The pathogenesis of malaria: a new perspective

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With 3.3 billion people at risk of infection, malaria remains one of the world's most significant health problems. Increasing resistance of the main causative parasite to currently available drugs has created an urgent need to elucidate the pathogenesis of the disease in order to develop new treatments. A possible clue to such an understanding is that the malaria parasite *Plasmodium falciparum* selectively absorbs vitamin A from the host and appears to use it for its metabolism; serum vitamin A levels are also reduced in children with malaria. Although vitamin A is essential in low concentration for numerous biological functions, higher concentrations are cytotoxic and pro-oxidant, and potentially toxic quantities of the vitamin are stored in the liver. During their life cycle in the host the parasites remain in the liver for several days before invading the red blood cells (RBCs). The hypothesis proposed is that the parasites emerge from the liver packed with vitamin A and use retinoic acid (RA), the main biologically active metabolite of vitamin A, as a cell membrane destabilizer to invade the RBCs throughout the body. The characteristic hemolysis and anemia of malaria and other symptoms of the disease may thus be manifestations of an endogenous form of vitamin A intoxication associated with high concentrations of RA but low concentrations of retinol (ROL). Retinoic acid released from the parasites may also affect the fetus and cause preterm birth and fetal growth restriction (FGR) as a function of the membranolytic and growth inhibitory effects of these compounds, respectively. Subject to testing, the hypothesis suggests that parasite vitamin A metabolism could become a new target for the treatment and prevention of malaria.

Keywords: Malaria, Retinoids, Liver, Parasites, Plasmodium, Anemia, Toxicity, Erythrocyte, Prematurity, Fetal growth restriction, Pathogenesis, Epidemiology

Introduction

About 3.3 billion people – half the world's population – are at risk of malaria due to the main causative parasite, *Plasmodium falciparum*.¹ Over 200 million cases of malaria occur each year, 90% of which occur in Africa, and 655 000 people died from the disease in 2010, making it one of the world's most important health problems. Increasing resistance of the parasite to currently available drugs has created an urgent need to discover new treatments.² However, this requires an improved understanding of the pathogenesis of malaria.³

Malarial infection begins when a person is bitten by an infected female anopheles mosquito and *Plasmodium* spp (species) parasites in the form of sporozoites are injected into the bloodstream. The sporozoites travel to the liver, multiplying asexually over the next 7–10 days. During this time there are no symptoms. The parasites, now in the form of merozoites, emerge from the liver cells in vesicles and travel through the heart to the capillaries of the lungs.

The vesicles eventually disintegrate, releasing the merozoites to enter the bloodstream where they invade and multiply in erythrocytes. When the cells burst, the parasites invade more erythrocytes. Clinical symptoms, including fever, occur in synchrony with the rupture of infected erythrocytes and the release of erythrocyte and parasite debris, including malarial pigment (hemozoin) and glyco-phosphatidylinositol, the putative 'malaria toxin'.^{4,5} In some infected blood cells, instead of replicating asexually, the merozoites develop into sexual forms (gametocytes), which circulate in the bloodstream and are ingested during mosquito bites. The ingested gametocytes develop in the mosquito into mature sex cells (gametes) which develop into ookinetes that actively burrow through the mid-gut wall of the mosquito and form oocysts, in which develop thousands of active sporozoites. The oocyst eventually bursts, releasing sporozoites that travel to the salivary glands of the mosquito. The cycle of human infection begins again when the mosquito bites another person.³

The associated symptoms of malaria include severe anemia, fever, thrombocytopenia, chills, headache,

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vomiting, muscle ache, anorexia, rigor, diarrhea, abdominal discomfort, cough, seizures, respiratory distress, hypoglycemia, metabolic acidosis, hyperlactemia, coma associated with increased intracranial pressure (cerebral malaria), retinopathy, and complications of pregnancy, including preterm birth and low birth weight due to fetal growth restriction (FGR). As noted, the symptoms are associated with the rupture of the infected erythrocytes and the release of putative malaria toxins, which activate peripheral blood mononuclear cells and stimulate the release of cytokines. It is believed that the balance between pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors, and effector molecules determines disease severity.⁶ Studies have reported increased IL-1B, IL-6, IL-8, and TNF-alpha in late-onset severe disease, and a low IL-10:TNF-alpha ratio. The role of cytokines, however, remains contradictory and unclear.³ Alterations in retinoids (vitamin A and its congeners) also occur in malaria, but the precise role of retinoids in the disease may be different and even opposite to the traditional focus on vitamin A deficiency and supplementation. This paper presents a new theory on the pathogenesis of malaria, suggesting that an endogenous form of hypervitaminosis A induced by the parasite contributes significantly to the signs and symptoms of the disease.

