The Journal of Immunology

RESEARCH ARTICLE | JUNE 01 2001

Peroxisome Proliferator-Activated Receptor γ-Retinoid X Receptor Agonists Increase CD36-Dependent Phagocytosis of *Plasmodium falciparum*-Parasitized Erythrocytes and Decrease Malaria-Induced TNF-α Secretion by Monocytes/Macrophages [1] FREE

Lena Serghides; ... et. al

J Immunol (2001) 166 (11): 6742–6748.
https://doi.org/10.4049/jimmunol.166.11.6742

Peroxisome Proliferator-Activated Receptor γ-Retinoid X Receptor Agonists Increase CD36-Dependent Phagocytosis of *Plasmodium falciparum*-Parasitized Erythrocytes and Decrease Malaria-Induced TNF-α Secretion by Monocytes/Macrophages[1]

Lena Serghides* and Kevin C. Kain[2]*†

Severe and fatal malaria is associated with the failure of host defenses to control parasite replication, excessive secretion of proinflammatory cytokines such as TNF-α, and sequestration of parasitized erythrocytes (PEs) in vital organs. The identification of CD36 as a major sequestration receptor has led to the assumption that it contributes to the pathophysiology of severe malaria and has prompted the development of antiadherence therapies to disrupt the CD36-PE interaction. This concept has been challenged by unexpected evidence that individuals deficient in CD36 are more susceptible to severe and cerebral malaria. In this study, we demonstrate that CD36 is the major receptor mediating nonopsonic phagocytosis of PEs by macrophages, a clearance mechanism of potential importance in nonimmune hosts at the greatest risk of severe malaria. CD36-mediated uptake of PEs occurs via a novel pathway that does not involve thrombospondin, the vitronectin receptor, or phosphatidylserine recognition. Furthermore, we show that proliferator-activated receptor γ-retinoid X receptor agonists induce an increase in CD36-mediated phagocytosis and a decrease in parasite-induced TNF-α secretion. Specific up-regulation of monocyte/macrophage CD36 may represent a novel therapeutic strategy to prevent or treat severe malaria. *The Journal of Immunology,* **2001, 166: 6742–6748.**

Young children and other nonimmune individuals are at the greatest risk of developing severe and complicated malaria leading to death. Despite decades of research, no specific treatments have been identified to prevent or improve the outcome of patients with cerebral or severe malaria (1). The central pathophysiologic events in severe *Plasmodium falciparum* malaria are the inability of the host defenses to control parasite replication, cytokine imbalance resulting in the excessive release of proinflammatory cytokines in response to infection, and the sequestration of parasitized erythrocytes (PEs)[3] in the microvasculature of vital organs (2–6). Several receptors have been implicated in the cytoadherence of PEs to vascular endothelium, including ICAM-1 (7–8), VCAM-1 (9–10), E-selectin (10), platelet/endothelial cell adhesion molecule-1/CD31 (11), chondroitin-4-sulfate (12), hyaluronic acid (13), thrombospondin (TSP) (14), $\alpha_v\beta_3$ (15), and CD36 (16–20).

CD36, an 88-kDa integral protein found on endothelial cells, adipocytes, platelets, monocytes, and macrophages (mϕs), has been shown to be a receptor preferentially recognized by most natural isolates of *P. falciparum* (7, 9, 21). The identification of CD36 as a major sequestration receptor has led to the assumption that it contributes to the pathophysiology of severe malaria and has prompted the development of antiadherence therapies to disrupt the CD36-PE interaction (7, 18, 22–23). However, its role in cerebral and severe malaria is unclear because little CD36 is expressed on cerebral microvasculature endothelial cells (5, 24), and studies have reported that significantly higher binding of PEs to CD36 occurs in cases of nonsevere malaria (25, 26). Furthermore, individuals deficient in CD36 were found to be more susceptible to severe and cerebral malaria (27). However, the mechanism by which CD36 may confer protection from severe disease is unknown.

In contrast to CD36, other adhesion receptors such as ICAM-1 are expressed in cerebral endothelial cells and may be up-regulated by inflammatory cytokines such as TNF-α (6, 8, 28). Elevated levels of proinflammatory cytokines such as TNF-α and IL-6 have been associated with severe and fatal malaria (8, 29–33). Furthermore, a genetic predisposition to overproduce TNF-α in response to infection has been proposed as a mechanism underlying the development of cerebral malaria (34–36). Collectively, these data suggest that the sequestration of PEs observed in cerebral malaria may result from up-regulated ICAM-1 and other adhesion molecules on the cerebral microvasculature due to excessive or unbalanced proinflammatory responses (6, 33, 34, 36).

Phagocytic cells are an essential first line of innate defense against malaria, facilitating control and resolution of infection by clearing PEs (37, 38). However, the molecular mechanisms by which these cells recognize PEs are not well understood. Most studies have focused on the phagocytosis of Ab-opsonized PEs

*Department of Medicine, University of Toronto, Toronto, Ontario, Canada; and †Tropical Disease Unit, Toronto General Hospital, Toronto, Ontario, Canada

Received for publication January 30, 2001. Accepted for publication March 19, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by the Medical Research Council of Canada (MT-13721), the Heart and Stroke Foundation of Canada (NA-3391), and the Ontario HIV Trials Network. L.S. is the recipient of a Medical Research Council of Canada Studentship. K.C.K. is supported by a Career Scientist Award from the Ontario Ministry of Health.

[2] Address correspondence and reprint requests to Dr. Kevin C. Kain, Tropical Disease Unit, EN G-224, Toronto General Hospital, 200 Elizabeth Street, Toronto, Ontario, Canada M5G 2C4. E-mail address: kevin.kain@uhn.on.ca

[3] Abbreviations used in this paper: PE, parasitized erythrocyte; TSP, thrombospondin; mϕ, macrophage; PPARγ, peroxisome proliferator-activated receptor γ; RXR, retinoid X receptor; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; RA, retinoic acid; MA, methoprene acid; 15d-PGJ$_2$, 15-deoxy-$\Delta^{12,14}$-PGJ$_2$.

0022-1767/01/$02.00

(39–41). However, it is uncertain what role opsonic clearance plays in the nonimmune individuals most at risk for severe and cerebral malaria. Phagocytes of the monocyte/Mϕ lineage are also the primary source of parasite-induced proinflammatory cytokine responses that have been linked to adverse clinical outcomes (6, 29–31). We have recently shown that CD36 mediates the uptake of nonopsonized PEs by human monocytes (42). Furthermore, activation of CD36 on monocytes, either by cross-linking the receptor or during nonopsonic phagocytosis, does not induce the release of the proinflammatory cytokine TNF-α (42).

Based on these observations, we hypothesized that monocytes/Mϕ CD36 may play a beneficial role during infection by mediating nonopsonic clearance of PEs and by not contributing to the release of proinflammatory cytokines associated with poor clinical outcomes. Furthermore, we hypothesized that clinical malaria might benefit from specific up-regulation of monocyte/Mϕ CD36, particularly in the nonimmune host, where opsonic phagocytosis would be expected to be less. To test this hypothesis, we have investigated the effect of up-regulating CD36 on the phagocytosis of nonopsonized PEs by aging monocytes in culture and by treating monocytes and Mϕs with peroxisome proliferator-activated receptor γ (PPARγ)-retinoid X receptor (RXR) agonists. The CD36 promoter contains a PPARγ-RXR binding site, and the PPARγ-RXR complex can modulate CD36 gene expression through direct promoter interaction (43). In addition, PPARγ-RXR agonists have anti-inflammatory properties; down-regulating LPS and PMA induced proinflammatory cytokine secretion (44, 45). If the anti-inflammatory effects of PPARγ-RXR agonists can be extended to malaria-induced inflammation, then these compounds may be beneficial in decreasing the excessive or unbalanced secretion of proinflammatory cytokines implicated in severe malaria (46). We present evidence that PPARγ-RXR agonist treatment results in an increase in CD36-mediated phagocytosis and a decrease in parasite-induced TNF-α secretion.

