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RESVERATROL, A COMPONENT OF RED WINE, IMPAIRS THE CYTOADHERENCE OF *PLASMODIUM FALCIPARUM*-INFECTED RED BLOOD CELLS BY REDUCING THE EXPRESSION OF PFEMP-1

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Sequestration of *Plasmodium falciparum*-infected red blood cells (IRBCs) is critical to parasite survival and is centrally involved in the pathogenesis of malaria. Adherence of IRBCs to microvascular endothelial cells (MVECs) enables parasites to avoid clearance from the bloodstream by the spleen. Cytoadherence is also implicated in microvascular inflammation and endothelial dysfunction. Rosetting is also believed to contribute to obstruction and ischemia-induced inflammation in microvessels. Both cytoadherence and rosetting are associated with severe and fatal *falciparum* malaria. *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), a family of parasite-encoded antigenically-variant proteins, mediates cytoadherence and rosetting and is encoded by *var* genes. The expression of *var* genes is regulated by parasite-encoded sirtuin 2 (PfSir2), a histone deacetylase. The polyphenol resveratrol (RV) activates PfSir2 and was recently shown to transcriptionally repress, in a differential manner, all three major sub-families of *var* genes. We thus hypothesized that RV impairs the cytoadherence and rosetting of IRBCs. To test this, we infected RBCs with the HB3 and FCR-3 *P. falciparum* lines in the presence of increasing concentrations of RV. After one cycle of parasite invasion and development to the trophozoite stage expressing PfEMP-1, we found that RV impaired (up to 57%) adherence to MVECs in a dose-dependent manner. Using the rosetting *P. falciparum* line 'varO', we found that RV also reduced (up to 40%) rosette frequencies in a dose-dependent manner. These findings were associated with moderate reductions in the levels of PfEMP-1 on the surface of IRBCs detected by flow cytometry. These reductions in cytoadherence, rosetting, and PfEMP-1 levels were not associated with decreased parasite viability. These data suggest the possibility that commercially-available RV - a component of red wine - may attenuate the virulence of *P. falciparum* by impairing cytoadherence and rosetting *in vivo*. Our findings thus provide a rationale investigating whether RV, in combination with antimalarial chemotherapy, could improve the survival of patients with severe malaria.

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PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES CONTAIN URIC ACID PRECIPITATES THAT ARE HIGHLY INFLAMMATORY

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Malaria life-threatening pathology is caused or exacerbated by excessive inflammatory responses in the host. Further understanding of the mechanisms involved in this process is needed to develop more effective therapies against malaria-induced pathology. We previously defined uric acid (UA) as a mediator of malaria-induced inflammation in mouse and human cells as reported previously. We have now discovered that *Plasmodium falciparum*-infected erythrocytes contain UA precipitates. Using both immunofluorescence with specific antibodies and lysate fractionation, we have detected UA precipitates in P. *falciparum*-infected erythrocytes in all cycle stages. UA precipitates are localized in the Plasmodium cytosol and are released into the medium upon schizont rupture. The inflammatory properties of UA precipitates (also named crystals) are well known because they are the causative agent of gout and are also considered a danger signal for the immune system. Direct release of UA precipitates in the blood upon schizont rupture may cause strong inflammatory responses during malaria infection. We found that addition of UA inhibitory drugs, allopurinol and uricase, reduced secretion of inflammatory cytokines (TNF, IL-1β and IL-6) from human peripheral blood mononuclear cells in response to Plasmodium-infected erythrocytes, suggesting that a decrease in UA levels in vivo may reduce the host inflammatory response and pathology. We obtained intracellular UA precipitates derived from Plasmodium-infected erythrocytes. These precipitates caused increased expression of the dendritic cell activation markers, CD40, CD80 and CD86 in vitro. This inflammatory effect was sensitive to uricase treatment, confirming their identity. This suggests that Plasmodium-derived UA activates the host inflammatory response and may contribute towards malaria pathology. Inhibiting UA formation may therefore decrease malaria-induced pathology, and this will establish the basis for developing specific therapies against this devastating disease.