Vitamin A and Malaria

Malarial infection is accompanied by reductions in serum vitamin A concentrations from ≥ 120 mmol/l to ≤ 70 μ mol/l (< 20 μ g/dl), a level usually taken to indicate deficiency in children.⁷

In rodent models of malaria there is an inverse correlation between parasitemia and host vitamin A.⁸ Reduced serum vitamin A levels are also found consistently in children with malaria.⁹ Such observations have led to the suggestion that *P. falciparum* uses vitamin A from the host for its metabolism;¹⁰ in fact, *P. falciparum* selectively absorbs vitamin A from host tissues. This selective uptake of vitamin A was shown by Mizuno *et al.*¹¹ in a study in which a standard isolate of the parasite was cultured with ³H-labeled vitamin A at concentrations of the vitamin normally present in human serum. The ³H-labeled vitamin A accumulated in the parasites in a parasitemia-dependent manner. Radioactivity levels detected in the parasites, found mostly in the cytoplasm, also increased with parasite maturation from the ring to the late trophozoite stage.

Vitamin A supplementation appears to have a protective effect against the disease. For instance, a randomized double-blind, placebo-controlled trial of vitamin A supplementation on morbidity prevention in children in a malaria-endemic area of Papua New

Guinea, showed that supplementation every three months for 13 months led to a 30% reduction in the number of confirmed *P. falciparum* febrile episodes, compared to the placebo group, and a 68% decrease in parasite density.¹² A more recent randomized controlled trial on the effect of a single dose of 200 000 IU of vitamin A with daily zinc supplementation for six months on children in Burkina Faso resulted in a significant 30% reduction in slide-confirmed malaria fevers.¹³ One mechanism suggested for the parasite density-lowering effect is an upregulation of the phagocytotic receptor CD36 and downregulation of cytokines such as TNF-alpha by binding of 9-cis-retinoic acid (RA) to the peroxisome proliferator-activated receptor gamma (PPARgamma) or Retinoid-X-Receptor (RXR).¹⁴ However, while vitamin A supplementation reduces the incidence of uncomplicated malaria by about one-third, it does not appear to reduce the risk of deaths that can be specifically attributed to malaria.⁹ The therapeutic potential of vitamin A is also complicated by the fact that retinol (ROL) antagonizes the antimalarial effect of artemisinin.¹⁵ Reversal of the effect of a major drug treatment for malaria suggests that vitamin A may in some way contribute to the disease.

Hamzah *et al.*¹⁶ investigated the role of retinoids as potential antimalarial agents, assessing the effect of all-trans-RA, 9-cis-RA, and 13-cis-RA, as well as ROL itself and its ester, retinyl palmitate, on 3H-hypoxanthine uptake by the laboratory-adapted strains of *P. falciparum*, 3D7 and K1. They also examined the influence of three specific RA receptor antagonists (ER 27191, Ro 41-5253, and AGN 194301) on retinoid-induced growth inhibition of 3D7. All-trans-RA, 9-cis-RA, and 13-cis-RA in concentrations ranging from 1×10^{-4} to 5×10^{-10} M each had antimalarial activity, but at lesser IC50 values than those of ROL. Retinyl palmitate had minimal effect on 3H-hypoxanthine uptake. Contrary to expectation, each retinoic acid receptor (RAR) antagonist inhibited 3H-hypoxanthine uptake and demonstrated IC50 values that were comparable to those of ROL. Retinoid antagonists were also weakly antagonistic to the antimalarial effects of ROL.