Materials and Methods

Reagents and parasite culture

Endotoxin-free RPMI 1640 culture medium was obtained from Life Technologies (Burlington, Canada). FCS was obtained from Wisent (Mississauga, Canada) and was heat inactivated at 55°C for 30 min before use. The anti-CD36 mAb FA6-152 was obtained from Immunotech (Marseille, France), the anti-$\alpha_v\beta_3$ mAb 23C6 from Serotec (Raleigh, NC), the anti-TSP mAb C6.7 from Medicorp (Montreal, Canada), and the anti-ICAM-1 mAb 15.2 from Santa Cruz Biotechnology (Balthesa, CA). Recombinant human TNF-α and monoclonal anti-human TNF-α were obtained from Genzyme (Mississauga, Canada). Ciglitazone, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2), and methoprene acid (MA) were obtained from Biomol (Plymouth Meeting, PA). PMA, DMSO, 9-*cis*-retinoic acid (RA), saline, trypsin, and sterile water were obtained from Sigma-Aldrich (Oakville, Canada). Ficoll-Paque, Percoll, and dextran T500 were obtained from Pharmacia (Peapack, NJ). Human IgG Fc fragments were obtained from Calbiochem (San Diego, CA). Lipids were purchased from Avanti Polar Lipids (Alabaster, AL).

Liposomes were prepared as described (47). Phosphatidylcholine (PC) liposomes were made at 100 mol % PC. Phosphatidylinositol (PI) and phosphatidylserine (PS) liposomes were made at 70 mol % PC and 30 mol % PS or PI.

Parasite cultures were maintained and synchronized as described (48–50). Parasites from two patient lines (P1 and P2) and the laboratory clone ItG were used. Parasite cultures were treated with mycoplasma removal agent (ICN Pharmaceuticals, Costa Mesa, CA) and tested negative for mycoplasma by PCR analysis before use. Culture supernatants were collected from parasite lines, aliquoted, and frozen for subsequent use.

Monocyte isolation and culture

Venus blood was collected from healthy volunteers, and monocytes were isolated as described (42). This procedure yields a platelet-free population of nonactivated monocytes that are >80% CD14 positive by flow cytometry, minimal baseline TNF-α secretion, and >98% viability by trypan blue exclusion. In some experiments, purified monocytes were adhered to glass coverslips in 12-well polystyrene plates (250,000 monocytes/well). Nonadherent cells were washed away, and the monocytes were cultured in RPMI 1640 with L-glutamine, HEPES, and 10% heat-inactivated FCS (RPMI 10) at 37°C and 5% CO_2. Monocytes, aged in culture for 5 days to derive Mϕs, were assayed for phagocytic ability or CD36 expression.

Detection of CD36 by flow cytometry

Human monocytes, THP-1 (a human monocytic cell line), and WR-1 cells (a transformed brain endothelial cell line; the kind gift of Dr. C. Ockenhouse, Walter Reed Army Institute of Research, Washington, DC) subjected to various treatments were stained with 1:100 dilution of the mAb FA6-152 (anti-CD36) for 30 min on ice followed by a 1:50 dilution of a secondary anti-mouse IgG-FITC conjugated Ab. An unstained control and secondary Ab-only stained controls were also performed. The monocytes were fixed in 1% paraformaldehyde/PBS and analyzed using the Epics ELITE flow cytometer and software (Beckman Coulter, Marseille, France).

Up-regulation of CD36 using PPARγ-RXR agonists and phagocytosis assay

Purified human monocytes or culture-derived Mϕs, plated on round glass coverslips in 12-well polystyrene plates (250,000 monocytes/well), were exposed to RPMI 10 containing 5 μM 15d-PGJ_2 plus 30 μM MA, 5 μM 15d-PGJ_2 plus 1 μM 9-*cis*-RA, ciglitazone (3–30 μM), or appropriate concentrations of DMSO as a control and incubated at 37°C 5% CO_2 for 22–24 h. THP-1 cells were treated in suspension.

The phagocytosis assay was performed as previously described (42). The monocytes were washed and incubated with 20 μg/ml Fc fragments for 30 min at room temperature (to block Fc receptors), followed by a 30-min incubation with 10 μg/ml of various mAbs or 0.1 mM of liposomes where appropriate. The monocytes were washed, and 500 μl of 2% hematocrit and 5–8% parasitemia of carefully synchronized trophozoite-stage parasites in RPMI 10 were layered on top. The final PE:monocyte/Mϕ ratio was 20:1. The assay was allowed to continue for 4 h at 37°C with gentle rotation. After 4 h, the coverslips were subjected to hypotonic lysis in ice-cold water for 30 s to remove nonphagocytosed PEs and were fixed and stained with Giemsa. The number of monocytes/Mϕs with fully internalized PEs was quantitated microscopically. At least 400 monocytes/Mϕs were counted per coverslip. Phagocytic index was defined as the percentage of monocytes/Mϕs with at least one internalized PE multiplied by the average number of PEs per monocyte/Mϕ for that coverslip (51).

TNF-α assay

Purified human monocytes were seeded in 24-well polystyrene plates (200,000 cells/well) and allowed to adhere for 1 h. Unattached cells were washed away, and the remaining cells were treated with PPARγ-RXR agonists or DMSO controls as described above, followed by the addition of 50 nM PMA, 1:10 dilution of various *P. falciparum* culture supernatants (mycoplasma free), or no additions. Following an 18- to 24-h incubation at 37°C, the supernatants were collected, cleared by centrifugation, and assayed for TNF-α using a sandwich ELISA (52).

TNF-α assays were also performed as described above using THP-1 cells kept in suspension at a concentration of 500,000 cells/ml.

Statistical analysis

All experiments were performed in duplicate or triplicate and repeated at least three times. There was some variation between experiments due to the use of different monocyte donors and parasite cultures. However, within each experiment, the results were consistent. Data are represented as mean $\pm$ SD, unless otherwise noted. Statistical significance was determined using the Student's *t* test.

Results

Culture-derived Mϕs express more CD36 and have increased phagocytic capacity for nonopsonized PEs

We have recently reported that CD36 is the major receptor on freshly isolated human monocytes mediating the uptake of nonopsonized PEs (42). However, our observations with freshly explanted monocytes may underestimate the potential for nonopsonic clearance of PEs in vivo. Tissue-resident Mϕs in the liver, spleen, and reticuloendothelial system that mediate the uptake of PEs would be expected to behave more like culture-derived Mϕs, which have been reported to express increased levels of CD36

(53). We investigated the effect of culture maturation of monocytes on CD36 expression and phagocytic capacity for nonopsonized PEs. CD36 surface levels increased on monocytes aged in culture for 5 days (Fig. 1*a*). In association with increased CD36 levels, the phagocytosis of nonopsonized PEs increased ~4-fold ($p < 0.01$; Fig. 1*b*). Phagocytosis occurred in a complement-free environment with Fc receptor blockade and no prior opsonization of PEs. The mAb blockade of CD36 (FA6-152; 10 μg/ml) resulted in a 50–70% inhibition of phagocytosis in both day 0 and day 5 monocytes ($p < 0.01$; Fig. 1*b*). There was no phagocytosis of uninfected erythrocytes by monocytes or culture-derived Mϕs.

