Title: PRODUCTS AND METHODS FOR ACTIVATING PPARγ-RXR AND UP-REGULATING MONOCYTE/MACROPHAGE CD36 FOR THE TREATMENT OF MALARIA

Abstract: This invention relates to therapeutic products to treat Plasmodium falciparum malaria. According to a first aspect of the invention, a composition for the treatment of malaria is disclosed. The composition includes, as an active ingredient, a PPARγ and/or a RXR agonist which upregulates monocyte/macrophage CD36 in admixture with a pharmaceutically acceptable carrier, excipient or diluent. According to second aspect of the invention, a method of treating or preventing severe infection by P. falciparum in a person is provided. The method includes the steps of: (a) forming a composition by combining a PPARγ and/or a RXR agonist with an acceptable carrier, excipient or diluent; and (b) administering an effective dose of the composition to a person infected with or at risk of infection with P. falciparum.
Products and Methods for Activating PPARγ-RXR and Up-regulating Monocyte/Macrophage CD36 for the Treatment of Malaria

FIELD OF THE INVENTION

This invention relates to compositions and methods for treating *Plasmodium falciparum* malaria. In particular, this invention relates to compositions and methods for activating PPARγ-RXR and up-regulating monocyte/macrophage CD36 for treating *Plasmodium falciparum* malaria.

BACKGROUND OF THE INVENTION

*Plasmodium falciparum* malaria is the world’s most important parasitic infection accounting for an estimated 300 to 500 million cases and 1.5 to 2.7 million deaths annually (WHO Bull. 1990, Miller 1994, Kain 1998). *P. falciparum* infection accounts for over 90% of the morbidity and mortality associated with malaria. Young children living in malaria-endemic areas and other non-immune individuals are at the greatest risk of developing severe complications such as cerebral malaria leading to death. Despite intensive research, no specific treatments have been identified to prevent or improve the outcome of patients with severe and cerebral malaria (White 1998). Severe malaria carries a high fatality rate (>15%) even for young, previously healthy individuals (White 1998). With escalating drug resistance and the lack of an effective vaccine, there is an urgent need for alternative therapeutic strategies particularly those designed to prevent or treat severe or cerebral malaria.

The central pathophysiologic events in falciparum malaria are the sequestration of parasitized erythrocytes ("PEs") in the microvascular beds of vital organs and the release of pro-inflammatory cytokines from cells of the monocytes/macrophage lineage.

CD36 is an 88kD cell surface glycoprotein, expressed on endothelial cells, platelets, monocytes and macrophages (Quendo 1989). CD36 is a major sequestration receptor, preferentially recognized by almost all wild isolates of *P. falciparum* (Ockenhouse 1991, Udomsangpetch 1997, Newbold 1997).

In contrast to CD36, the expression of ICAM-1 and other sequestration receptors, is up-regulated by pro-inflammatory cytokines such as TNFα (Miller 1994, Berendt 1989). A strong correlation has been observed between elevated TNFα levels and disease severity and poor prognosis in *falciparum* malaria (Grat 1989, Allan 1994, Kwiatkowski 1990). In addition, the host’s genetic predisposition to over secrete TNFα in response to *falciparum* malaria may determine an individuals susceptibility to cerebral malaria (Turner 1994, Berendt 1989, McGuire 1994).

Phagocytic cells are an essential first line of defense against malaria, facilitating the control and resolution of the infection by clearing PEs (Shear 1989, Urquhart 1994). Phagocytes of the monocyte/macrophage lineage are also the primary source of parasite-induced TNFα (Grat 1989, Allan 1994, Kwiatkowski 1990, Miller 1994). Several studies have concentrated on the phagocytosis of opsonized PEs (Cappadaro, Staunton
1992, Turrini 1992). However, the relevance of opsonic clearance of PEs in the non-immune individual is unclear. In vitro evidence exists that monocytes bind PEs in the absence of antibody (Ruangjirachuporn 1992, Ockenhouse 1989, Staunton 1992) and engulf PEs even when complement and Fc-receptor pathways are blocked (Turrini 1992).

CD36 is the major sequestration receptor preferentially recognized by almost all wild isolates of *P. falciparum* and its gene promoter contains a PPARγ-RXR binding site. PPARγ is a member of the nuclear hormone receptor superfamily, and as a heterodimer with the retinoid X receptor (RXR) activates transcription of target genes by binding to DR-1 (direct repeat with 1 nucleotide spacer) type hormone response elements (Kliwer 1992, Tontonoz 1994, Shao 1998). PPARγ is primarily expressed in adipose tissue, mammary and colonic epithelium and in myelomonocytic cells (Tontonoz 1998). The PPARγ-RXR complex can modulate CD36 gene expression through direct promoter interaction with the PPARγ-RXR binding site (Tontonoz 1998). In addition, PPARγ agonists have been shown to suppress monocyte secretion of pro-inflammatory cytokines induced by phorbol esters but not be LPS (Jiang 1998). Several PPARγ-RXR ligands are now known. These include the prostanoid 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), the thiazolidinedione (TZD) class of antidiabetic drugs, members of the non-steroidal-anti-inflammatory drugs (NSAIDs), L-tyrosine-based PPARγ ligands (Henke 1998, Vamecq 1999), Vitamin A and its metabolites and synthetic RXR ligands such as LG1069 and LG100268.

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 anti-adherence therapies to disrupt the CD36-PE interaction (Ockenhouse JID 1989 and 1991, Baruch 1997; Cooke BM 1998). However unlike
ICAM-1, little if any CD36 is expressed on cerebral endothelial cells. CD36 is known to be well-expressed in microvascular endothelial cells from non-vital sites such as skin, muscle and sites rich in resident macrophages such as liver and spleen (Turner 1994). Virtually all natural *P. falciparum* isolates bind CD36, but only a small proportion of infected individuals develop severe or cerebral malaria. Collectively, these observations suggest that the CD36-PE interaction represents a parasite-host adaptation, evolved for improved survival of the parasite (sequestration in non-vital vascular beds) with limited damage to the host (parasite replication balanced by host clearance).

Although the molecular basis by which PEs bind CD36 is incompletely understood, several groups have sought to inhibit the CD36-PE interaction in an attempt to prevent the cytoadherence of PEs to endothelial cells and the development of severe malaria.

In WO 971968 (Duffy *et al.*), the inventors use a cytoadherence protein (sequestrin) specific to *Plasmodium falciparum* which binds to CD36. The inventors indicated that the use of sequestrin or a fragment of sequestrin might prevent severe malaria.

In WO 9306849 (Ockenhouse *et al.*), the inventors blocked the binding of malarially infected erythrocyte to CD36 by using an agent capable of binding to the CD36 binding site. The inventors suggested that by blocking CD36 binding, the complications arising from malaria can be ameliorated.

In WO 9306848 (Springer *et al.*), the inventors inhibited CD36 binding by malaria infected erythrocytes by administering an antibody covalently attached to CD36 or CD36 fragment. Binding was alleged to bring the antibody into close proximity to the malaria infected erythrocytes to promote cell death by stimulating phagocytosis via the
Fc receptor.

Each of these approaches focuses on reversing parasite sequestration by inhibiting the binding of PEs to CD36 on endothelial cells.

However, these approaches have several shortcomings. Our data indicates that CD36 plays a beneficial role during infection by aiding in the clearance of parasites as a phagocytic receptor on monocytes/macrophages and that CD36-mediated phagocytosis does not lead to the release of pro-inflammatory cytokines such as TNFα, which contribute to severe malaria and adverse clinical outcomes. These observations have important therapeutic implications. First, current strategies to disrupt CD36-PE interactions may be deleterious if they inhibit CD36-mediated PE phagocytosis and displace PEs from CD36 in non-vital sites, to the receptors of the cerebral vasculature. Second, clinical malaria will benefit from selective upregulation of monocyte/macrophage CD36, particularly in the non-immune host where opsonic phagocytosis would be expected to be less.