These complex observations on the role of vitamin A in malaria call for a new perspective on the data. On one hand, *P. falciparum* selectively absorbs vitamin A from the host, low-blood concentrations of ROL are associated with severe malaria, and vitamin A supplementation partially protects against malarial infection. These points suggest that vitamin A has an overall beneficial role in malaria. On the other hand, ROL antagonizes the antimalarial effect of artemisinin and retinoid receptor antagonists are

as effective as ROL in inhibiting parasite growth, suggesting an opposite role for vitamin A. These apparent contradictions can be resolved if we consider that vitamin A, while essential in low concentration for numerous biological functions, is toxic at higher concentrations; in addition, the merozoite-stage parasite spends several days in the liver, the major storage organ for vitamin A, before invading the erythrocytes. This suggests that serum ROL concentrations are reduced in malaria infection, in part from selective absorption of vitamin A by the parasite, and perhaps to a greater extent from impaired hepatic secretion of vitamin A, since disturbed liver function is recognized in uncomplicated malaria^{17,18} and especially in severe malaria.¹⁸ The absorbed vitamin A, however, may be used by the parasite in ways that contribute to the disease, as suggested below.

Hypothesis of Disease Pathogenesis

Here it is proposed that malarial symptoms are caused by an endogenous form of vitamin A intoxication induced by the parasites following their egress from the liver. On this hypothesis, the parasites emerge from the liver packed with vitamin A and use RA as a cell membrane destabilizer to invade the red blood cells (RBCs), causing hemolysis and anemia. Other symptoms of the disease – e.g., fever, headache, muscle aches, gastrointestinal symptoms, seizures, coma, respiratory distress, and retinopathy – may similarly reflect parasite-induced vitamin A toxicity in the brain and other organs, following the transport of RBCs throughout the body and the release of RA (or RAs) into the circulation. It is further suggested that RA released from the parasites also enters the fetus and causes preterm birth and/or intrauterine growth restriction and low birth weight as a function of the membrane destabilizing and growth inhibitory effects of retinoids. This prediction, that low serum ROL concentrations in malaria are accompanied by increased host- and parasite-derived concentrations of RA, has not yet been tested in patients with malaria. If the hypothesis is correct, compounds that alter the metabolism of RA or block its expression may be effective as treatments, not only by attacking the parasite and its nutrition but by blocking the toxic effects of RA released by *P. falciparum* and other Plasmodium species on the human host.

Retinoids

Retinoids (vitamin A and its natural and synthetic congeners) are fat-soluble signaling molecules that are mainly derived from the diet as preformed vitamin A or as provitamin A carotenoids, and are essential for multiple biological functions, including embryonic development, vision, gene expression, tissue differentiation and growth, immune function, and mucus secretion.¹⁹ Retinoic acid is produced

from free ROL via a process that involves hydrolysis of retinyl esters in the liver, the release of ROL into the circulation, and its delivery to the target tissues bound to retinol-binding protein (RBP). Retinol is oxidized to retinal (aldehyde) via alcohol dehydrogenase, and RA is synthesized from retinaldehyde via an aldehyde dehydrogenase reaction. Retinoic acid exerts its effects by binding to and activating two types of nuclear protein receptors: the RARs and RXRs (rexinoids, RXRs), both of which exist as three distinct gene products (alpha, beta, and gamma). These receptors control the expression of numerous target genes by binding to specific DNA sequences termed RA response elements (RAREs).^{20,21} About 80% of the body stores of vitamin A are contained in the liver, in quantities sufficient to last the average adult about two years without the need for additional intake.²² Serum ROL levels remain stable due to a carefully regulated transport system that ensures the target tissues receive the necessary amounts of ROL despite major fluctuations in dietary intake.²³