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VAR2CSA ELICITS BROAD REACTIVE ANTI-ADHESIVE ANTIBODIES

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Pregnancy Associated Malaria (PAM) has harmful consequences for both the mother and foetus, primarily due to the accumulation of infected erythrocytes (iE) in the placenta. A member of the *Plasmodium falciparum* erythrocyte membrane protein 1 (*Pf*EMP-1) family, called VAR2CSA, is a variant surface antigen (VSA) which mediates adherence of iE to a placental receptor - chondroitin sulphate A (CSA). Women with PAM-related placental infection develop VSAPAM-specific anti-CSA adhesive antibodies after successive pregnancies that protect them from the severe consequences of PAM. Identifying which part of the VAR2CSA protein elicits broad reactive anti-CSA adhesive antibodies will provide a breakthrough for developing an anti-PAM vaccine. A cohort of 1000 pregnant women, recruited before 24weeks of pregnancy, was followed until delivery with the aim of accurate quantification of the effects of PAM on foetal and maternal health in Korogwe, North-eastern Tanzania. The overall aim of this longitudinal study is to optimize strategies for preventive intermittent treatment and facilitate development of a vaccine against PAM. Parasite isolates collected from pregnant women were cultured to late trophozoite and schizont stages and then tested for their ability to transcribe and express VAR2CSA by using qRT-PCR and flow cytometry, respectively. Antibodies raised in rats against different VAR2CSA Duffy binding like (DBL) domains of the FCR3 strain were assessed for their ability to inhibit adhesion of the PAM-derived *P. falciparum* iE to CSA *in vitro* using a static inhibition of binding assay (IBA). Based on qRT-PCR and flow cytometric analyses, we show that parasite isolates from pregnant women transcribe and express VAR2CSA on the surface of iE. The IBA results show that antibodies targeting particular VAR2CSA DBL domains inhibit the adhesion of most clinical isolates tested. In conclusion, immunization of rats with particular recombinant VAR2CSA protein domains based on the FCR3 sequence elicits broad reactive anti-adhesive

antibodies. Our findings bring us closer to identifying which part of the VAR2CSA protein may be used as a basis for developing an anti-PAM vaccine candidate.

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ANTIBODIES AGAINST VAR2CSA OF PFEMP1 DBL2X AND DBL3X DOMAINS INHIBITED ADHESION OF IE TO CHONDROITIN SULFATE A

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Over 500 million cases of clinical malaria occur annually. Malaria is the leading cause of infant mortality in under-developed countries. During pregnancy, *Plasmodium falciparum* infected erythrocytes (IE) bearing a preferentially expressed VAR2CSA surface protein sequester on placental syncytiotrophoblast by binding to Chondroitin Sulfate A (CSA). This phenomenon occurs mostly in primigravidae resulting in maternal anemia, low birth weight and in severe cases death of the fetus. However, after multiple pregnancies, multigravidae women develop blocking antibodies against VAR2CSA protein. VAR2CSA is a member of PfEMP1; a family of structurally related proteins with its extracellular portion made up of six Duffy-Binding-Like (DBL) domains and four Cysteine-rich Inter-Domain Regions. We refolded and purified recombinant DBL2X, DBL3X, and CSA binding sub-domain 3 of DBL3X (DBL3X-S3) and sub-domain 3 of DBL2X (DBL2X-S3) from *E. coli* inclusion bodies. DBL2X-S3 and DBL3X-S3 bind with higher specificity and lower affinity to CSA expressed on CHO-K1 cells compared to DBL2X and DBL3X which bind with lower specificity and higher affinity, respectively. Rat and rabbit antibodies raised against the DBL domains recognized homologous parasite IE and some heterologous parasite IE expressing alternative alleles of VAR2CSA. Preliminary results obtained with combinations of antibodies against these DBL domains suggest additive inhibition of IE binding to CSA expressed on CHO-K1 cells. Several of the rat and rabbit antibodies raised against these DBL domains showed limited inhibition of maternal field isolate binding to CSA, however, further studies are required. Taken together, these individual DBL domains of approximately 25-30 kDa can be produced in large quantities and scale in *E. coli*, hence favoring them as viable vaccine candidates for PAM.