Therefore, there is a need for a composition and method for treating P. falciparum malaria, which results in increased phagocytic clearance of PEs and decreased secretion of TNFα from monocytes/macrophages. Collectively, increased malaria clearance, decreased TNFα and sequestration of parasites in vital organs, would be expected to prevent or treat severe malaria.

To help design pharmaceutical compositions and therapies for severe P. falciparum malaria, one must understand the function of monocyte/macrophage CD36 in the phagocytosis of PEs. Once the function of CD36 is understood, products can be selected or designed to modulate function. This would lead to improved methods of treating or preventing severe P. falciparum malaria.
SUMMARY OF THE INVENTION

Accordingly it is an object of the invention to provide compositions and methods for the treatment of *P. falciparum* malaria which result in increased phagocytic clearance of PEs and which do not result in elevated levels of TNFα.

The inventors have determined the molecular mechanisms of *P. falciparum* phagocytosis. Monocyte/macrophage CD36 participates in a novel phagocytic pathway for PEs that is distinct from that utilized in the clearance of apoptotic cells. Further, the up-regulation of monocyte/macrophage CD36 with PPARγ-RXR agonists or ligands results in increased phagocytic clearance of PEs, and decreased secretion of pro-inflammatory cytokines (such as TNFα) by monocytes and macrophages in response to malaria glycosylphosphatidylinositol (GPI) toxins.

Therefore, broadly stated, the present invention relates to products which can be used to upregulate the monocyte/macrophage CD36, contributing to the phagocytosis of *P. falciparum*-infected erythrocytes, and to downregulate pro-inflammatory responses to malaria toxins. These products can be used to treat or prevent severe *P. falciparum* malaria.

According to a first aspect of the invention, a composition for the treatment of malaria is provided. The composition comprises one or more members of a group consisting of a PPARγ agonist, a RXR agonist and natural or synthetic ligands thereof. Included within the group are prostaglandin A, prostaglandin D, prostanoid 15-deoxy-Δ^{12,14}-prostaglandin J2 (15d-PGJ2), thiazolidinedione (TZD) class of antidiabetic drugs, non-steroidal-anti-inflammatory drugs, L-tyrosine-based agonists, cytokines, lipoproteins and their components, polyunsaturated fatty acids, methoprene acid, vitamin A, and vitamin A metabolites, and compounds which are inhibitors of antagonists of PPARγ or
RXR and a mimetic of any of the foregoing. For the thiazolidinedione (TZD) class of antidiabetic drugs, one could use ciglitazone, troglitazone, pioglitazone, rosiglitazone, englitazone, and a mimetic of any of the foregoing. For the non-steroidal-anti-inflammatory drugs (NSAIDS), one could use indomethacin, flufenamic acid, fenoprofen, ibuprofen. The L-tyrosine-based agonist could be GW1929. Cytokines would include IL-4, GM-CSF, M-CSF. Lipoproteins and their components used could be selected from one or more members of a group consisting of oxidized LDL, 13-hydroxyoctadecadienoic acid [13-HODE], 15-hydroxyeicosatetraenoic acid [15-HETE]. The vitamin A metabolite could be selected from one or more members of a group consisting of 9-cis-retinoic acid, BRL49653, LG100268, LG 1069, LGD49653 and a mimetic of any of the foregoing. The PPARγ-RXR agonist could be derived from natural or engineered ligands.

Another aspect of the invention is a method for treating or preventing severe infection by *P. falciparum* malaria in a person comprising administering to a patient in need thereof a composition as described above..

Yet another aspect of the invention is a method for identifying a product for the treatment or prevention of malaria, the method comprising the steps of:

(a) contacting a product with a PPARγ-RXR agonist;

(b) determining whether the product up-regulates CD36 or decreases TNFα secretion in response to *falciparum* malaria (the up-regulation indicates that the product is useful for the treatment or prevention of malaria).

Yet another aspect of the invention is a kit for the treatment of malaria comprising a product which activates PPARγ-RXR or upregulates monocyte/mφ CD36 in admixture with a pharmaceutically acceptable carrier, excipient or diluent. The kit would
include the products described above.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

**DETAILED DESCRIPTION OF THE INVENTION**

The experimental data supporting the finding that phagocytosis of *P. falciparum* is mediated by CD36 is detailed below.

**Materials and Methods:**

Fetal calf serum was from Wisent (Mississauga, Canada) and was heat inactivated at 55°C for 30 minutes prior to use. FA6-152 was from Immunotech (Marseille, France), the anti-α,β₃ monoclonal 23C6 was from Serotec (Raleigh, NC), the anti-TSP monoclonal C6.7 was from Medicorp (Montreal, Canada), the anti-ICAM monoclonal 15.2 was from Santa Cruz Biotech (Balthesa, CA). Recombinant human TNFα and monoclonal anti-human TNFα were from Genzyme (Mississauga, Canada). Ciglitzone, 15d-Δ¹²,¹⁴-PGI₂, and methoprene acid were from BIOMOL (Plymouth, PA). E. coli 0111:B4 endotoxin (LPS), PMA, DMSO, 9-cis-retinoic acid, saline, trypsin, and sterile water were from Sigma-Aldrich (Oakville, Canada). Ficoll-Paque, Percoll, and Dextran T500 were from Pharmacia (Peapack, NJ). Human IgG Fc fragments were from Calbiochem (San Diego, CA). Lipids were purchased from Avanti Polar Lipids (Alabaster, AL).

All liposome were prepared as described (Fadok . 1998). PC liposomes were
made at 100 mol % PC. PI and PS liposomes were made at 70 mol % PC and 30 mol %
PS or PI.

*Parasite Cultures:*

Parasite cultures were maintained in erythrocytes from A+ donors and RPMI-1640 supplemented with 10% autologous human serum. Cultures were kept in a candle jar at 37°C and 5% CO₂. Parasites were synchronised using sorbitol lysis as described (Lambros 1979). Parasites of the ItG line were used in addition to two patient lines designated PT1 and PT2. All parasite cultures were treated with mycoplasma removal
agent (ICN) and tested negative for mycoplasma by PCR analysis, prior to use.

Culture supernatants were collected from all three parasite lines, aliquoted, and
frozen for subsequent use.

*Monocyte Isolation:*

Human monocytes were isolated from the blood of healthy volunteers as
previously described (McGilvray 2000). Briefly, the buffy coat fraction from whole
blood in sodium heparin was brought up to an osmolality of 360mOsm by the addition of
sterile 9% saline. The buffy coat was then centrifuged over a 40/55 Percoll gradient, the
monocyte layer was collected and washed 3 times with cold RPMI. This procedure
yields a platelet-free population of non-activated monocytes >80% CD14 positive by
flow cytometry, minimal baseline TNFα secretion, >98% viability by trypan blue
exclusion.
Detection of CD36 expression by flow cytometry:

Human monocytes, treated for 22-24 hours with PPARγ and RXR agonists (initially dissolved in DMSO) or controls containing the same concentration of DMSO but without a PPARγ or RXR agonist, were stained with 1:100 dilution of the mAb FA6-152 (anti-CD36) for 30 min on ice followed by a 1:100 dilution of a secondary anti-mouse IgG-FITC conjugated antibody. Unstained and a secondary antibody only stained controls were also performed. The monocytes were fixed in 1% paraformaldehyde/PBS and analyzed using the EPICS ELITE flow cytometer (Beckman-Coulter) for CD36 expression. Flow data was analyzed using the ELITE software (Beckman-Coulter).