While retinoids in low concentrations act as growth factors, higher concentrations are pro-oxidant, cytotoxic, mutagenic, and teratogenic, especially when unbound to protein. Vitamin A toxicity is due mainly to excessive dietary intake or to self-medication, when the liver becomes saturated with vitamin A and stored retinyl esters spill over into the circulating blood. Retinyl esters react more randomly with cell membranes than the physiologically-sequestered RBP and hence are a major form of vitamin A toxicity.^{24,25} Large single or short-term doses of preformed vitamin A can induce a condition of acute toxicity characterized by nausea, vomiting, headache, fatigue, vertigo, blurred vision, increased intracranial pressure, irritability, and lack of muscular coordination. The symptoms of chronic vitamin A poisoning are varied and can be manifested by central nervous system effects, liver abnormalities, growth arrest in children, bone and skin changes, and other adverse effects. Even low intakes of vitamin A in early pregnancy (7800 µg/day) are associated with congenital malformations.²⁶ Retinoid toxicity can also occur in cholestasis, when the secretion of RBP is inhibited, vitamin A accumulates in the liver, and biliary material (including metabolites of ROL, retinyl acetate, retinal, or RA) spills over into the circulation and is reabsorbed.²⁷

Possible Role of Retinoid Toxicity in Malaria Pathogenesis

Clinical manifestations of severe falciparum malaria typically include severe anemia and respiratory distress. Anemia is the primary clinical manifestation of severe malaria in children, with mortality rates that can exceed 30% among children aged less than

5 years. Asexual blood stage parasites produce 8–20 new merozoites every 48 hours, causing parasite numbers to increase rapidly to levels as high as 10 trillion (10^{13}) per host.²⁸ Host cell invasion is controlled by the sequential secretion of proteins from two types of specialized organelles: micronemes and rhoptries.^{29,30} The pathophysiological processes that contribute to severe anemia include destruction of parasitized and non-parasitized RBCs, inefficient or suppressed erythropoiesis, and dyserythropoiesis associated with impaired innate immunity.³ However, the precise mechanisms responsible for parasite entry, which requires localized destabilization of the host cytoskeleton and takes places in a matter of seconds, are unknown.³¹

It is hypothesized that the signs and symptoms of malaria are due to the effects of vitamin A accumulated by the parasites in the host liver. It is proposed that the parasites use the vitamin A, in the form of RA, to invade the RBCs; RA is then distributed via the transport of RBCs throughout the body in toxic concentrations. Based on this hypothesis, hemolysis and anemia occur due to the membranolytic actions of RA released from the parasites to invade the RBCs. Retinoids, including ROL and RA, can inhibit cell growth and interact directly with membranes to increase their permeability and fluidity, causing hemolysis of erythrocytes and increased secretion of enzymes from lysosomes.³² Cells exposed briefly to ROL show marked swelling, while longer exposures cause cell death; cultured human lymphoblastoid cells exposed to ROL and RA undergo a time- and dose-dependent decrease in viability accompanied by cell swelling.³³ Retinoids also arrest the growth of cultured lymphocytes due to a membrane destabilizing effect.³⁴ Retinol-induced erythrocyte hemolysis appears to be caused by physical damage to the membrane micelle induced by the penetration of ROL molecules rather than by oxidative disruption of erythrocyte membrane lipids initiated by ROL oxidation.³⁵ Retinoic acid fosters the phagocytosis of parasitized erythrocytes, which is stimulated by phosphatidylserine (PS) exposure at the cell surface. Exposure to RA and a specific RAR agonist, 4-(*E*-2-[5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl]-1-propenyl) benzoic acid (TTNPB), significantly increased erythrocyte annexin binding, an effect reflecting cell shrinkage, and significantly increased cytosolic Ca^{2+} -activity, a known trigger of PS exposure. Infection of erythrocytes with *P. falciparum* also increased PS exposure, an effect that was facilitated by the presence of TTNPB.³⁶

The fact that RA and TTNPB trigger phosphatidylserine exposure and cell shrinkage of erythrocytes, which are typical features of suicidal erythrocyte death or eryptosis, supports the present hypothesis that the

malarial parasite itself uses RA accumulated in the host liver to invade erythrocytes. Other signs and symptoms may be similarly caused by generalized and localized endogenous forms of host- and parasite-generated vitamin A poisoning in the affected tissues.

