants of HZ-fed monocytes also inhibited BFUE growth whereas supernatants of latex-fed or RBC-fed monocytes had no effect (Giribaldi *et al.*, 2004). Inhibition of erythroid growth and thrombopoiesis was reproduced dose-dependently by HNE supplementation, found to generate adducts with crucial GM-CSF-receptor (Skorokhod *et al.*, 2004).

Stimulatory effects of HZ

1. Stimulation of production of pro-inflammatory molecules

Elevated serum concentrations of pro-inflammatory cytokines, MIP-1alpha and macrophage migration inhibitory factor (MIF) have been found in malaria patients, correlated with disease severity. Several *in vitro* studies have shown that phagocytosis of HZ by human monocytes induced release of several of the above factors. Those data confirm the importance of HZ as a stimulatory factor of monocytes in malaria. Preliminary data by our group (Giribaldi G, unpublished) have shown cytokine and MIP-1alpha upregulation by 15-HETE.

2. Activation of metallo-proteinase 9

It has been recently shown in our group (Prato *et al.*, 2005) that HZ-fed human monocytes displayed increased metalloproteinase-9 (MMP-9) activity and protein/mRNA expression. MMP-9 functions by proteolytically shedding pro-forms of cytokines such as TNF-alpha and IL-1beta in the blood, by disrupting the subendothelial matrix and enhancing extravasation of blood cells. Activation and induction of MMP-9 were reproduced dose-dependently by 15-HETE (Prato M, unpublished).

Mechanism of HZ action

In HZ and parasitized RBC a complex mixture of monohydroxy derivatives of arachidonic (HETE) and linoleic (HODE) acid, and large amounts of the terminal aldehyde HNE have been determined by our group (Schwarzer *et al.*, 2003). No evidence of lipoxygenase activity was found in parasites, while the large number of isomers, their racemic structure and generation by incubation of arachidonic acid with HZ indicated their non-enzymatic origin *via* hemecatalysis (Schwarzer *et al.*, 2003). Phagocytosed HZ ferries those lipid derivatives into the phagocyte, while ingested HZ further produces the same compounds (Schwarzer *et al.*, 2003). Mechanistically, we have provided evidence that specific HETE, HODE or HNE generated by HZ were responsible for the abrogation of oxidative burst and other inhibitory effects mediated by HZ phagocytosis (see above). HNE, which avidly reacts with thiols and amino groups of proteins to form stable Michael adducts or Schiff base crosslinks (Skorokhod *et al.*, 2005), seems to play an important mechanistic role. Work in progress will determine in detail localization of protein-HNE adducts in the various HZ-affected systems.

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