Upregulation of CD36 using PPARγ agonists and phagocytic assay:

Purified human monocytes suspended in RPMI-10 were plated on round glass coverslips in a 24 well polystyrene plate (250,000 monocytes per well), and allowed to adhere for 1 hour. Non-adherent cells were washed away, the supernatant was removed and replaced with RPMI-10 containing 5μM 15d-PGJ2 plus 30μM methoprene acid, or 5μM 15d-PGJ2 plus 5μM 9-cis-retinoic acid, or 30μM ciglitazone alone, or appropriate concentrations of DMSO as a control, and incubated at 37°C 5% CO₂ for 22-24 hours. The monocytes were then washed and incubated with 20μg/mL Fc fragments for 30 min at room temperature (in order to block Fc receptors) followed by a 30 min incubation with 5-10μg/mL FA6-152 when appropriate. The monocytes were washed and 500μL of 2% Hct, 5-8% parasitemia of carefully synchronised parasites in RPMI-10 prepared as described (McGilvray 2000), were layered on top. The assay was allowed to continue for 4 hours, at 37°C with gentle rotation.

Other antibody inhibition studies were performed with monocytes plated in
RPMI-10, treated with Fc fragments (as described above) and exposed to 10µg/mL of monoclonal C6.7 (anti-TSP), 23C6 (anti-αvβ3), or FA6-152 (anti-CD36) for 30 min at room temperature. The monocytes were washed and PEs were added as above.

5 **TNFα Assay:**

Purified human monocytes were seeded in 24 well polystyrene plates at 200,000 cells per well, and allowed to adhere for 1 hour. Unattached cells were washed away, and the remaining cells were treated with PPARγ and RXR agonists, or DMSO controls as described above, followed by the addition of 1µg/ml LPS, 50nM PMA, 1:10 dilution of various falciparum culture supernatants, or no additions. Following a 18-24 hour incubation at 37°C, the supernatants were collected and cells spun down and removed. The cleared supernatants were subjected to a sandwich ELISA for the detection of human TNFα. TNFα assays were also performed as above using THP-1 cells, kept in suspension at a concentration of 500,000 cells/ml, in place of purified human monocytes.

*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 strains. However, within each experiment the results were very consistent. Statistical significance was determined using ANOVA followed by Tukeys analysis. A P-value of ≤ 0.05 was required for significance.

Phagocytic index represents the number of monocytes with at least one internalized PE divided by the total number of monocytes counted. Experiments were
performed in duplicate and repeated at least three times. The variation in phagocytic index between experiments is due to different donors and day to day parasite variation. The phagocytic index within experiments was very consistent.

5 Results:

*Up-regulation of CD36 on human monocytes results in increased non-opsonic phagocytic clearance of* *P. falciparum-infected erythrocytes.*

Adherent human peripheral monocytes treated with the arachidonic acid metabolite 15d-PGJ2, a PPARγ agonist, and the RXR agonist methoprene acid for 24 hours expressed 40 to 60% higher levels of CD36 compared to similarly treated DMSO controls. Similar results were obtained using the RXR agonist 9-cis-retinoic acid (9-cis-RA) in combination with 15d-PGJ2, and treatment with ciglitazone alone (a member of the TZD family of drugs). Surface presence of CD36 was determined by staining with the anti-CD36 monoclonal antibody FA6-152 and assessed by flow cytometry. Similar up-regulation was observed in treated THP-1 cells.

Monocytes were treated with the combination of 15d-PGJ2 and methoprene acid or 15d-PGJ2 and 9-cis-retinoic acid led to an ~ 40% to 60% increase in CD36 and an ~ 40% to 60% increase in the phagocytic clearance of non-opsonized falciparum infected erythrocytes over control monocytes (P < 0.05). Treatment with ciglitazone resulted in an ~ 60% increase in phagocytic clearance of PEs over controls (P<0.05). In each case, phagocytosis was inhibited (40 to 70%) by pre-incubation with the anti-CD36 monoclonal antibody FA6-152.

In phagocytosis experiments, Fc receptors were blocked by pre-treating monocytes with Fc fragments (20μg/mL) and complement mediated phagocytosis was
avoided by using heat-inactivated serum.

Adherent monocytes were treated for 24 hours with 30μM ciglitazone alone, a combination of 5μM 15d-PGJ2 (PG) and 30μM methoprene acid (MA), a combination of 5μM 15d-PGJ2 and 1μM 9-cis-retinoic acid (9RA), or vehicle alone (DMSO). In parallel phagocytosis assays, CD36 was blocked by incubation with FA6-152. Treatment with 15d-PGJ2 and methoprene acid or 9-cis-retinoic acid resulted in a significant increase in the phagocytic clearance of PEs over control. This phagocytosis was blocked by incubating monocytes with FA6-152 prior to the assay. Treatment with the TZD drug ciglitazone also resulted in increased phagocytic clearance of PEs which was inhibited by CD36 blockade.

*Non-opsonic phagocytic clearance of P. falciparum infected erythrocytes is CD36 dependent but does not utilize the αβ3-TSP-CD36 phagocytic mechanism.*

To verify that the observed phagocytosis was due to a CD36-PE interaction, CD36 on monocytes was blocked by incubation with 10μg/mL of FA6-152 (anti-CD36 monoclonal antibody) prior to the addition of PEs. Incubation with FA6 resulted in a 40-70% inhibition of phagocytosis of PEs by PPARγ-RXR agonist-treated monocytes. CD36 receptor blockade with FA6 inhibited phagocytosis of PEs in both control and PPARγ-RXR agonist-treated monocytes to similar levels.

Previous studies examining the phagocytosis of apoptotic cells, have shown that monocyte/mΦ CD36 co-operates with the vitronectin receptor (αβ3) and thrombospondin (TSP) to clear apoptotic neutrophils and other cells (Savill 1995). To determine whether CD36 was co-operating in a similar manner to phagocyte PEs, CD36, αβ3, and TSP were blocked by incubation of monocytes with the monoclonal antibodies FA6-152, 23C6 (anti-αβ3) and C6.7 (anti-TSP) alone and in combination prior to exposure to PEs.
In single antibody inhibition studies only FA6 resulted in a reduction of phagocytosis. The anti-α,β3 and anti-TSP antibodies had no effect on phagocytosis of PE, alone or in combination. Furthermore no synergistic inhibition was observed since treatment with a combination of all three antibodies, FA6-152, 23C6 and C6.7, inhibited phagocytosis to the same level as treatment with FA6-152 alone.

Blocking CD36 on monocytes by pre-treatment with 10ug/mL FA6-152 resulted in a 60% decrease in phagocytosis of PEs over controls. Blockade of the vitronectin receptor, and TSP with 23C6 (anti-α,β3) and C6.7 (anti-TSP) had no effect on the level of phagocytosis of PEs. Co-treatment with all three antibodies resulted in a decrease in phagocytosis that was similar to blockade by FA6-152 alone.

Failure to block phagocytosis with anti-α,β3 and anti-TSP antibodies suggested that the mechanism of CD36-mediated phagocytosis of PEs is distinct from the cooperative mechanism between α,β3-TSP-CD36 described for the clearance of apoptotic cells (Savill 1995).

Trypsinization of the PEs abolishes non-opsonic clearance

Although the PE ligand(s) that interacts with CD36 has not been definitively characterized, it has been demonstrated that this ligand is trypsin sensitive (Barauch 1997). We investigated whether the phagocytosis of PEs is dependent upon the trypsin-sensitive ligand on the infected erythrocyte surface. A 30 minute trypsinization of the PEs prior to exposure to monocytes, reduced their phagocytic clearance by over 75%. The level of phagocytosis observed with trypsinized PEs was similar to that seen after CD36 receptor blockade with the anti-CD36 monoclonal FA6-152.