- Fever, fatigue, and malaise may represent generalized, non-specific effects of retinoid toxicity, as seen in the RA syndrome associated with the use of RA (13-*cis*-RA or isotretinoin) as a treatment for acute promyelocytic leukemia.³⁷
- Respiratory distress associated with interstitial pulmonary infiltrates, pleural and pericardial effusion, and dyspnea is seen in over 80% of patients with the RA syndrome.³⁷
- Thrombocytopenia, one of the most common complications of severe malaria,³⁸ is also a feature of hypervitaminosis A. For instance, hypervitaminosis A in infants causes severe thrombocytopenia and anemia, which appears to be due to a direct inhibitory effect on the growth of all bone marrow cellular components and may be mediated by the upregulation of cyclin-dependent kinase inhibitors.³⁹
- Headache and intracranial hypertension in malaria may be equivalent to pseudotumor cerebri (PTC), a feature of acute hypervitaminosis A.⁴⁰
- Retinopathy and papilledema in malaria could also be associated with hypervitaminosis A. Exposure to isotretinoin can cause retinopathy and optic nerve abnormalities,⁴¹ and RA contributes to light-induced retinopathy in mice via plasma membrane permeability and mitochondrial poisoning, leading to caspase activation and mitochondria-associated cell death.⁴²
- Malaria in pregnancy contributes to a substantial number of maternal deaths as well as infant deaths resulting from preterm birth, FGR and low birth weight.⁴³ The present hypothesis suggests that retinoids released from the parasites enter the fetus and cause preterm birth and/or FGR and subsequent low birth weight due to the membranolytic and growth-inhibiting effects of excess circulating retinoids, respectively. This hypothesis is supported by the results of a study by the author and colleagues using an animal model of pre-eclampsia, in which severe gestational diabetes and FGR were induced by the drug streptozotocin.⁴⁴ In this study, FGR was associated with markedly increased liver enzymes and alterations in retinoid profiles suggesting retinoid toxicity, e.g., the percentage of retinyl esters as a fraction of total vitamin A, normally <10%,²⁴ was 23% versus 11% in the untreated control group. The ratio of retinyl esters to ROL and the concentration of RA were also significantly increased compared to control values. If the present hypothesis is correct, the putative malaria toxin could be RA or a related metabolite of vitamin A.

Further Implications of the Retinoid Toxicity Hypothesis for Malaria

In mice made moderately diabetic with streptozotocin, infection with *P. yoelii* malaria or injection of extracts from malaria-parasitized RBCs has been found to normalize the associated hyperglycemia.⁴⁵ There is growing evidence of an association between

type 2 diabetes mellitus and alterations in retinoid metabolism, including an increase in circulating RBP, the transport protein for ROL; moreover, exercise lowers the concentration of retinoid and improves glucose metabolism.^{46,47} The present hypothesis suggests that the normalization of hyperglycemia in malaria-infected diabetic mice is due to the selective absorption of vitamin A by the parasites and its reduction below a critical threshold.

Co-infection with helminths also protects against severe malaria.^{48,49} This may be tentatively explained on the present hypothesis as follows. The parasitic worm *Onchocerciasis volvulus*, like *P. falciparum*, selectively absorbs and concentrates vitamin A, such that the ROL concentration in *O. volvulus* is about eight times higher than that in the surrounding tissues of the host.⁵⁰ The present hypothesis suggests that *O. volvulus* reduces the availability of vitamin A for the malaria parasite in the early sporozoite or blood stage of the lifecycle, which starves and weakens the parasite, perhaps reducing the numbers of parasites reaching the liver and thereby lessening symptom severity.

How does the Merozoite Avoid the Host Immune System?

The merozoite stage of the malaria parasite lifecycle begins within host hepatocytes. The merozoites reach the circulation via hepatic sinusoids, followed by erythrocyte invasion and the appearance of disease symptoms.⁵¹ The mechanism by which the merozoites escape the host immune system before erythrocyte invasion is unknown. Using a mouse model of malaria, Sturm *et al.*⁵² showed that the parasites kill and detach their host hepatocytes, after which parasite-filled vesicles (merosomes) emerge into the sinusoidal lumen. The parasites simultaneously inhibit the release of PS onto the outer leaflet of the host plasma membrane, which enhances phagocytosis. Thus, hepatocyte-derived merosomes ensure both the migration of parasites into the blood stream and their protection from host immunity.