Mϕ phagocytosis of nonopsonized PEs is a CD36-specific process

CD36 participates in cooperation with $\alpha_v\beta_3$ and TSP in the phagocytic removal of apoptotic cells (54–55). We investigated whether these or other recognized PE receptors such as ICAM-1 contributed to nonopsonic phagocytosis of PEs by culture-derived Mϕs. Receptor blockade of $\alpha_v\beta_3$ or TSP on culture-derived Mϕs using mAbs (23C6 and C6.7, respectively) (54–55) did not decrease PE phagocytosis (Fig. 2*a*). Blocking $\alpha_v\beta_3$, TSP, and CD36 in combination resulted in a decrease in PE phagocytosis similar to that observed with CD36 blockade alone (Fig. 2*a*). Receptor blockade of ICAM-1, an important sequestration receptor for PEs expressed on both endothelial cells and monocytes/Mϕs, had no significant inhibitory effect on PE phagocytosis (Fig. 2*a*).

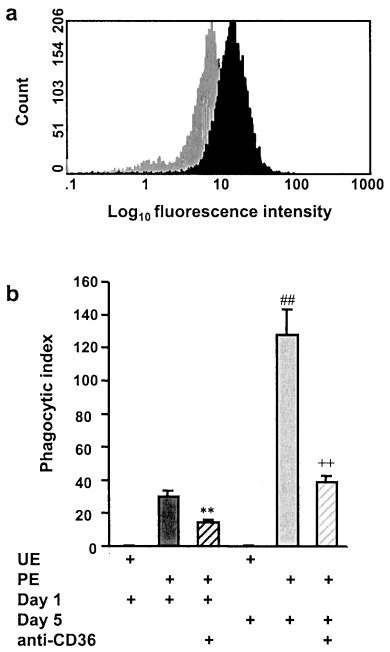

FIGURE 1. Culture-derived Mϕs express more CD36 and have increased phagocytic capacity. *a*, The CD36 mean $\log_{10}$ fluorescence of freshly isolated monocytes is shown in gray, and that for monocytes aged in culture for 5 days is shown in black. The flow cytometry histograms shown are typical of four independent experiments. Secondary-only stained controls were equivalent for both fresh and day-5 monocytes. *b*, Nonopsonized PEs were incubated with adhered, Fc receptor-blocked, freshly isolated monocytes and day-5 culture-derived Mϕs. Where indicated (▨), CD36 was blocked by preincubation with 10 μg/ml FA6-152 (anti-CD36 mAb). Phagocytic assays were performed as described in the *Materials and Methods*. Phagocytic index is defined as the percentage of monocytes/Mϕs with at least one internalized PE multiplied by the average number of internalized PEs per monocytes/Mϕ. There was no phagocytosis of uninfected erythrocytes. Experiments were performed in duplicate or triplicate and repeated at least three times. **, $p < 0.01$, day 1 vs day 1 plus CD36 blockade; ##, $p < 0.01$, day 5 vs day 1; ++, $p < 0.01$, day 5 vs day 5 plus CD36 blockade (all by Student's *t* test, $n = 9$/group).

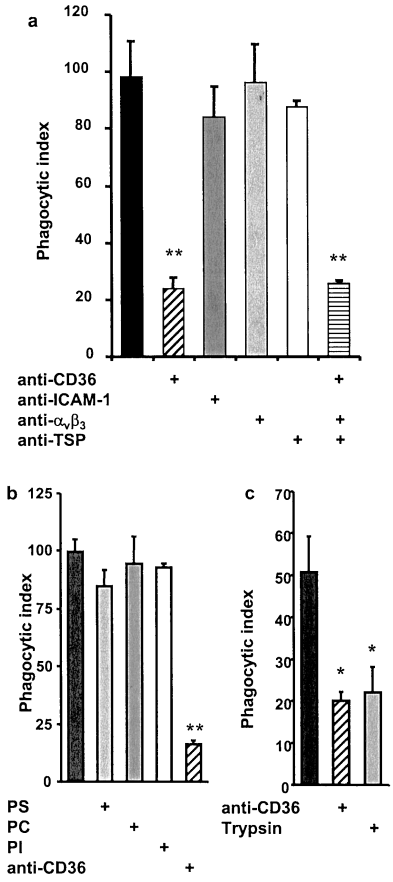

FIGURE 2. Phagocytosis of nonopsonized PEs by culture-derived Mϕs is a CD36-specific process. *a*, Fc receptor-blocked culture-derived Mϕs were pretreated for 30 min with 10 μg/ml of the mAbs FA6-152 (anti-CD36), 15.2 (anti-ICAM-1), 23C6 (anti-$\alpha_v\beta_3$), C6.7 (anti-TSP), or a combination of FA6-152, 23C6, and C6.7. Receptor blockade was followed by a standard phagocytosis assay (see *Materials and Methods*). **, $p < 0.01$ (Student's *t* test, $n = 3$/group), control vs FA6-152-treated and control vs FA6-152 plus 23C6 plus C6.7-treated. *b*, Fc receptor-blocked culture-derived Mϕs were pretreated for 30 min with 0.1 mM PS-containing liposomes, PC-containing liposomes, PI-containing liposomes, or with 10 μg/ml of mAb FA6-152 (anti-CD36) before exposure to nonopsonized PEs (see *Materials and Methods*). **, $p < 0.01$ (Student's *t* test, $n = 3$/group), control vs FA6-152-treated Mϕs. *c*, Fc receptor-blocked culture-derived Mϕs were exposed to either nonopsonized PEs or to nonopsonized PEs that were trypsin treated for 30 min. *, $p < 0.05$ (Student's *t* test, $n = 3$/group), control vs trypsinized PEs and control vs FA6-152 (anti-CD36)-treated. The above were performed in duplicate or triplicate and are representative of data collected in at least three independent experiments.

CD36 has been shown to interact with PS, and PS-containing liposomes have been reported to inhibit CD36-mediated apoptotic cell phagocytosis (51). To investigate the possible involvement of PS in the phagocytic uptake of nonopsonised PEs, we exposed culture-derived Mϕs to PS-containing liposomes before phagocytosis. PS-containing liposomes had no inhibitory effect on nonopsonic PE phagocytosis (Fig. 2*b*), nor did PC- or PI-containing liposomes (Fig. 2*b*).

The ligand for CD36 on the PE is a trypsin-sensitive protein, *P. falciparum* erythrocyte membrane protein 1 (56). Removal of the CD36 ligand by mild trypsinization of the PEs before phagocytosis resulted in a decrease in phagocytosis similar to that observed with CD36 receptor blockade (Fig. 2*c*).

Up-regulating CD36 in monocytes and Mϕs results in increased uptake of nonopsonized PEs

Our observations that culture-derived Mϕs express more CD36 and have increased phagocytic capacity for PEs prompted us to test the hypothesis that pharmacologic up-regulation of CD36 would increase phagocytic uptake of nonopsonized PEs.