Treatment of monocytes and THP-1 cells with PPARγ-RXR agonists inhibits P. falciparum-induced TNFα secretion.
Elevated levels of TNFα have been consistently correlated with severity of disease and a poor prognosis leading to the conclusion that excessive secretion of TNFα by monocyte/mφs in response to parasite products may promote severe and cerebral malaria (Grau 1989; McGuire 1994; Allan 1995). Jiang and colleagues have previously demonstrated that PPARγ agonists can reduce PMA-induced but not LPS-induced pro-inflammatory cytokine secretion from monocytes. We examined whether PPARγ agonists would also inhibit *P. falciparum*-induced TNFα secretion from monocytes and THP-1 cells.

We have previously reported that thoroughly washed and well synchronised mature stage PEs do not induce TNFα release from monocytes during a 4 hour co-incubation (McGilvray 2000). In contrast, monocytes and THP-1 cells exposed to parasite culture supernatants secrete TNFα. This is in agreement with the induction of TNFα from monocyte/mφs by parasite glycosylphosphatidylinositol (GPI) toxins released during schizont rupture (Schofield 1993). PMA-induced TNFα secretion was inhibited by 15d-PGJ2 and 9-cis RA treatment, while LPS-induced TNFα secretion was unaffected by monocyte pre-treatment with these compounds. Co-treatment of monocytes or THP-1 cells with 15d-PGJ2 and 9-cis-RA, plus a 1:10 dilution of parasite culture supernatants over 24 hours resulted in a significant decrease in TNFα production compared to equivalent controls (30-50% decrease). PPARγ agonist-induced inhibition of TNFα was not limited to the established parasite line ItG, but also occurred with two wild isolates tested, SB and MB.

TNFα levels produced by THP-1 cells exposed to 1ug/ml LPS, 50nM/ml PMA, or a 1:10 dilution of various *P. falciparum* culture supernatants (see methods for details) were observed. The decrease in TNFα levels seen in the PPARγ-RXR-treated cells
exposed to PMA and the culture supernatants were significant.

TNFα levels produced by human monocytes treated as the THP-1 cells above were observed. A significant decrease in TNFα levels was seen in monocytes treated with PPARγ agonists and exposed to PMA, and P. falciparum culture supernatants.

An increase in CD36 expression seen in human monocytes treated with 1μM 9-cis-RA for 48 hours was observed. CD36 expression was determined by flow cytometric analysis of FA6-152 stained monocytes. An increase in CD36 expression seen in THP-1 cells treated with 9-cis-RA as above was also observed.

*Culture-derived macrophages expressed more CD36 and have increased phagocytic capacity for non-opsonised PEs.*

CD36 is the major receptor on freshly isolated human monocytes mediating the uptake of non-opsonised PEs (McGilrvay 2000). However our observations with freshly explanted monocytes underestimate the potential for non-opsonic clearance of PEs in vivo. Tissue resident macrophages (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 (Huh 1996). We investigated the effect of culture maturation of monocytes on CD36 expression and ability to phagocytose non-opsonised PEs. CD36 surface levels increased on monocytes aged in culture for 5 days. In association with increased CD36 levels, the phagocytosis of non-opsonised PEs increased approximately 4-fold. Phagocytosis occurred in a complement-free environment, with Fc receptor blockade and with no prior opsonization of PEs. Monoclonal antibody (mAb) blockade of CD36 (FA6-152, 10μg/ml) resulted in a 50%-70% inhibition of phagocytosis in both day 0 and day 5 monocytes (P<0.05).
There was no phagocytosis of uninfected erythrocytes (UEs) by monocytes or culture-derived mφs.

*Macrophage phagocytosis of non-opsonised PEs is a CD36 specific process.*

CD36 has previously been reported to participate, in co-operation with αb3 and thrombospondin (TSP), in the phagocytic removal of apoptotic cells (Savill. 1989, Ren 1995). We investigated whether these, or other recognized PE receptors such as ICAM-1, contributed to non-opsonic phagocytosis of PEs by monocyte-derived mφs. Receptor blockade of αb3 or TSP on monocyte-derived mφs using monoclonal antibodies (23C6 and C6.7 respectively (Savill 1992, Ren 1995) did not decrease PE phagocytosis. Blocking αb3, TSP and CD36 in combination resulted in a decrease in PE phagocytosis similar to that observed with CD36 blockade alone. Receptor blockade of ICAM-1, an important sequestration receptor for PEs, had no significant inhibitory effect on PE phagocytosis.

CD36 has been shown to interact with phosphatidyl-serine (PS) and PS-containing liposomes have been reported to inhibit CD36-mediated apoptotic cell phagocytosis (Fadok 1998). Exposing monocyte-derived mφs to PS-containing liposomes prior to phagocytosis had no inhibitory effect on non-opsonic PE phagocytosis; nor did phosphatidyl-choline (PC) or phosphatidyl-inositol (PI) containing liposomes.

The ligand for CD36 on the PE is a trypsin sensitive protein, PfEMP-1 (Baruch 1996). Removal of the CD36 ligand by mild trypsinization of the PEs prior to phagocytosis resulted in a decrease in phagocytosis similar to that observed with CD36 receptor blockade.
Fc-receptor blocked culture-derived macrophages were pre-treated for 30 minutes with 10μg/ml of the monoclonal antibodies FA6-152 (anti-CD36), 15.2 (anti-ICAM-1), 23C6 (anti-ανβ3), C6.7 (anti-TSP), or a combination of FA6-152, 23C6, and C6.7. Receptor blockade was followed by a standard phagocytosis assay (see methods). Phagocytic index was quantitated microscopically. P<0.01 (Student's t) comparing control to FA6-152 treated, and control to FA6-152 plus 23C6 plus C6.7 treated. There was no significant difference in phagocytosis between CD36 blockade alone and combination blockade of CD36, ανβ3 and TSP.

Fc-receptor blocked culture-derived macrophages were pre-treated for 30 minutes with 0.1mM PS containing liposomes, PC containing liposomes or PI containing liposomes, or with 10μg/ml of monoclonal antibody FA6-152 prior to exposure to non-opsonised PEs (see methods). Phagocytic index was quantitated microscopically. Liposome preparations did not inhibited phagocytosis of PEs.

*Up-regulating CD36 in monocytes and macrophages resulted in increased clearance of non-opsonised PEs.*

Our observations that CD36 is a major phagocytic receptor for non-opsonized PEs (McGilvray., 2000) and that monocyte-derived mφs express more CD36 and have increased phagocytic capacity for PEs, prompted us to determine whether pharmacologic up-regulation of CD36 would increase phagocytic removal of non-opsonised PEs.

We treated monocytes treated with the PPARγ agonists 15d-PGJ2 (5μM) or ciglitazone (3μM-100μM) a member of the thiazolidinedione (TZD) family of drugs, and the RXR agonists methoprene acid (MA, 10μM) or 9-cis-retinoic acid (9-cis-RA, 1μM) and assessed CD36 surface levels by flow cytometry. Monocytes treated overnight with
15d-PGJ2 and a RXR agonist or ciglitazone alone expressed 40-60% higher levels of CD36 compared to similarly treated controls. Similar up-regulation was observed in treated THP-1 cells. This increase in surface level of CD36 was associated with a 2-fold or greater increase in the phagocytic clearance of non-opsonised PEs over controls (P < 0.01). Treated monocytes that internalized at least one PE increased 40-60%, and 30% more PEs were internalized per phagocytic positive monocyte compared to 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. Inhibition levels ranged from ~50-70% (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.