According to the present hypothesis, this process may be due to the uptake of hepatic ROL by the parasites, followed by the localized release of high concentrations of RA or other polar metabolites, causing the destruction of host hepatocytes and the simultaneous depletion of retinoid in surrounding host cells, leading to loss of potency of immune system defense mechanisms. Sturm *et al.*⁵² reported that host cell death did not occur by classical necrosis or apoptosis but by the activation of cysteine proteases. This was suggested by the observation that the treatment of infected hepatocytes with the cysteine protease inhibitor E64 completely inhibited parasitophorus vacuole membrane (PVM) destruction

and cytochrome c release. Examination of infected hepatocytes early after detachment revealed condensation of the cell nuclei, suggesting that cell death was occurring. This was supported by loss of mitochondrial membrane potential, the release of cytochrome c from the mitochondria of infected cells, and alterations in the shape of the mitochondria. Consistent with the retinoid hypothesis, all-trans RA disturbs mitochondrial function long before detectable signs of cell death.⁵³ It was also noted that, like *P. falciparum* erythrocyte merozoites, hepatic merozoites actively accumulate intracellular Ca^{2+} released from the damaged stores of the dying host to higher levels than those of host cells. This process could be one aspect of the selective uptake of retinoid by the parasite from surrounding host cells, since cellular calcium homeostasis interacts with signaling systems involved in the control of RA-dependent transcriptional activity.⁵⁴ The observation that early Plasmodium liver stages confer resistance to apoptosis to the host cell⁵⁵ could be related to the active depletion of ROL in those cells by the parasite. Indeed, dead parasites do not protect host cells from undergoing cell death.⁵⁶ In summary, it is postulated that the process by which Plasmodium liver stage parasites manipulate their host cells to ensure the safe delivery of merozoites into the blood stream is related to alterations in host retinoid metabolism and immunity induced by selective retinoid uptake and release by the parasite.

Implications of the Model for Understanding Current Treatments

Low levels of arginine, the precursor for nitric oxide (NO), are common in patients with malaria and are associated with endothelial dysfunction. Administering arginine intravenously reverses hypoargininemia and endothelial dysfunction. Carbon monoxide shares certain functional properties with NO and protects against cerebral malaria in mice. Arginine therapy may therefore prove beneficial in severe malaria in addition to conventional antimalarial treatment.⁵⁷ Consistent with the retinoid toxicity hypothesis, the potential efficacy of NO in the treatment of malaria could be due to the fact that RA and NO are inversely related;⁵⁸ hypoargininemia could therefore reflect increased RA. This suggests that arginine or NO therapy could be effective in malaria by inhibiting the actions of RA.

The quinolines (including chloroquine, primaquine, mefloquine, and quinine) are among the most successful yet poorly understood classes of drugs, with important uses in the treatment of systemic lupus erythematosus and HIV as well as malaria. The 8-amino-quinolines such as primaquine and tafenoquine are the only drugs that target the liver stage malaria parasites.²⁸ The therapeutic effectiveness of these drugs may lie in their ability to affect vitamin A

metabolism, specifically by inhibiting the conversion of ROL to RA, the latter of which may be critical for the nutrition of the malaria parasite, as well as by increasing ROL to concentrations that are toxic to the parasites. As noted, RA is produced from the oxidation of ROL to retinal via the action of an alcohol dehydrogenase, and from retinaldehyde via an aldehyde dehydrogenase reaction. Thus the quinolines may be acting as dehydrogenase inhibitors. This is supported by the fact that aldehyde dehydrogenase 1 (ALDH1) is selectively inhibited by quinolones; other known inhibitors of ALDH1 are also lethal to or inhibit the growth of *P. falciparum* *in vitro*.⁵⁹

Implications for New Treatments

The treatment of malaria currently depends on a few available compounds and these are vulnerable to the emergence of compound-resistant parasites and mosquitoes.²⁸ Subject to obtaining positive results based on tests of the proposed model, new treatments for malaria could be developed using agents that target the metabolism or expression of retinoids. Such treatments would be expected to eliminate the parasite by affecting its nutritional status as well as treating the manifestations of the disease, i.e. by inhibiting the clinical effects of host- and parasite-derived RA.