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DISPARITIES IN ACCESS TO SANITATION IN BOLIVIA

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Bolivia is the only country in Latin America that is falling short of Millennium Development Goal #7 target for sanitation. Understanding where access to sanitation is the lowest, and the socio-economic factors associated with lack of access to sanitation, aid in identification of the populations most in need. Bolivia's population is estimated to be up to two-thirds indigenous Amerindian, and these groups dominate the rural population. Among the rural population, 57% (≈1,894,000 people) do not have access to a toilet or latrine. Previous studies have demonstrated that children in rural Bolivia are at greater risk of morbidity, malnutrition and impaired development associated with diseases linked to inadequate water, sanitation and hygiene. This analysis provides an in-depth assessment of disparities in access to sanitation by comparing the relative influence of location, socioeconomic factors (household construction materials, number of household members), educational status and gender for major ethnic groups in Bolivia using the most recent data from the nationally representative Demographic and Health Survey (DHS). The language that the head of household reported as learning to speak first was selected to indicate ethno-linguistic group. Across the 3 major indigenous ethno-linguistic groups of Bolivia, the primary correlates with access to sanitation differ: among the Aymara people (20% of total population, 46% household sanitation coverage within group), rural location is the strongest correlate with low sanitation coverage; among the Quechua (27% of total population, 48% household sanitation coverage within group), rudimentary household construction materials are most strongly associated with lack of household sanitation; and among the Guaraní and other Llano region groups (1% of total population, 53% household sanitation coverage within group), larger household size is associated with less access to sanitation. These differences in the primary correlates with lack of household sanitation across the ethno-linguistic groups of Bolivia can inform regional sanitation programs by identifying the populations with the greatest need and helping implementers to better target population selection and sanitation intervention strategies to be more effective for the geographic and social context of their programs.

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AN EVENT-BASED MODEL FOR ENVIRONMENTAL TRANSMISSION OF *GIARDIA* INFECTION

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Diarrheal illness is a major burden in the developing world, with a median of ~3.2 episodes per year in children under 5 years. Giardia is a major contributor to this burden. Where resources are insufficient for distribution of clean drinking water and removal of human waste, many less expensive antidiarrheal interventions have been investigated. These include latrine construction, handwashing with soap, and various methods for household water treatment (HWT). Published trials of these interventions often claim reductions in diarrheal illness of 30% or more. However, most trials are short-term, and nearly all are subject to bias. Furthermore, characteristics of interventions and communities vary greatly, and interventions are seldom maintained after the trial is over. Since long-term trials require much time and money, a simulation approach may be helpful for assessing the effectiveness of interventions in various contexts. A simulation model describing Giardia transmission in an isolated, underdeveloped community was programmed using Octave 3.0. It uses the Gillespie event-based algorithm to stochastically track susceptible, exposed, and infectious individuals and the number of cysts in the water source. Dose-response modeling determines exposure outcomes from ingestion of contaminated water. The model also includes an HWT intervention that reduces the number of cysts in drinking water for community members who use it.

Results (preliminary): If there is no intervention, the model equilibrates at a hyperendemic state, with ~90% of the population infected. If the entire community uses an HWT intervention that reduces cysts in drinking water by 99.0%, the prevalence gradually declines to ~8% after 1 year. If only 75% of the population uses the intervention, ~36% of the population is infected after 1 year. If everyone in the community uses the intervention on 95% of their drinking water, but continues to drink untreated water 5% of the time, the model equilibrates at ~67% infected after ~150 days. Highly consistent use of HWT may be necessary to control giardiasis in hyperendemic communities. Further refinements of the model may alter these conclusions. Household structure and additional transmission routes (e.g., contaminated hands) will be included in future versions of the model, allowing simulation of additional interventions (e.g., handwashing and sanitation).