**PPARγ and RXR agonists up-regulate CD36 expression in human monocytes and increase their phagocytic capacity for non-opsonised PEs.**

Adherent human monocytes were treated with increasing doses of ciglitazone (3-100μM), or appropriate controls for 24 hours. CD36 surface levels were analysed by flow cytometry following staining with FA6-152. Mean fluorescence of ciglitazone-treated was compared to control-treated monocytes. Phagocytic index was determined as described in the methods. Phagocytic index of ciglitazone-treated was compared to control-treated monocytes.

Increases in monocyte surface levels of CD36 and non-opsonic phagocytosis demonstrated a dose-response relationship to treatment with ciglitazone (3-100μM).

We also demonstrated that 9 cis-retinoic acid alone up-regulates macrophage CD36 levels and CD36 dependent phagocytosis of non-opsonised *P. falciparum* parasitised erythrocytes.
Monocytes were plated on glass coverslips, aged in culture for 4 days, and then treated with 1uM 9-cis-RA or appropriate controls for 48 hours.

CD36 expression was determined by staining the macrophages with the monoclonal FITC conjugated anti-CD36 antibody FA6-152, followed by flow cytometric analysis. Control and 9-cis-RA treated macrophages were exposed to a 20:1 ratio of non-opsonised *P. falciparum* parasitised erythrocytes for 4 hours at 37C. Fc receptors were blocked using Fc fragments. All serum used was heat inactivated to remove complement. In some cases CD36 was blocked using 10ug/ml of the anti-CD36 monoclonal antibody FA6-152. Following the 4 hours incubation, non-internalised PEs were removed by hypotonic lysis, and the coverslips were fixed and stained. Phagocytosis was quantitated microscopically. The phagocytic index was significantly higher in 9-cis-RA treated verses control macrophages, P<0.014, N=8, Student’s t. CD36 blockade significantly reduced the phagocytic index of both control (P<0.018, N=8, Student’s t) and 9-cis-RA treated macrophages (P<0.0003, N=8, Student’s t).

*P. falciparum*-induced TNFα secretions were also reduced by 9-cis-retinoic acid.

Freshly isolated monocytes were co-treated for 24 hours with 1uM 9-cis-RA or appropriate controls plus either 100nM PMA, or 100ul/ml of culture supernatants of the *P. falciparum* clone ITG or of a patient isolate. Supernatants were collected and analysed for TNFα by ELISA. Treatment with 9-cis-RA significantly reduced the level of TNFα secreted in response to culture supernatants as compared to controls.

The ability of PPARγ and RXR agonists to increase CD36 expression and phagocytic capacity was not limited to freshly isolated monocytes. Monocyte-derived mφs treated with 15d-PGJ2 plus 9-cis-RA had increased CD36 surface levels and a corresponding increase in non-opsonic phagocytosis with a ~70% increase in ingested
PEs in treated macrophages. Similar to untreated mφs, phagocytosis of non-opsonized PEs by PPARγ-RXR-treated mφs was inhibited by mAb blockade of CD36 and by cleaving the CD36 ligand from the PEs and not by receptor blockade of αvβ3, TSP, and ICAM-1, or by pre-incubation with PS, PC, or PI containing liposomes.

Culture-derived macrophages were treated with 5μM 15d-PGJ2 plus 1μM 9-cis-retinoic acid or DMSO controls for 24 hours, and then were used in PE phagocytosis assays. P<0.01 (Student’s t) comparing phagocytic index in control vs. treated macrophages.

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

Elevated levels of pro-inflammatory cytokines such as TNFα have been correlated with disease severity and a poor prognosis suggesting that excessive secretion of TNFα by monocytes/mφs in response to parasite products promote severe and cerebral malaria (Grau 1989, Allan 1995, McGuire 1994). PPARγ agonists have been shown to reduce PMA-induced but not LPS-induced pro-inflammatory cytokine secretion from monocytes (Jiang 1998). We examined whether PPARγ agonists would inhibit *P. falciparum*-induced TNFα 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 TNFα release from monocytes (McGilvray 2000). However, human monocytes and THP-1 cells exposed to parasite culture supernatants containing parasite glycosylphosphatidylinositol (GPI) toxins released during schizont rupture (Schofield 1993), do secrete TNFα. Monocyte TNFα secretion induced by PMA, was significantly inhibited by co-treatment of cells with PPARγ-RXR agonists, while LPS-induced TNFα secretion was unaffected by these
compounds. Co-treatment of monocytes or THP-1 cells with 15d-PGJ2 and 9-cis-RA, plus a 1:10 dilution of parasite culture supernatants resulted in a significant decrease in TNFα production compared to controls. PPARγ agonist-induced inhibition of TNFα was not limited to the laboratory clone ITG, but also occurred with wild isolates (P1 and P2).

Treating THP-1 cells with increasing doses of 15d-PGJ2 (5-15μM) resulted in a dose response inhibition of PMA and parasite-induced TNFα production.

THP-1 cells were exposed to 1μg/ml LPS, 50nM/ml PMA, or a 1:10 dilution of various P. falciparum culture supernatants (ITG, P1 and P2) and TNFα production was assayed (see methods for details). Cells included DMSO treated controls and cells treated with 5μM 15d-PGJ2 plus 1μM 9-cis-retinoic acid. Significant decreases in TNFα secretion were observed in the PPARγ-RXR treated cells exposed to PMA and the culture supernatants (P<0.05; Student’s t). Experiments were performed in triplicate and data obtained was representative of at least 3 independent experiments.

Adherent human monocytes were treated as the THP-1 cells above and assayed for TNFα production. A significant decrease in TNFα secretion was observed in monocytes treated with PPARγ agonists and exposed to PMA and P. falciparum culture supernatants (P<0.05, Student’s t). Experiments were performed in triplicate and data obtained was representative of at least 3 independent experiments.

Discussion:

Despite decades of research, no new treatments have been identified that improve the outcome of patients with severe or cerebral malaria (White 1998). We demonstrated that monocyte/mφ CD36 participates in a novel phagocytic pathway for parasitized erythrocytes (PEs) that is distinct from that utilized in the clearance of apoptotic cells. Further, we demonstrated that up-regulation of monocyte/mφ CD36 resulted in increased
phagocytic clearance of PEs and decreased TNFα secretion by monocytes in response to malaria GPI toxins. Together these findings demonstrate that pharmacologic up-regulation of monocyte/mφ CD36 with PPARγ-RXR agonists represents a novel therapeutic strategy or adjunctive therapy to prevent or treat severe malaria.

In this study, we confirmed that the majority of non-opsonic monocyte/mφ phagocytosis is dependent on CD36. We demonstrated that up-regulation of CD36 expression on monocytes via activation of the transcription activator heterodimer PPARγ-RXR by natural and engineered ligands, results in increased phagocytic uptake of non-opsonized PEs. This increase in phagocytic uptake is CD36-dependent since it can be inhibited by FA6-152 an anti-CD36 monoclonal antibody and since it is abrogated by cleavage of the PE ligand for CD36 (Barauch 1997). Although we do not exclude the possibility of a co-receptor co-operating with CD36 in the phagocytosis of PEs, αβ3 and TSP do not appear to be involved as co-receptors. αβ3 and TSP have been shown to co-operate with CD36 in the phagocytic clearance of apoptotic cells (Savill 1989).

However, in contrast to apoptotic cells, antibodies against αβ3 and TSP do not inhibit the phagocytosis of PEs, either singly or in combination. Nor do these antibodies synergistically inhibit PE phagocytosis when used together with anti-CD36 antibodies. Thus, CD36-mediated phagocytosis of PEs is distinct from that mediating phagocytosis of apoptotic cells.

Our data support a protective role for CD36 in the host-parasite interaction. Although the great majority of wild isolates of falciparum malaria adhere to CD36, only ~1-2% of infected patients develop severe malaria or die. CD36 expression in the brain is very low to absent (Turner 1994) and thus cytoadherence to CD36 is unlikely to account for cerebral sequestration. However, several other receptors support
sequestration on falciparum infected erythrocytes, and many of these such as ICAM-1 and CD31 are expressed on brain endothelium and their expression is up-regulated by exposure to pro-inflammatory cytokines such as TNFα.