With regard to alternative treatments suggested by the hypothesis, a randomized, double-blind, placebo-controlled phase I/II trial of rosiglitazone, a PPARgamma agonist (4 mg twice daily for 4 days), showed that the drug significantly enhanced parasite clearance times compared with controls. Rosiglitazone also reduced inflammatory biomarkers to malarial infection and was described as well-tolerated as an adjunct to the standard therapy for nonsevere *P. falciparum* malaria.⁶⁰ Several studies indicate that PPARgamma ligands regulate differentiation and induce cell growth arrest and apoptosis in a variety of cancer types.⁶¹ It is of special interest that the first RARalpha antagonist to be developed, Ro41-5253,⁶² is a PPARgamma agonist in that it binds and activates PPARgamma, albeit weakly.⁶³ This suggests that expression of PPARgamma is effectively equivalent to RARalpha antagonism and the latter is a natural consequence of PPARgamma activation or expression. These observations support the retinoid toxicity hypothesis and suggest that RAR antagonists themselves could be useful in the treatment of malaria.

Other compounds with anti-retinoid actions could be explored as potential treatments for malaria. For instance, the cytolytic effects of ROL on biological membranes are counteracted by tocopherol, which may act as a membrane stabilizer through interaction with polyunsaturated fatty acid residues of

phospholipid molecules.⁶⁴ The presence of tocopherol (200 µM) as well as taurine (5–20 mM) and zinc (50–100 µM) were reported to protect cultured human lymphoblastoid cells from retinol-induced injury, and the combination of all three compounds afforded complete protection, possibly through a membrane-stabilizing action unrelated to lipid peroxidation.³³ Taurine, zinc, and tocopherol could be tested together as antimalarial agents, as well as in combination with RAR antagonists.

The retinoid toxicity hypothesis has implications for the development of malaria vaccines, the development of which has been hampered by an incomplete understanding of the pathogenesis of the disease; in particular, uncertainty regarding the immunological processes that provide protection after vaccination with irradiated sporozoites.⁶⁵ Immunity from severe malaria is acquired rapidly, after only 1–2 episodes of severe disease.⁶⁶ Given that vitamin A can function as an adjuvant, i.e. by promoting vaccine antibody responses,⁶⁷ it is possible that vitamin A supplementation functions as a vaccine or vaccine adjuvant in malaria, ‘inoculating’ the host from the presumably more toxic ‘dose’ of retinoid from the parasite by prior exposure to a lesser dose. Studies showing that vitamin A partially protects against malaria infection^{9,68} could therefore be interpreted as indicating that, in this role, vitamin A functions as a vaccine adjuvant rather than as a nutritional supplement to prevent vitamin A deficiency. The mechanism of adjuvant action may involve feedback inhibition of the production of vitamin A, since administered RA can inhibit the endogenous synthesis of RA. For instance, Barua *et al.*⁶⁹ found that a single oral dose of RA (0.167 mM) in corn oil given to six healthy human subjects led to a mean decline in serum ROL of approximately 20% within 1 hour and lasted for 24 hours.

Guilbride⁷⁰ has proposed that malaria vaccines fail because of live parasites in the skin and the presence of regulatory T cells (Tregs), which inhibit the immune response and block vaccine function. The retinoid toxicity hypothesis suggests that Tregs are activated in the skin of patients with malaria due to high concentrations of RA released by the parasites. In support of this suggestion, RA enhances the de novo differentiation of naïve CD4+ cells to Foxp3+ Tregs and suppresses Th17 development.⁷¹

It remains for future work to explore the theoretical and practical implications of these concepts. In the meantime, the present hypothesis could be tested initially by determining whether, as predicted, low serum concentrations of ROL are associated with high concentrations of RA both in animal models and in patients with malaria compared to non-infected controls. Subject to the outcomes of such tests,

parasite retinoid metabolism could become a new target for the treatment and prevention of malaria.

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