Peroxisome Proliferator-Activated Receptor γ and Retinoid X Receptor Agonists Have Minimal Effects on the Interaction of Endothelial Cells with *Plasmodium falciparum*-Infected Erythrocytes

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Peroxisome proliferator-activated receptor γ -retinoid X receptor (PPAR γ -RXR) agonists had minimal effects on the surface levels of CD36, intercellular cell adhesion molecule-1, or platelet-endothelial cell adhesion molecule-1 and had no effect on the cytoadherence of infected erythrocytes to either human umbilical vein endothelial cells or human microvascular endothelial cells or on malaria-induced interleukin-6 secretion from these cells. PPAR γ -RXR agonists do not significantly modify malaria-infected erythrocyte-endothelial cell interactions in vitro.

Severe *Plasmodium falciparum* malaria is associated with high parasite burdens, excessive inflammatory responses to malaria glycosylphosphatidylinositol (GPI) (5, 13), and the sequestration of infected erythrocytes (IEs) in vital organs (9). Many endothelial cell receptors have been implicated in sequestration including CD36, intercellular cell adhesion molecule-1 (ICAM-1), and platelet-endothelial cell adhesion molecule-1 (PECAM-1) (6). Almost all IEs adhere to CD36. CD36 is expressed on endothelial cells but also on monocytes/macrophages, where it has been implicated in the innate clearance of IEs (8). These observations suggest that malaria-CD36 interactions may represent coevolutionary adaptations that limit inflammatory responses that are deleterious to both the parasite and the host and have led to the hypothesis that increasing monocytes/macrophage CD36 may result in increased parasite clearance and reduced malaria immunopathology (16). CD36 is transcriptionally regulated by the nuclear receptor peroxisome proliferator-activated receptor γ -retinoid X receptor (PPAR γ -RXR) (18). PPAR γ -RXR agonists have been shown to increase CD36-mediated phagocytosis of IEs (12, 14, 15, 17) and to reduce GPI-induced tumor necrosis factor alpha (TNF- α) production (14, 15) and have been proposed as potential therapeutics for malaria. However, before this can be explored it is important to investigate their effects on endothelial cell-parasite interactions. Furthermore, since this class of compounds is in common use, it is important to understand their effects on host-malaria interactions. Here we examine the effects of PPAR γ -RXR agonists on the expression of CD36, ICAM-1, and PECAM-1, IE cytoadherence, and GPI-induced cytokine response by using human umbilical vein endothelial

cells (HUVECs) and human microvascular endothelial cells (MDECs).

We used flow cytometry to examine the effects of 48-h treatment with the PPAR γ agonist troglitazone and 9-*cis*-retinoic acid, a vitamin A metabolite and RXR agonist, on the expression of CD36, ICAM-1, and PECAM-1 on HUVECs and MDECs (Clonetics) with the monocytic cell line U937 as a control. PPAR γ agonists have been shown to increase CD36 expression in monocytic cells (10, 14, 15, 18), while their impact on ICAM-1 expression varies according to cell type (1, 2). 9-*cis*-Retinoic acid, as expected, significantly increased CD36 levels on U937 cells (Fig. 1A); however, it did not significantly modulate the level of CD36, ICAM-1, or PECAM-1 on either MDECs or HUVECs (Fig. 1 to 3). Troglitazone increased CD36 levels on U937 cells (Fig. 1A) as previously reported (10, 14, 15, 18), but CD36, ICAM-1, and PECAM-1 levels on MDECs were unaffected (Fig. 1 to 3). Although troglitazone did not affect CD36 levels on HUVECs, it did cause a significant increase in both ICAM-1 and PECAM-1 levels (Fig. 1 to 3).

Since severe malaria is associated with elevated levels of TNF- α that may upregulate cell adhesion molecules, we also examined concomitant treatment of endothelial cells with PPAR γ -RXR agonists and TNF- α . TNF- α did not significantly alter CD36 levels nor the effect of PPAR γ -RXR agonists in any of the cell types (Fig. 1D to F). TNF- α caused an increase in ICAM-1 expression on MDECs, HUVECs, and U937s (Fig. 2D to F) (7). Although troglitazone significantly enhanced ICAM-1 and PECAM-1 expression in TNF- α -treated MDECs (Fig. 2E and 3C), it inhibited expression in HUVECs (Fig. 2F and 3D). It has been reported that troglitazone has a dual action on ICAM-1 expression in HUVECs (1). In the absence of cytokine stimulation, troglitazone enhanced ICAM-1 expression, while in the presence of TNF- α , troglitazone caused a decrease in ICAM-1 (1, 3, 19). Troglitazone has also been shown to inhibit TNF- α -stimulated vascular cell adhesion mol-