High TNFα levels correlate with a poor clinical outcome. This may be due, in part, to the TNFα-induced up-regulation of these sequestration receptors in endothelium in the brain. TNFα secretion during falciparum malaria infection has been associated with a parasite-derived glycosylphosphatidylinositol (GPI) "toxin" which stimulates TNFα from monocytes/mφs by a pathway that differs from that of LPS-induced TNFα production. We demonstrated that PPARγ-RXR agonists inhibit parasite GPI-induced TNFα secretion by monocytes and THP-1 cells.

Jiang and colleagues have recently shown that PPARγ-RXR agonists inhibit pro-inflammatory cytokine secretion from PMA-activated but not LPS-activated monocytes (Jiang 1998) suggesting that parasite-derived GPI may induce TNFα secretion by a similar pathway as PMA. These observations have important clinical implications and our data support the conclusion that adjunctive therapy with PPARγ-RXR agonists will reduce amounts of TNFα secreted in response to malaria infection. This will decrease expression of ICAM-1 and other endothelial cell receptors on brain endothelial cells and divert sequestration away from this and other vital organs.

Demonstrating that CD36 expressed on monocytes/mφs mediates non-opsonic phagocytic removal of PEs assigns a protective role for CD36 in a malaria infection. The use of PPARγ agonists such as 15d-PGJ2 and ciglitazone to up-regulate CD36 on monocytes/mφs and thus increase non-opsonic clearance of PEs represents a novel therapeutic strategy for falciparum malaria. Although we have only examined the effect of PPARγ-RXR agonists such as ciglitazone, on the up-regulation of CD36 in monocytic
cells, these compounds may also up-regulate CD36 in other cells. CD36 is primarily expressed by monocytes and by mφs of the reticuloendothelial system (RES), especially the liver and spleen, where the majority of tissue resident macrophages reside and by endothelial cells of the RES and such organs as the skin, muscle, and heart.

Sequestration of PEs in the RES should enhance their clearance. Similarly, up-regulating CD36 in microvascular beds of non-vital organs such as skin or muscle would not endanger a patient.

TZD drugs are currently in clinical trials for the management of type II diabetes. These agents have been well tolerated in these studies. The commercial availability of TZD compounds will expedite the in-vivo testing of their effectiveness in enhancing phagocytic clearance of parasitized erythrocytes and reducing TNFα levels. TZDs and or other PPARγ-RXR ligands will represent a new adjunctive short term therapy for severe falciparum infection (Vamecq 1999, Henke 1998).

CD36 may also been implicated in the outcome of malaria in pregnancy. Semi-immune women who become pregnant often have poor clinical outcomes with falciparum malaria despite having controlled many previous falciparum infections with minimal symptoms (Duffy 1996). This has been attributed to the presence of parasite isolates which bind chondroitin sulfate A (CSA), a sequestration receptor primarily found in the placenta. Of note, parasite lines which bind CSA are unique among falciparum isolates in that they do not also bind CD36. Our data demonstrates that the inability to control CSA-binding parasites may be attributable, in part, because they do not adhere to CD36 and thus avoid CD36-mediated phagocytic clearance and decreased pro-inflammatory cytokine responses.

We also demonstrated that CD36 is the major receptor mediating the
phagocytosis of non-opsonized parasitized erythrocytes (PEs) by monocytes and culture-derived macrophages (mφs). This clearance mechanism is of potential relevance to those at greatest risk of severe disease including non-immune patient populations and semi-immune individuals infected with a falciparum PfEMP-1 variant to which they lack an opsonizing or specific immune response. CD36-PE uptake occurs via a novel phagocytic pathway that is distinct from the co-operative αvβ3-TSP-CD36 mechanism involved in the uptake of apoptotic cells and does not appear to involve phosphatidyl-serine recognition. Furthermore, we demonstrated that up-regulation of CD36 expression on monocytes and mφs by activation of the transcription activator heterodimer PPARγ-RXR by natural and engineered ligands, increases phagocytic uptake of non-opsonized PEs and PPARγ-RXR activation decreases monocyte secretion of TNFα in response to malaria GPI toxins. Therefore, specific pharmacologic up-regulation of monocyte/mφ CD36 with PPARγ-RXR agonists represents a therapeutic strategy to prevent, or improve the outcome of, severe malaria.

Since most natural parasite isolates bind CD36, it has been considered a target for anti-sequestration therapy (Ockenhouse 1998, Baruch 1997, Cooke 1998). However, our data 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 finding. Although almost all wild isolates of falciparum-malaria adhere to CD36, only a minority of infected non-immune patients develop cerebral or severe malaria. CD36 expression in the brain is low to absent (Turner 1994) and cytoadherence to CD36 is unlikely to account for cerebral sequestration. CD36 is however well-expressed in microvascular endothelial cells from sites such as skin and muscle (Turner 1994). CD36-mediated cytoadherence directs parasites to these non-vital sites and away from cerebral
microvasculature. Sequestration in peripheral sites such as skin facilitates transmission (Silamut 1999) while at the same time does not compromise host survival. These findings are supported by Newbold and colleagues, who have reported that significantly higher binding to CD36 occurs in cases of non-severe disease (Newbold 1999). More recent population data has linked CD36 deficiency with an increased susceptibility to severe and cerebral malaria (Aitman 2000). Taken together, we conclude that the CD36-PE interaction is a parasite-host adaptation, evolved for improved survival of the parasite with consequent reduced injury to the host (down-regulated pro-inflammatory response; parasite replication balanced by host clearance).

Several other receptors support sequestration of PEs, and many of these such as ICAM-1 and CD31 are expressed on brain endothelium and are up-regulated by pro-inflammatory cytokines such as TNFα (Berdnt 1989, Treutiger 1997, Schofield 1996, Kwiatkowski 1990, Brown 1999, Day 1999, Dobbie 1999). TNFα secretion during infection has been associated with a parasite-derived GPI “toxin” which stimulates TNFα release from monocytes and mφs. We demonstrated that PPARγ-RXR agonists inhibit parasite-induced TNFα secretion by monocytes and THP-1 cells. PPARγ agonists inhibit pro-inflammatory cytokine secretion from PMA-activated but not LPS-activated monocytes (Jiang 1998) GPI induces TNFα secretion by a similar pathway. In addition, recent work has shown that PPARγ agonists also inhibit TNFα-induced ICAM-1 expression (Pasceri 2000).

These observations have important clinical implications. Adjunctive therapy with PPARγ-RXR agonists reduces excessive pro-inflammatory cytokine secretion and ICAM-1 up-regulation 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, macrophages, adipocytes, myocytes, and endothelial cells of the skin and reticuloendothelial system, especially the liver and spleen (Turner 1994). Increased sequestration in these sites would not be expected to endanger infected patients, since binding within the reticuloendothelial system should theoretically enhance clearance and binding to CD36 in non-vital sites might confer protection against severe and cerebral disease (Newbold 1999, Aitman 2000, McGilvray 2000). We determined whether these agents up-regulate CD36 in microvascular endothelium of vital organs such as the brain. We have observed that treatment of the immortalized human brain endothelial cell line WR-1 (kind gift of Dr. C. Ockenhouse, Walter Reed Army Institute of Research) with PPARγ-RXR agonists did not increase CD36 expression. The effect of PPARγ activation in endothelial cells from a variety of sites was observed to ensure that CD36-mediated cytoadherence in vital organs will not be increased.