The nuclear receptor PPARγ, acting in combination with the RXR, has been shown to up-regulate CD36 expression in monocytic cells (43). We treated monocytes with the PPARγ agonists 15d-PGJ_2 (5 μM) or ciglitazone (3–100 μM), a member of the thiazolidinedione family of drugs, and the RXR agonists MA (10 μM) or 9-*cis*-RA (1 μM) to determine whether PPARγ-RXR activation increased CD36 surface levels and CD36-mediated phagocytic capacity. Monocytes treated overnight with 15d-PGJ_2 and a RXR agonist or ciglitazone alone expressed 40–60% higher levels of CD36 compared with similarly treated controls as determined by flow cytometry (Fig. 3*a* and data not shown). Similar up-regulation was observed in treated THP-1 cells (data not shown). This increase in surface level of CD36 was associated with a 2-fold or greater increase in the phagocytic uptake of nonopsonized PEs over controls ($p < 0.01$; Fig. 3, *b* and *c*). Treated monocytes that internalized at least one PE increased 40–60%, and 30% more PEs were internalized per phagocytic-positive monocyte compared with controls (1.51 ± 0.21 vs 1.16 ± 0.13, PEs per monocyte ± SD; $n = 16$, $p < 0.05$). In each case, phagocytosis was inhibited by mAb blockade of CD36 ($p < 0.01$; Fig. 3, *b* and *c*). Inhibition levels ranged from ~50 to 70% (mean = 61.15%) in control and ~65–90% (mean = 72.9%) in treated monocytes. CD36-independent phagocytosis did not differ significantly between treated and control monocytes.

Increases in monocyte surface levels of CD36 and nonopsonic phagocytosis demonstrated a dose-response relationship to treatment with ciglitazone (3–100 μM; Fig. 3*d*).

The ability of PPARγ and RXR agonists to increase CD36 expression and phagocytic capacity was not limited to freshly isolated monocytes. Culture-derived Mϕs treated with 15d-PGJ_2 plus 9-*cis*-RA showed increased CD36 surface levels ~40% over control Mϕs and a corresponding increase in nonopsonic phagocytosis (~70% increase in ingested PEs in treated Mϕs; $p < 0.01$; Fig. 4*a*). Similar to untreated Mϕs, phagocytosis of nonopsonized PEs by PPARγ-RXR agonist-treated Mϕs was inhibited by mAb blockade of CD36 and by cleaving the CD36 ligand from the PEs and not by receptor blockade of $\alpha_v\beta_3$, TSP, and ICAM-1 or preincubation with PS-, PC-, or PI-containing liposomes (Fig. 4, *b–d*).

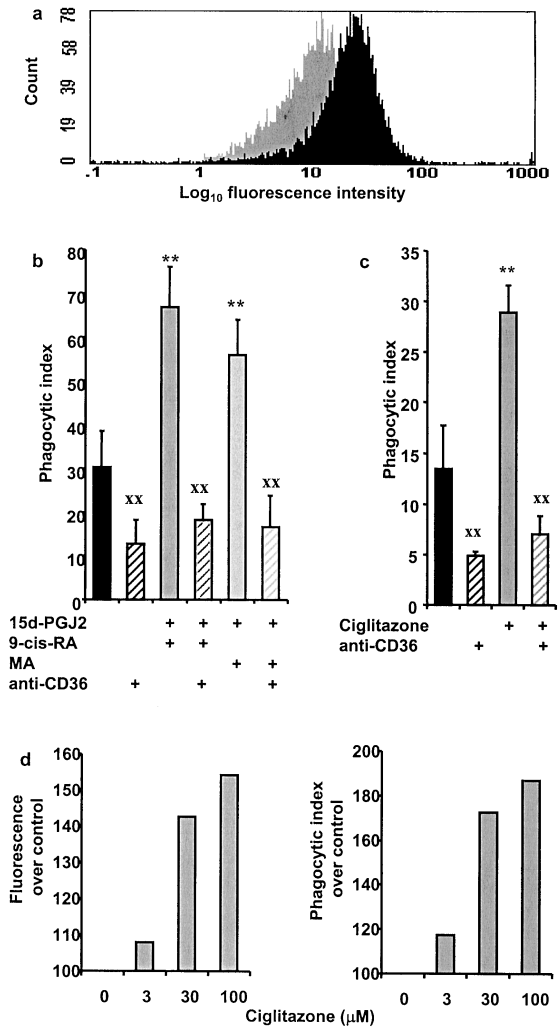

FIGURE 3. PPARγ and RXR agonists up-regulate the CD36 expression of human monocytes and increase their phagocytic capacity for nonopsonized PEs. *a*, Monocytes were treated for 24 h with 5 μM 15d-PGJ_2 plus 1 μM 9-*cis*-RA or with DMSO as a control. CD36 mean $\log_{10}$ fluorescence of control monocytes is shown in gray and that of treated monocytes in black. The histograms are representative of data collected in three or more independent experiments. Secondary-only stained controls were equivalent for both control and treated monocytes. *b–c*, Monocytes were treated with 5 μM 15d-PGJ_2 plus 1 μM 9-*cis*-RA (*b*), 5 μM 15d-PGJ_2 plus 10 μM MA (*b*), or 30 μM ciglitazone (*c*) for 24 h and then used in PE phagocytosis assays as described in *Materials and Methods*. Fc receptors were blocked in all assays and, where indicated, CD36 was blocked by preincubation with 10 μg/ml FA6-152 (anti-CD36; ▨). Data shown are means ± SD of at least three independent experiments. **, $p < 0.01$, controls vs treated; xx, $p < 0.01$, Fc vs CD36 blockade by FA6-152 (Student's *t* test, n = 9/group; ciglitazone, n = 6/group). *d*, Monocytes were treated with increasing doses of ciglitazone (3–100 μM) or appropriate controls for 24 h. CD36 mean $\log_{10}$ fluorescence of ciglitazone-treated as compared with control-treated monocytes is shown in the *left panel*. Phagocytic index of ciglitazone-treated as compared with control-treated monocytes is shown in the *right panel*.

PPARγ agonists reduce P. falciparum*-induced TNF-α from human monocytes*

Elevated levels of proinflammatory cytokines such as TNF-α have been associated with disease severity and a poor prognosis, suggesting that excessive secretion of TNF-α by monocytes/Mϕs in response to parasite products may promote severe and cerebral malaria (29, 30, 34). PPARγ agonists have been shown to reduce LPS and PMA-induced proinflammatory cytokine secretion from monocytes (44, 45). We examined whether PPARγ agonists would inhibit *P. falciparum*-induced TNF-α secretion from human monocytes and THP-1 cells.

We have previously demonstrated that CD36-mediated phagocytosis of washed well-synchronized mature stage PEs does not induce TNF-α release from monocytes (42). However, human monocytes and THP-1 cells exposed to parasite culture supernatants containing parasite GPI toxins released during schizont rupture (57) do secrete TNF-α (Fig. 5, *a* and *b*). Monocyte TNF-α secretion induced by PMA was significantly inhibited by cotreatment of cells with PPARγ-RXR agonists ($p < 0.05$). Cotreatment of monocytes or THP-1 cells with 15d-PGJ_2 and 9-*cis*-RA plus a 1:10 dilution of parasite culture supernatants resulted in a significant decrease in TNF-α production compared with controls ($p < 0.05$; Fig. 5, *a* and *b*). PPARγ-RXR agonist-induced inhibition of TNF-α was not limited to the laboratory clone ItG but also occurred with wild isolates (P1 and P2).