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FIG. 1. PPAR γ -RXR agonists and CD36 surface expression. Illustrated are CD36 surface levels on U937, MDECs, and HUVECs analyzed by flow cytometry at 48 h posttreatment. Fluorescence intensity is normalized (to a value of 100) to the dimethyl sulfoxide (DMSO)-treated (control) cells in panels A to C and to the TNF- α -treated cells in panels D to F. CD36 levels on U937 (A and D), MDECs (B and E), and HUVECs (C and F) are shown. DMSO-treated cells are shown in black, 9-*cis*-retinoic acid (9RA; 1 μ M)-treated cells in dark grey, troglitazone (TRO; 25 μ M)-treated cells in light grey, and TNF- α (10 ng/ml)-treated cells in hatched bars. The mean fluorescence intensity with standard deviations for the DMSO-treated cells is shown numerically. The bars represent normalized mean fluorescence intensity with standard deviations for at least three independent experiments. *, $P < 0.05$; **, $P < 0.01$; versus DMSO (A-C) and versus TNF- α (D-F), by analysis of variance with post-hoc Tukey's.

FIG. 3. PPAR γ -RXR agonists and PECAM-1 surface expression. Illustrated are PECAM-1 levels analyzed by flow cytometry at 48 h posttreatment. Fluorescent intensity is normalized (to a value of 100) to the dimethyl sulfoxide (DMSO)-treated cells (control) in panels A to B and to the TNF- α -treated cells in panels C to D. PECAM-1 levels on MDECs (A and C) and HUVECs (B and D) are shown. DMSO (control)-treated cells are shown in black, 9-*cis*-retinoic acid (9-RA; 1 μ M) in dark grey, troglitazone (TRO; 25 μ M) in light grey, and TNF- α (10 ng/ml) in hatched bars. The mean fluorescence intensity with standard deviations for the DMSO-treated cells is shown numerically. The bars represent normalized mean fluorescence intensity with standard deviations for at least three independent experiments. *, $P < 0.05$; **, $P < 0.01$; versus DMSO (A-B) and versus TNF- α (C-D) by analysis of variance with post-hoc Tukey's.

ecule-1 and E-selectin in HUVECs (3, 11) but not PECAM-1 as observed here.

We have previously reported that PPAR γ -RXR agonists enhance monocyte/macrophage uptake of nonopsonized IEs. However, the potential benefits of enhanced clearance may be limited if PPAR γ -RXR agonists also cause a significant increase in endothelial cell cytoadherence. We investigated the

FIG. 4. PPAR γ -RXR agonists and cytoadherence of *P. falciparum*-infected erythrocytes to endothelial cells. Shown is cytoadherence to endothelial cells treated with 9-*cis*-retinoic acid (9-RA; 1 μ M), troglitazone (TRO; 25 μ M), or control (dimethyl sulfoxide [DMSO]) for 48 h, followed by formalin fixation and exposure to 100,000 purified trophozoites of the clone ITG. The data are normalized (to a value of 100) to the DMSO-treated cells and are the means with standard deviations of three independent experiments performed in duplicate. The mean number of infected erythrocytes adhering per field with standard deviations is shown numerically. Cytoadherence to MDECs (A) and HUVECs (B) is shown. The black bars represent cells cotreated with TNF- α (10 ng/ml), the grey bars represent cells not exposed to TNF- α . The hatched bars represent either CD36 (diagonal) or ICAM-1 (horizontal) monoclonal antibody blockade (10 μ g/ml). *, $P < 0.05$; **, $P < 0.01$; control versus antibody blockade. #, $P < 0.05$; ##, $P < 0.01$; DMSO versus 9RA or TRO treated. Cotreatment with TNF- α and 9-*cis*-retinoic acid or troglitazone decreased cytoadherence, although not significantly. The data were analyzed by analysis of variance with post-hoc Tukey's.

FIG. 2. PPAR γ -RXR agonists and ICAM-1 surface expression. Illustrated are ICAM-1 levels analyzed by flow cytometry at 48 h posttreatment. Fluorescent intensity is normalized (to a value of 100) to the dimethyl sulfoxide (DMSO)-treated cells (control) in panels A to C and to the TNF- α -treated cells in panels D to F. ICAM-1 levels on U937 (A and D), MDECs (B and E), and HUVECs (C and F) are shown. DMSO (control)-treated cells are shown in black, 9-*cis*-retinoic acid (9RA; 1 μ M) in dark grey, troglitazone (TRO; 25 μ M) in light grey, and TNF- α (10 ng/ml) in hatched bars. The mean fluorescence intensity with standard deviations for the DMSO-treated cells is shown numerically. The bars represent normalized mean fluorescence intensity with standard deviations for at least three independent experiments. **, $P < 0.01$; versus DMSO (A-C) and versus TNF- α (D-F). #, $P < 0.05$; ##, $P < 0.01$; TNF- α versus DMSO treated, by analysis of variance with post-hoc Tukey's.