Shankar and colleagues reported that vitamin A supplementation decreased the parasitemia and morbidity of falciparum-malaria in children, particularly among the youngest and most likely to be non-immune (Shankar 1999). The mechanism by which vitamin A improved outcome was unknown. We demonstrated that PPARγ agonists with 9-cis-retinoic acid (the sole natural ligand for RXR and a metabolite of vitamin A) resulted in increased CD36 expression and decreased TNFα secretion. Although the PPARγ-RXR heterodimer is most active when both partners are ligand bound, activation can occur with an RXR ligand alone (Kliwer 1992) and our unpublished observations). Thus, the protective effect of vitamin A observed is, at least in part, attributable to PPARγ-RXR activation and up-regulation of CD36. Additional evidence comes from the
work of Foote and colleagues who mapped a genetic locus that influences malaria parasitemia and mortality in mice to a region of chromosome 9 that encodes retinol binding proteins involved in the metabolism of vitamin A and the generation of RXR ligands (Foote 1997). Thus, vitamin A metabolites activating the PPARγ-RXR heterodimer increase CD36-mediated clearance and decrease pro-inflammatory cytokine release, providing a putative mechanism for the protective role of vitamin A in malaria.

In summary, this invention establishes that CD36 is a major receptor mediating non-opsonic clearance of PEs, which is important in non-immune hosts who are at greatest risk of severe and cerebral malaria. We also demonstrated that up-regulation of monocyte/mφ CD36 via PPARγ-RXR activation resulted in an increase in the CD36-mediated phagocytosis of PEs and a decrease in parasite-induced TNFα production. Pharmacologic up-regulation of monocyte/mφ CD36 represents a novel way to immunomodulate host defense and a new strategy to prevent or treat severe falciparum-malaria. Since several PPARγ agonists are approved for human use, and can be used in clinical trials for this purpose (Toronto 1998, Kersten 2000)

We are now initiating randomized placebo controlled trials of PPARγ-RXR agonists in the treatment of falciparum malaria. The first phase of this work is a pilot study examining an FDA approved TZD compound (rosiglitazone) in the management of uncomplicated falciparum malaria. Adults with uncomplicated falciparum malaria are being randomized to standard antimalarial therapy plus placebo or standard therapy plus rosiglitazone (once daily) for 5 days. Measured endpoints will be fever and parasite clearance times, and TNFα levels. Safety and tolerance is being assessed including blood glucose and liver function tests.

Providing there are no untoward effects in this trial, we will initiate randomized
placebo controlled trials to examine the ability of PPARγ-RXR agonists to treat severe falciparum malaria. Treatment trials will examine consecutive patients with severe falciparum malaria (as defined by the World Health Organization [Warrell 1990]) randomized to standard therapy plus placebo or standard therapy plus rosiglitazone (once daily for 5 days) or other suitable orally or parenterally administered PPARγ-RXR agonist (reviewed in Vamecq 1999, Henke 1998, Brown 1999). Primary endpoint will be survival rates in each of the treatment arms. Secondary endpoints will be fever and parasite clearance times, TNFα levels, and long term morbidity in survivors. This trial will have a power of 80% for detecting a 50% decrease in mortality between the treatment groups and labeling it as statistically significant with a two tailed α of 0.05 (n = 219 per group).

In view of results described above, products which activate PPARγ-RXR or upregulate monocyte/mφ CD36 can be selected and designed in the manufacture of pharmaceutical compositions for the treatment of malaria. The pharmaceutical compositions can be administered to patients by methods known to those skilled in the art, such as oral administration, aerosol administration, direct lavage and parenteral injection. Dosages to be administered depend on patient needs, on the desired effect and on the chosen route of administration. Synthetic or natural ligands of PPARγ-RXR or genes (DNA) encoding these products or CD36, may also be introduced using in vivo delivery vehicles such as liposomes, infection or transfection with live or plasmid recombinant vectors, and cationic lipids. They may also be introduced into these cells using physical techniques such as microinjection and electroporation or chemical methods such as coprecipitation and incorporation of DNA into liposomes.

The pharmaceutical compositions can be prepared by known methods for the
preparation of pharmaceutically acceptable compositions which can be administered to patients, and such that an effective quantity of the products are combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, USA 1990).

On this basis, the pharmaceutical compositions could include an active ingredient, in association with one or more pharmaceutically acceptable vehicles, such as carriers, excipients or diluents, and contained in buffered solutions with a suitable pH and isoosmotic with the physiological fluids. The methods of combining suitable products with the vehicles is well known to those skilled in the art. The composition could include a targeting agent for the transport of the active compound to specified sites within tissue.

*In vitro* such peptides or vectors may be administered by infection, microinjection, electroporation and by other methods known in the art.

We recommend oral medications for administration of the PPARγ-RXR ligands, peptides or peptide mimetics. For those unable to take oral medications, *in vivo* parenteral administration of the PPARγ-RXR ligands, peptides or peptide mimetics is preferred with subdermal or intramuscular administration most preferred. Intravenous administration or use of implanted milliosmol pumps (available from Alza) may also be used.

When used for parenteral administration, the pharmaceutical compositions of the present invention may be formulated in a variety of ways. Aqueous solutions of composition of the present invention may be encapsulated in polymeric beads, liposomes,
nanoparticles or other injectable depot formulations known to those of skill in the art. (Examples thereof may be found, for example, in Remington's Pharmaceutical Sciences, 18th Edition, 1990.) Doses are selected to provide effective activation of PPARγ-RXR and upregulation of monocyte/mφ CD36.

Compositions including a liquid pharmaceutically inert carrier such as water may also be considered for both parenteral and oral administration. Other pharmaceutically compatible liquids may also be used. The use of such liquids is well known to those of skill in the art. (Examples thereof may be found, for example, in Remington's Pharmaceutical Sciences, 18th Edition, 1990.)

The dose level and schedule of administration may vary depending on the particular product used, the method of administration, and such factors as the age and condition of the patient.

As discussed previously, parenteral administration is preferred for ill patients, but formulations may also be considered for other means of administration such as orally, per rectum, and transdermally. The usefulness of these formulations may depend on the particular compound used and the particular patient receiving the compound.

Oral formulations of products may optionally and conveniently be used in compositions containing a pharmaceutically inert carrier, including conventional solid carriers, which are conveniently presented in tablet or capsule form. Formulations for rectal or transdermal use may contain a liquid carrier that may be oily, aqueous, emulsified or contain certain solvents suitable to the mode of administration. Suitable formulations are known to those of skill in the art. (Examples thereof may be found, for example, in Remington's Pharmaceutical Sciences, 18th Edition, 1990.)
Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated to those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. All modifications coming within the scope of the following claims are claimed.

All publications, patents and patent applications referred to in this application are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.
References:


adhesion molecule 1 (ICAM-1) is an endothelial cytoadherence receptor for 

Posttranscriptional regulation of macrophage tissue factor expression by 


reverses the diabetic phenotype of the Zucker diabetic fatty rat. Diabetes 48;1415-24 
(1999).

Luzzatto L, Arese P. 1998. Early phagocytosis of glucose-6-phosphate 
dehydrogenase (G6PD)-deficient erythrocytes parasitized by Plasmodium falciparum 
may explain malaria protection in G6PD deficiency. Blood 92(7):2527-34.

isolates from Thailand: Evidence for chondroitin sulfate as a cytoadherence 

PFEMP-1 blocks and reverses adhesion of malaria-infected red blood cells to CD36 

on the adherent behaviour of Plasmodium falciparum-infected erythrocytes. 
Parasitology 113:317.


27. Howard R.J. and Gilladoga A.D. Molecular studies related to the pathogenesis of


36. Knight J.C. *et al*. A polymorphism that affects OCT-1 binding to the TNF promoter


and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis, J Clin Invest 90:1513.