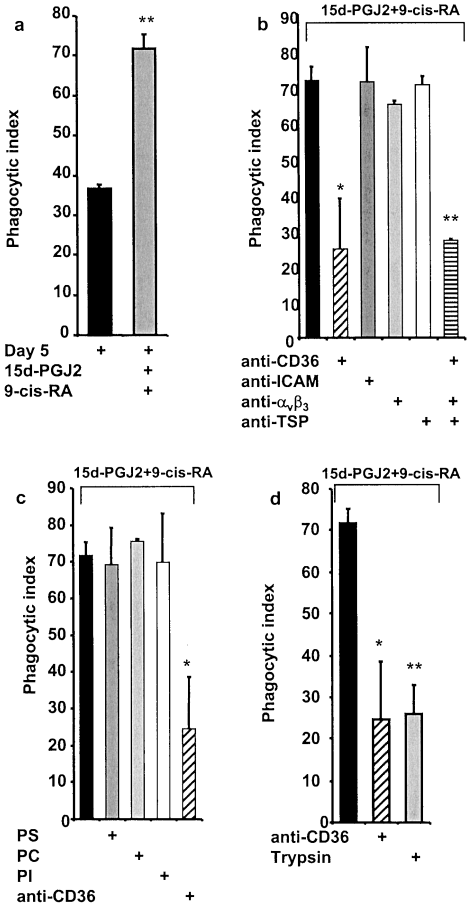

FIGURE 4. PPARγ and RXR agonists increase CD36-dependent phagocytosis of nonopsonized PEs by culture-derived Mϕs. *a*, Mϕs were treated with 5 μM 15d-PGJ_2 plus 1 μM 9-*cis*-RA (▨) or DMSO controls (■) for 24 h and were then used in PE phagocytosis assays. **, $p < 0.01$ (Student's *t* test, $n = 3$/group), control vs treated Mϕs. The data are representative of three independent experiments. *b–d*, These experiments were performed on culture-derived Mϕs that were pretreated with 5 μM 15d-PGJ_2 plus 1 μM 9-*cis*-RA for 24 h. *b*, Fc receptor-blocked Mϕs were treated for 30 min with 10 μg/ml of the following mAbs: FA6-152 (anti-CD36), 15.2 (anti-ICAM-1), 23C6 (anti-$\alpha_v\beta_3$), C6.7 (anti-TSP), a combination of FA6-152, 23C6, and C6.7, or they were left untreated (Fc receptor-blocked only; ■). Nonopsonic phagocytosis assays were then performed as previously described. *, $p < 0.05$, Fc only- vs FA6-152-treated Mϕs; **, $p < 0.01$, Fc only- vs FA6-152, 23C6, and C6.7-treated Mϕs; by Student's *t* test, $n = 3$/group. *c*, Mϕs were subjected to no treatment, to treatment with 0.1 mM of PS-containing liposomes, PC-containing liposomes, or PI-containing liposomes, or to treatment with 10 μg/ml of mAb FA6-152 (anti-CD36) before phagocytosis assays. *, $p < 0.05$ (Student's *t* test, $n = 3$/group), control vs FA6-152-treated Mϕs. *d*, Fc receptor-blocked Mϕs were exposed to nonopsonized PEs or nonopsonized PEs that were pretreated with trypsin for 30 min. FA6-152 (anti-CD36)-treated monocytes exposed to nonopsonized PEs are also shown. **, $p < 0.01$, trypsinized vs nontrypsinized PEs; *, $p < 0.05$ Fc only- vs FA6-152-treated Mϕs (Student's *t* test, $n = 3$/group).

Treating THP-1 cells with increasing doses of 15d-PGJ_2 (5–15 μM) resulted in a dose-response inhibition of PMA and parasite-induced TNF-α production (data not shown).

Discussion

In this study, we demonstrate that CD36 is the major receptor mediating the phagocytosis of nonopsonized PEs by monocytes and culture-derived Mϕs. This clearance mechanism is of potential relevance to those at the greatest risk of severe disease, including

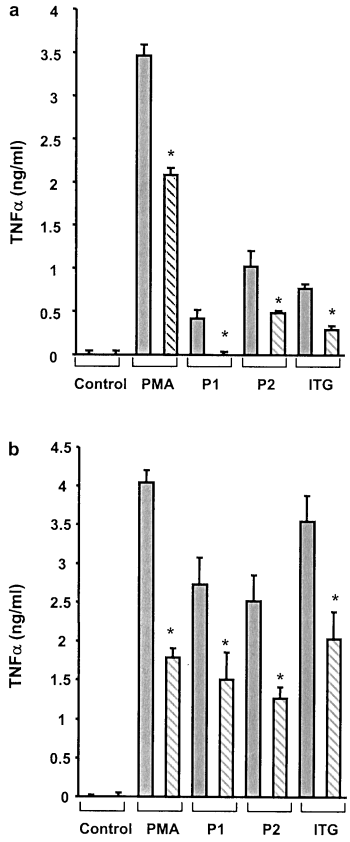

FIGURE 5. Treatment with PPARγ agonists reduces *P. falciparum*-induced TNF-α secretion from THP-1 cells and human monocytes. *a*, THP-1 cells were exposed to no treatment, 50 ng/ml PMA, or a 1:10 dilution of various *P. falciparum* culture supernatants (ItG, P1, and P2), and TNF-α production was assayed (see *Materials and Methods* for details). ▨, DMSO-treated controls; ▧, cells treated with 5 μM 15d-PGJ_2 plus 1 μM 9-*cis*-RA. Experiments were performed in triplicate, and data shown are representative of at least three independent experiments. *, $p < 0.05$ (Student's *t* test, $n = 6$ per group). *b*, Adherent human monocytes were treated as described above and assayed for TNF-α production. ▧, Monocytes treated with 5 μM 15d-PGJ_2 and 1 μM 9-*cis*-RA; ▨, DMSO-treated controls. Experiments were performed in triplicate, and data shown are representative of at least three independent experiments. *, $p < 0.05$ (Student's *t* test, $n = 6$/group).

nonimmune patient populations. CD36-PE uptake occurs via a novel phagocytic pathway that is distinct from the cooperative $\alpha_v\beta_3$-TSP-CD36 mechanism involved in the uptake of apoptotic cells and does not appear to involve PS recognition. Furthermore, we demonstrate that natural and synthetic PPARγ-RXR agonists increase monocytes/Mϕ CD36-mediated phagocytosis of nonopsonized PEs and decrease monocyte secretion of TNF-α in response to malaria toxins.

Because most natural parasite isolates bind CD36, it has been considered a target for antisequestration therapy (18, 22, 23). Despite the role of CD36 in cytoadherence, our data putatively assign a protective role for CD36 and suggest that strategies to block CD36-PE interactions may be deleterious to the host. Several additional lines of evidence support this hypothesis. Although almost all wild isolates of *P. falciparum* malaria adhere to CD36, only a minority of infected nonimmune patients develop cerebral or severe malaria. CD36 expression in the brain is low to absent (5), and cytoadherence to CD36 is unlikely to account for cerebral sequestration. However, CD36 is well expressed in microvascular endothelial cells from sites such as skin and muscle (5). CD36-mediated cytoadherence may direct parasites to these nonvital sites

and away from cerebral microvasculature. Sequestration in peripheral sites such as skin might be expected to facilitate transmission (58) while at the same time not compromise host survival. This hypothesis is supported by the work of Newbold and colleagues, who have reported that significantly higher binding to CD36 occurs in cases of nonsevere disease (25). More recent population data has linked CD36 deficiency with an increased susceptibility to severe and cerebral malaria (27). Taken together, our data and the above observations support an emerging model of the CD36-PE interaction as a complex parasite-host adaptation, resulting in improved survival of the parasite with consequent reduced injury to the host (down-regulated proinflammatory response and parasite replication balanced by host clearance).