FIG. 5. PPAR γ -RXR agonists and IL-6 production by endothelial cells. IL-6 levels in picograms per milliliter produced by MDECs (A-C) or HUVECs (D-F) exposed to LPS (1 μ g/ml), PMA (100 nM), or *P. falciparum* culture supernatants (GPI; 100 μ l/ml) are shown. IL-6 levels were assessed by enzyme-linked immunosorbent assay. MDECs and HUVECs were treated with either troglitazone (25 μ M; TRO), 9-*cis*-retinoic acid (1 μ M; 9RA), or dimethyl sulfoxide (DMSO) as a control. Shown are means with standard errors of the means of at least two independent experiments. *, $P < 0.05$; **, $P < 0.01$; versus DMSO, by analysis of variance with post-hoc Tukey's.

effect of 48-h troglitazone, 9-*cis*-retinoic acid, or control treatment, with or without concomitant TNF- α treatment, on IE cytoadherence to MDECs and HUVECs, as previously described (20). Both MDECs and HUVECs supported the cytoadherence of IEs, although MDECs, which express CD36, did so more avidly. Cytoadherence to control-treated MDECs was significantly inhibited by both CD36 and ICAM-1 antibody blockade (Fig. 4A). Cotreatment with TNF- α and 9-*cis*-retinoic acid or troglitazone did not significantly increase cytoadherence to MDECs.

Cytoadherence to HUVECs was significantly enhanced by troglitazone but not by 9-*cis*-retinoic acid. Cytoadherence was significantly inhibited only by antibody blockade of ICAM-1 (Fig. 4B). TNF- α treatment caused a significant increase in cytoadherence to HUVECs, correlating with the increase seen in ICAM-1 levels.

PPAR γ -RXR agonists have been shown to have anti-inflammatory properties (4), and we previously demonstrated that they can inhibit GPI-induced TNF- α secretion from monocytes (14, 15). We next investigated whether PPAR γ -RXR agonists modulate endothelial cell-secreted cytokines in response to GPI, as previously described (14, 15).

Lipopolysaccharide (LPS), phorbol myristate acetate

(PMA), and malaria GPI induced interleukin-6 (IL-6) production from MDECs and HUVECs, although with differing kinetics (Fig. 5). 9-*cis*-Retinoic acid decreased PMA-induced IL-6 in both MDECs and HUVECs and LPS-induced IL-6 in HUVECs. Troglitazone caused a small decrease in LPS-induced IL-6 in MDECs and an increase in PMA-induced IL-6 at the early time points in HUVECs. In contrast, GPI-induced IL-6 was unaffected by either 9-*cis*-retinoic acid or troglitazone treatment of either cell type (Fig. 5C and F). Thus, PPAR γ -RXR agonists appeared to have little influence on the malaria-stimulated IL-6 response from endothelial cells.

Both troglitazone and 9-*cis*-retinoic acid have marked effects on monocyte/macrophage-parasite interactions. They significantly increase CD36-mediated IE phagocytosis and cause a significant reduction in malaria-induced TNF- α (14, 15). These properties might be expected to have beneficial effects on malaria pathophysiology if they result in enhanced parasite clearance and reduced proinflammatory responses to infection. However, it is important to assess their effects beyond monocytic cells. Our data suggest that PPAR γ -RXR agonists have minimal effects on endothelial cell-parasite interactions. 9-*cis*-Retinoic acid does not appear to influence endothelial cell-parasite interactions, including cytoadherence and malaria-in-

duced cytokine production. Troglitazone only has minor effects and these were seen primarily with HUVECs. Although HUVECs are widely used in in vitro studies, their in vivo relevance is unclear, since cytoadherence to these cells is not a component of malaria pathology in vivo. MDECs are a more relevant cell type, and cytoadherence to these cells appears to be unaffected by troglitazone or 9-*cis*-retinoic acid. This is encouraging, as it implies that PPAR γ -RXR-mediated enhancement of monocyte/macrophage phagocytosis and modulation of malaria-induced inflammatory responses may not be offset by negatively modulating endothelial cell-parasite interactions. However, an important caveat is that our study did not examine brain endothelium and that additional examination of endothelial cells from a variety of organs may be informative.

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