WE CLAIM:

1. A composition for the treatment of malaria, comprising a product which activates PPARγ-RXR or upregulates monocyte CD36 in admixture with a pharmaceutically acceptable carrier, excipient or diluent.

2. The composition of claim 1, wherein the product comprises a PPARγ-RXR agonist.

3. The composition of claim 1, wherein the product is selected from one or more members of a group consisting of a PPARγ agonist, a RXR agonist and natural or synthetic ligands thereof.

4. The composition of claim 3, wherein the product is selected from one or more members of a group consisting of prostaglandin A, prostaglandin D, prostanoid 15-deoxy-Δ^{12,14}-prostaglandin J2 (15d-PGJ2), thiazolidinedione (TZD) class of antidiabetic drugs, non-steroidal-anti-inflammatory drugs, L-tyrosine-based agonists, cytokines, lipoproteins and their components, polyunsaturated fatty acids, methoprene acid, vitamin A, and vitamin A metabolites, and compounds which are inhibitors of antagonists of PPARγ or RXR and a mimetic of any of the foregoing.

5. The composition of claim 4, wherein the thiazolidinedione (TZD) class of antidiabetic drugs are selected from one or members of a group consisting of ciglitazone, troglitazone, pioglitazone, rosiglitazone, englitazone, and a mimetic of any of the foregoing.

6. The composition of claim 4, wherein the non-steroidal-anti-inflammatory drugs (NSAIDS) are selected from one or more members of a group consisting of indomethacin, flufenamic acid, fenoprofen, ibuprofen.
7. The composition of claim 4, wherein the L-tyrosine-based agonist is GW1929.

8. The composition of claim 4, wherein the cytokines are selected from one or more members of a group consisting of IL-4, GM-CSF, M-CSF.

9. The composition of claim 4, wherein the lipoproteins and their components are selected from one or more members of a group consisting of oxidized LDL, 13-hydroxyoctadecadienoic acid [13-HODE], 15-hydroxyeicosatetraenoic acid [15-HETE].

10. The composition of claim 4, wherein the vitamin A metabolite is selected from one or more members of a group consisting of 9-cis-retinoic acid, BRL49653, LG100268, LGD49653 and a mimetic of any of the foregoing.

11. The composition of claim 2, wherein the PPARγ-RXR agonist is derived from natural or engineered ligands.

12. A method for treating or preventing severe infection by P. falciparum malaria in a person comprising administering to a patient in need thereof a composition as claimed in claims 1-11.

13. The method of claim 12, wherein the composition causes an increase in the CD36 mediated phagocytosis of parasitized erythrocytes.

14. The method of claim 12, wherein the composition causes a decrease in parasite-induced TNFα production.

15. A method for treating or preventing malaria in a mammal, comprising the steps of:

(a) forming a composition by combining one or more of a PPARγ agonist, an RXR agonist and a PPARγ -RXR agonist with an acceptable carrier,
excipient or diluent; and

(b) administering an effective dose of the composition to the mammal.

16. The method of claim 15, wherein the product is selected from one or more members of a group consisting of prostaglandin A, prostaglandin D, prostanoid 15-deoxy-\(\Delta^{12,14}\)-prostaglandin J2 (15d-PGJ2), thiazolidinedione (TZD) class of antidiabetic drugs, non-steroidal-anti-inflammatory drugs, L-tyrosine-based agonists, cytokines, lipoproteins and their components, polyunsaturated fatty acids, methoprene acid, vitamin A, and vitamin A metabolites, and compounds which are inhibitors of antagonists of PPAR\(\gamma\) or RXR and a mimetic of any of the foregoing.

17. The method of claim 16, wherein the thiazolidinedione (TZD) class of antidiabetic drugs are selected from one or members of a group consisting of ciglitazone, troglitazone, pioglitazone, rosiglitazone, englitazone, and a mimetic of any of the foregoing.

18. The method of claim 16, wherein the non-steroidal-anti-inflammatory drugs (NSAIDS) are selected from one or more members of a group consisting of indomethacin, flufenamic acid, fenoprofen, ibuprofen.

19. The method of claim 16, wherein the L-tyrosine-based agonist is GW1929.

20. The method of claim 16, wherein the cytokines are selected from one or more members of a group consisting of IL-4, GM-CSF, M-CSF.

21. The method of claim 16, wherein the lipoproteins and their components are selected from one or more members of a group consisting of oxidized LDL, 13-hydroxyoctadecadienoic acid [13-HODE], 15-hydroxyeicosatetraenoic acid [15-
22. The method of claim 16, wherein the vitamin A metabolite is selected from one or more members of a group consisting of 9-cis-retinoic acid, BRL49653, LG100268, LGD49653 and a mimetic of any of the foregoing.

23. The method of claim 16, wherein the PPARγ-RXR agonist is derived from natural or engineered ligands.

24. A method of up-regulating CD36 in a mammal, comprising the steps of:
   (a) forming a composition by combining one or more of a PPARγ agonist, a RXR agonist and a PPARγ-RXR agonist with an acceptable carrier, excipient or diluent; and
   (b) administering an effective dose of the composition to the mammal.

25. A method for identifying a product for the treatment or prevention of malaria, the method comprising the steps of:
   (a) contacting a product with a PPARγ-RXR agonist;
   (b) determining whether the product up-regulates CD36, wherein the up-regulation indicates that the product is useful for the treatment or prevention of malaria.

26. A kit for the treatment of malaria comprising a product which upregulates monocyte/mφ CD36 in admixture with a pharmaceutically acceptable carrier, excipient or diluent.

27. The kit of claim 26, wherein the product comprises a PPARγ-RXR agonist.

28. The kit of claim 26, wherein the product comprises a PPARγ agonist and or a RXR agonist.
29. The kit of claim 26, wherein the product is selected from one or more members of a group consisting prostaglandin A, prostaglandin D, prostanoid 15-deoxy-\(\Delta^{12,14}\)-prostaglandin J2 (15d-PGJ2), thiazolidinedione (TZD) class of antidiabetic drugs, non-steroidal-anti-inflammatory drugs, L-tyrosine-based agonists, cytokines, lipoproteins and their components, polyunsaturated fatty acids, methoprene acid, vitamin A, and vitamin A metabolites, and compounds which are inhibitors of antagonists of PPAR\(\gamma\) or RXR and a mimetic of any of the foregoing.

30. The kit of claim 26, wherein the thiazolidinedione (TZD) class of antidiabetic drugs are selected from one or members of a group consisting of ciglitazone, troglitazone, pioglitazone, rosiglitazone, englitazone, and a mimetic of any of the foregoing.

31. The kit of claim 26, wherein the non-steroidal-anti-inflammatory drugs (NSAIDS) are selected from one or more members of a group consisting of indomethacin, flufenamic acid, fenoprofen, ibuprofen.

32. The kit of claim 26, wherein the L-tyrosine-based agonist is GW1929.

33. The kit of claim 26, wherein the cytokines are selected from one or more members of a group consisting of IL-4, GM-CSF, M-CSF.

34. The kit of claim 26, wherein the lipoproteins and their components are selected from one or more members of a group consisting of oxidized LDL, 13-hydroxyoctadecadienoic acid [13-HODE], 15-hydroxyeicosatetraenoic acid [15-HETE].

35. The kit of claim 26, wherein the vitamin A metabolite is selected from one or
more members of a group consisting of 9-cis-retinoic acid, BRL49653, LG100268, LGD49653 and a mimetic of any of the foregoing.

36. The kit of claim 26, wherein the PPARγ-RXR agonist is derived from natural or engineered ligands.

37. The kit of claim 26, wherein the RXR agonist comprises an agent selected from one or more members of a group consisting of methoprene acid, vitamin A, and vitamin A metabolites, and a mimetic of any of the foregoing.