Several other receptors including ICAM-1 and CD31 support sequestration of PEs, are expressed on brain endothelium, and are up-regulated by proinflammatory cytokines (8, 11, 28, 31–33, 59). Secretion of inflammatory cytokines during infection has been associated with a parasite-derived GPI toxin, which stimulates the release of TNF-α, IL-1, and IL-6 from monocytes and Mϕs. GPI from *P. falciparum* has also been shown to be a potent inducer of inducible NO synthase and NO output, presumably via the activation of transcription factors such as NF-κB and c-*rel* (28, 46). NO up-regulates ICAM-1 and VCAM-1 expression and has been implicated in the etiology of cerebral malaria (reviewed in Ref. 46). PPARγ agonists including 15d-PGJ_2 and thiazolidinediones have been reported to inhibit the induction of inflammatory genes via both PPARγ-dependent and -independent mechanisms, an effect mediated, at least partially, by inhibition of NF-κB (44–45, 60–63). PPARγ agonists down-regulate TNF-α and IL-6 and inhibit activation of inducible NO synthase in response to LPS and PMA (44–45, 63). If the anti-inflammatory effects of PPARγ agonists can be demonstrated for malaria GPI-induced inflammation, then these compounds may decrease excessive induction of inflammatory cytokines implicated in the etiology of severe malaria (46). Here we show that PPARγ-RXR agonists inhibit parasite-induced TNF-α secretion by monocytes and THP-1 cells. PPARγ agonists have also been shown to inhibit TNF-α-induced ICAM-1 expression (64). Collectively, these observations have clinical implications and suggest that adjunctive therapy with PPARγ-RXR agonists might reduce excessive or unbalanced proinflammatory responses to infection and might inhibit the up-regulation of endothelial cell receptors associated with severe malaria.

Whether the use of PPARγ agonists to up-regulate CD36 on monocytic cells will also up-regulate CD36 in other cells will depend on tissue-specific expression of PPARγ and CD36. CD36 is primarily expressed by monocytes, Mϕs, adipocytes, myocytes, and endothelial cells of the skin and reticuloendothelial system, especially the liver and spleen (5). Increased sequestration in these sites would not be expected to directly contribute to excess mortality, because binding within the reticuloendothelial system should theoretically enhance clearance, and binding to CD36 in nonvital sites has been proposed to confer protection against severe and cerebral disease (25, 27, 42). However, it will be important to determine whether these agents up-regulate CD36 in microvascular endothelium of vital organs such as the brain. In preliminary studies, we have observed that treatment of the immortalized human brain endothelial cell line WR-1 with PPARγ-RXR agonists did not increase CD36 expression (data not shown). However, it will be important to directly examine the effect of PPARγ activation in endothelial cells from a variety of sites to ensure that CD36-mediated cytoadherence in vital organs will not be increased.

Previous studies have reported that Mϕ ingestion of opsonized PEs or large amounts of hemazoin results in impaired phagocytic function and decreased expression of MHC class II Ag (36, 65, 66) These data suggest that hemozoin loading of Mϕs may impair both nonspecific and specific immune responses. Despite these considerations, the great majority of *P. falciparum*-infected individuals do not progress to severe malaria and control their infections due, at least in part, to the activity of circulating and tissue-resident monocytes/Mϕs. Whether CD36-mediated PE phagocytosis will lead to similar Mϕ impairment is currently under investigation. However, even if the phagocytic function of the circulating pool of monocytes is decreased, these cells are replaced during the course of clinical infection by fresh phagocytes. In fact, the recovery phase of human malaria is coincident with an increased capacity for its phagocytic clearance (67).

In summary, we present evidence establishing CD36 as a major receptor mediating nonopsonic uptake of PEs, which is of potential importance in nonimmune hosts who are at the greatest risk of severe and cerebral malaria. We also demonstrate that PPARγ-RXR agonists induce an increase in the CD36-mediated phagocytosis of PEs and a decrease in parasite-induced TNF-α production. Specific up-regulation of monocyte/Mϕ CD36 may represent a novel way to immunomodulate host defense and a new strategy to prevent or treat severe *P. falciparum* malaria. Because several PPARγ agonists are approved for human use, this hypothesis can be directly tested (43, 68).

Acknowledgments

We are grateful to the blood donor volunteers, especially W. Soukoreff, A. C. Labbe, Y. Yau, T. Smith, S. Huntley, J. Dlugosz, and S. Nathoo.

References

1. White, N. J. 1998. Not much progress in treatment of cerebral malaria. *Lancet 353:594.*
2. Warrell, D. A. 1987. Pathophysiology of severe *falciparum* malaria in man. *Parasitology 94:S53.*
3. MacPherson, G. G., M. J. Warrell, N. J. White, S. Looareesuwan, and D. A. Warrell. 1985. Human cerebral malaria: a quantitative ultrastructural analysis of parasitized erythrocyte sequestration. *Am. J. Pathol. 119:385.*
4. Howard, R. J., and A. D. Gilladoga. 1989. Molecular studies related to the pathogenesis of cerebral malaria. *Blood 74:2603.*
5. Turner, G. D., H. Morrison, M. Jones, T. M. Davis, S. Looareesuwan, I. D. Buley, K. C. Gatter, C. I. Newbold, S. Pukritayakamee, and B. Nagachinta. 1994. An immunohistochemical study of the pathology of fatal malaria. *Am. J. Pathol. 145:1057.*
6. Miller, L. H., M. F. Good, and G. Milon. 1994. Malaria pathogenesis. *Science 264:1878.*
7. Ockenhouse, C. F., M. Ho, N. N. Tandon, G. A. Van Seventer, S. Shaw, N. J. White, G. A. Jamieson, J. D. Chulay, and H. K. Webster. 1991. Molecular basis of sequestration in severe and uncomplicated *Plasmodium falciparum* malaria: differential adhesion of infected erythrocytes to CD36 and ICAM-1. *J. Infect. Dis. 164:163.*
8. Berendt, A. R., D. L. Simmons, J. Tansey, C. I. Newbold, and K. Marsh. 1989. Intercellular adhesion molecule 1 (ICAM-1) is an endothelial cytoadherence receptor for *Plasmodium falciparum*. *Nature 341:57.*
9. Udomsangpetch, R., P. H. Reinhardt, T. Schollaardt, J. F. Elliot, P. Kubes, and M. Ho. 1997. Promiscuity of clinical *Plasmodium falciparum* isolates for multiple adhesion molecules under flow conditions. *J. Immunol. 158:4358.*
10. Ockenhouse, C. F., T. Tegoshi, Y. Maeno, C. Benjamin, M. Ho, K. E. Kan, Y. Thway, K. Win, and R. R. Lobb. 1992. Human vascular endothelial cell adhesion receptors for *Plasmodium falciparum*-infected erythrocytes: roles for endothelial leukocyte adhesion molecule 1 and vascular cell adhesion molecule 1. *J. Exp. Med. 176:1183.*
11. Treutiger, C. J., A. Heddini, V. Fernandez, W. A. Muller, and M. Wahlgren. 1997. PECAM-1/CD31, an endothelial receptor for binding *Plasmodium falciparum*-infected erythrocytes. *Nat. Med. 3:1405.*
12. Chaiyaroj, S. C., P. Angkasekwinai, A. Buranakiti, S. Looareesuwan, S. J. Rogerson, and G. V. Brown. 1996. Cytoadherence characteristics of *Plasmodium falciparum* isolates from Thailand: evidence for chondroitin sulfate as a cytoadherence receptor. *Am. J. Trop. Med. Hyg. 55:76.*
13. Beeson, J. G., S. J. Rogerson, B. M. Cooke, J. C. Reeder, W. Chai, A. M. Lawson, M. E. Molyneux, and G. V. Brown. 2000. Adhesion of *Plasmodium falciparum*-infected erythrocytes to hyaluronic acid in placental malaria. *Nat. Med. 6:86.*
14. Roberts, D. D., J. A. Sherwood, S. L. Spitalnik, L. J. Panton, R. J. Howard, V. M. Dixit, W. A. Frazier, L. H. Miller, and V. Ginsburg. 1985. Thrombospondin binds *falciparum* parasitized erythrocytes and may mediate cytoadherence. *Nature 318:64.*

15. Siano, J. P., K. K. Grady, P. Millet, and T. M. Wick. 1998. *Plasmodium falciparum*: cytoadherence to $\alpha_v\beta_3$ on human microvascular endothelial cells. *Am. J. Trop. Med. Hyg. 59:77.*
16. Oquendo, P., E. Hundt, J. Lauler, and B. Seed. 1989. CD36 directly mediates cytoadherence of *Plasmodium falciparum* parasitized erythrocytes. *Cell 58:95.*
17. Ockenhouse, C. F., and J. D. Chulay. 1988. *Plasmodium falciparum* sequestration: OKM5 antigen (CD36) mediates cytoadherence of parasitized erythrocytes to a myelo-monocytic cell line. *J. Infect. Dis. 157:584.*
18. Ockenhouse, C. F., C. Magowan, and J. D. Chulay. 1989. Activation of monocytes and platelets by monoclonal antibodies or malaria-infected erythrocytes binding to the CD36 surface receptor in vitro. *J. Clin. Invest. 84:468.*
19. Panton, L. J., J. H. Leech, L. H. Miller, and R. J. Howard. 1987. Cytoadherence of *Plasmodium falciparum*-infected erythrocytes to human melanoma cell lines correlates with surface OKM5 antigen. *Infect. Immun. 55:2754.*
20. Barnwell, J. W., C. F. Ockenhouse, and, D. M. Knowles, II. 1985. Monoclonal antibody OKM5 inhibits the in vitro binding of *Plasmodium falciparum*-infected erythrocytes to monocytes, endothelial, and C32 melanoma cells. *J. Immunol. 135:3494.*
21. Newbold, C. I., P. Warn, G. Black, A. Berendt, A. Craig, B. Snow, M. Msobo, N. Peshu, and K. Marsh. 1997. Receptor specific adhesion and clinical disease in *Plasmodium falciparum* malaria. *Am. J. Trop. Med. Hyg. 57:389.*
22. Baruch, D. I., X. C. Ma, H. B. Singh, X. Bi, B. L. Pasloske, and R. J. Howard. 1997. Identification of a region of PfEMP1 that mediates adherence of *Plasmodium falciparum* infected erythrocytes to CD36: conserved function with variant sequence. *Blood 90:3766.*
23. Cooke, B. M., C. L. Nicoll, D. I. Baruch, and R. L. Coppel. 1998. A recombinant peptide based on PfEMP-1 blocks and reverses adhesion of malaria-infected red blood cells to CD36 under flow. *Mol. Microbiol. 30:83.*
24. Aikawa, M., M. Iseki, J. W. Barnwell, D. Taylor, M. M. Oo, and R. J. Howard. 1990. The pathology of human cerebral malaria. *Am. J. Trop. Med. Hyg. 43:30.*
25. Newbold, C., A. Craig, S. Kyes, A. Rowe, D. Fernandez-Reyes, and T. Fagan. 1999. Cytoadherence, pathogenesis and the infected red cell surface in *Plasmodium falciparum*. *Int. J. Parasitol. 29:927.*
26. Traore, B., K. Muanza, S. Looareesuwan, S. Supavej, S. Khusmith, M. Danis, P. Viriyavejakul, and F. Gay. 2000. Cytoadherence characteristics of *Plasmodium falciparum* isolates in Thailand using in vitro human lung endothelial cells model. *Am. J. Trop. Med. Hyg. 62:38.*
27. Aitman, T. J., L. D. Cooper, P. J. Norsworthy, F. N. Wahid, J. K. Gray, B. R. Curtis, P. M. McKeigue, D. Kwiatkowski, B. M. Greenwood, R. W. Snow, A. V. Hill, and J. Scott. 2000. Malaria susceptibility and CD36 mutation. *Nature 405:1015.*
28. Schofield, L., S. Novakovic, P. Gerold, R. T. Schwarz, M. J. McConville, and S. D. Tachado. 1996. Glycosylphosphatidylinositol toxin of *Plasmodium* up-regulates intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin expression in vascular endothelial cells and increases leukocyte and parasite cytoadherence via tyrosine kinase-dependent signal transduction. *J. Immunol. 156:1886.*
29. Grau, G. E., T. E. Taylor, M. E. Molyneux, J. J. Wirima, P. Vassalli, M. Hommel, and P. H. Lamber. 1989. Tumour necrosis factor and disease severity in children with *falciparum* malaria. *N. Engl. J. Med. 320:1586.*
30. Allan, R. J., P. Beattie, C. Bate, M. B. Van Hensbroek, S. Morris-Jones, B. M. Greenwood, and D. Kwiatkowski. 1995. Strain variation in tumor necrosis factor induction by parasites from children with acute *falciparum* malaria. *Infect. Immun. 63:1173.*
31. Kwiatkowski, D., A. V. Hill, I. Sambou, P. Twumasi, J. Castracane, K. R. Manogue, A. Cerami, D. R. Brewster, and B. M. Greenwood. 1990. TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. *Lancet 336:1201.*
32. Brown, H., G. Turner, S. Rogerson, M. Tembo, J. Mwenechanya, M. Molyneux, and T. Taylor. 1999. Cytokine expression in the brain in human cerebral malaria. *J. Infect. Dis. 180:1742.*
33. Day, N. P., T. T. Hien, T. Schollaardt, P. P. Loc, L. V. Chuong, T. T. Chau, N. T. Mai, N. H. Phu, D. X. Sinh, N. J. White, and M. Ho. 1999. The prognostic and pathophysiologic role of pro- and anti-inflammatory cytokines in severe malaria. *J. Infect. Dis. 180:1288.*
34. McGuire, W., A. V. Hill, C. E. Allsopp, B. M. Greenwood, and D. Kwiatkowski. 1994. Variation in the TNF-α promoter region associated with susceptibility to cerebral malaria. *Nature 371:508.*
35. Knight, J. C., I. Udalova, A. V. Hill, B. M. Greenwood, N. Peshu, K. Marsh, and D. Kwiatkowski. 1999. A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria. *Nat. Genet. 22:145.*
36. Wahlgren, M. 1999. Creating deaths from malaria. *Nat. Genet. 22:120.*
37. Shear, H. L., R. Srinivasan, T. Nolan, and C. Ng. 1989. Role of IFN-γ in lethal and non-lethal malaria in susceptible and resistant murine hosts. *J. Immunol. 143:2038.*
38. Urquhart, A. D. 1994. Putative pathophysiological interactions of cytokines and phagocytic cells in severe human *falciparum* malaria. *Clin. Infect. Dis. 19:117.*
39. Cappadoro, M., G. Giribaldi, E. O'Brien, F. Turrini, F. Mannu, D. Ulliers, G. Simula, L. Luzzatto, and P. Arese. 1998. Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by *Plasmodium falciparum* may explain malaria protection in G6PD deficiency. *Blood 92:2527.*
40. Staunton, D. E., C. F. Ockenhouse, and T. A. Springer. 1992. Soluble intercellular adhesion molecule 1-immunoglobulin G1 immunoadhesion mediates phagocytosis of malaria-infected erythrocytes. *J. Exp. Med. 176:1471.*
41. Turrini, F., H. Ginsburg, F. Bussolino, G. P. Pescarmona, M. V. Serra, and P. Arese. 1992. Phagocytosis of *Plasmodium falciparum*-infected human red blood cells by human monocytes: involvement of immune and nonimmune determinants and dependence on parasite developmental stage. *Blood 80:801.*
42. McGilvray, I. D., L. Serghides, A. Kapus, O. D. Rotstein, and K. C. Kain. 2000. Non-opsonic monocyte/macrophage phagocytosis of *Plasmodium falciparum*-parasitized erythrocytes: a role for CD36 in malarial clearance. *Blood 96:3231.*
43. Torontoz, P., L. Nagy, J. G. Alvarez, V. A. Thomazy, and R. M. Evans. 1998. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell 93:241.*
44. Jiang, C., A. T. Ting, and B. Seed. 1998. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature 391:82.*
45. Chawla, A., Y. Barak, L. Nagy, D. Liao, P. Tontonoz, and R. M. Evans. 2001. PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat. Med. 7:48.*
46. Clark, I. A., and L. Schofield. 2000. Pathogenesis of malaria. *Parasitol. Today 16:451.*
47. Fadok, V. A., D. J. Laszlo, P. W. Noble, L. Weinstein, D. W. Riches, and P. M. Henson. 1993. Particle digestibility is required for induction of the phosphatidylserine recognition mechanism used by murine macrophages to phagocytose apoptotic cell. *J. Immunol. 151:4274.*
48. Trager, W., and J. B. Jensen. 1976. Human malaria parasites in continuous culture. *Science 193:673.*
49. Lambros, C., and J. P. Vanderberg. 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J. Parasitol. 65:418.*
50. Serghides, L., I. C. Crandall, E. Hull, and K. C. Kain. 1998. The *Plasmodium falciparum*-CD36 interaction is modified by a single amino acid substitution in CD36. *Blood 92:1814.*
51. Fadok, V. A., M. L. Warner, D. L. Bratton, and P. M. Henson. 1998. CD36 is required for phagocytosis of apoptotic cells by macrophages that use either a phosphatidylserine receptor or the vitronectin receptor ($\alpha_v\beta_3$). *J. Immunol. 161:6250.*
52. Brisseau, G. F., A. P. Dackiw, P. Y. Cheung, N. Christie, and O. D. Rotstein. 1995. Posttranscriptional regulation of macrophage tissue factor expression by antioxidants. *Blood 85:1025.*
53. Huh, H. Y., S. F. Pearce, L. M. Yesner, J. L. Schindler, and R. L. Silverstein. 1996. Regulated expression of CD36 during monocyte-to-macrophage differentiation: potential role of CD36 in foam cell formation. *Blood 87:2020.*
54. Savill, J., N. Hogg, Y. Ren, and C. Haslett. 1992. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J. Clin. Invest. 90:1513.*
55. Ren, Y., R. L. Silverstein, J. Allen, and J. Savill. 1995. CD36 gene transfer confers capacity for phagocytosis of cells undergoing apoptosis. *J. Exp. Med. 181:1857.*
56. Baruch, D. I., J. A. Gormely, C. Ma, R. J. Howard, and B. L. Pasloske. 1996. *Plasmodium falciparum* erythrocyte membrane protein 1 is a parasitized erythrocyte receptor for adherence to CD36, thrombospondin, and intercellular adhesion molecule 1. *Proc. Natl. Acad. Sci. USA 93:3497.*
57. Schofield, L., L. Vivas, F. Hackett, P. Gerold, R. T. Schwarz, and S. Tachado. 1993. Neutralizing monoclonal antibodies to glycosylphosphatidylinositol, the dominant TNF-α-inducing toxin for *Plasmodium falciparum*: prospects for the immunotherapy of severe malaria. *Ann. Trop. Med. Parasitol. 87:617.*
58. Silamut, K., N. H. Phu, C. Whitty, G. D. Turner, K. Louwrier, N. T. Mai, J. A. Simpson, T. T. Hien, and N. J. White. 1999. A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. *Am. J. Pathol. 155:395.*
59. Dobbie, M. S., R. D. Hurst, N. J. Klein, and R. A. Surtees. 1999. Upregulation of intercellular adhesion molecule-1 expression on human endothelial cells by tumour necrosis factor-α in an in vitro model of the blood-brain barrier. *Brain Res. 830:330.*
60. Moore, K. J., E. D. Rosen, M. L. Fitzgerald, F. Randow, L. P. Andersson, D. Altshuler, D. S. Milstone, R. M. Mortensen, D. M. Spiegelman, and M. W. Freeman. 2001. The role of PPARγ in macrophage differentiation and cholesterol uptake. *Nat. Med. 7:41.*
61. Rossi, A., P. Kapahi, G. Natoli, T. Takahashi, Y. Chen, M. Karin, and M. G. Santoro. 2000. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IκB kinase. *Nature 403:103.*
62. Straus, D. S., G. Pascual, M. Li, J. S. Welch, M. Ricote, C. Hsiang, L. L. Sengchanthalangsy, G. Ghosh, and C. K. Glass. 2000. 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits multiple steps in the NF-κB signaling pathway. *Proc. Natl. Acad. Sci. USA 97:4844.*
63. Ricote, M., A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass. 1998. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage function. *Nature 391:79.*
64. Pasceri, V., H. D. Wu, J. T. Willerson, and E. T. Yeh. 2000. Modulation of vascular inflammation in vitro and in vivo by peroxisome proliferator-activated receptor-γ activators. *Circulation 101:235.*
65. Schwarzer, E., F. Turrini, D. Ulliers, G. Giribaldi, H. Ginsburg, and P. Arese. 1993. Impairment of macrophage functions after ingestion of *Plasmodium falciparum*-infected erythrocytes or isolated malarial pigment. *J. Exp. Med. 177:1033.*
66. Schwarzer, E., M. Alessio, D. Ulliers, and P. Arese. 1998. Phagocytosis of the malarial pigment, hemozoin, impairs expression of major histocompatibility complex class II antigen, CD54, and CD11c in human monocytes. *Infect. Immun. 66:1601.*
67. Leitner, W. W., and U. Krzych. 1997. *Plasmodium falciparum* malaria blood stage parasites preferentially inhibit macrophages with high phagocytic activity. *Parasite Immunol. 19:103.*
68. Kersten, S., B. Desvergne, and W. Wahli. 2000. Roles of PPARs in health and disease. *Nature 405:421.*