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SPECIAL ARTICLE

Autoimmunity, phospholipid-reacting antibodies and malaria immunity

LR Gomes[1,*], YC Martins[1,2,*], MF Ferreira-da-Cruz[1] and CT Daniel-Ribeiro[1]
[1]Laboratory for Malaria Research, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro and Center for Malaria Research and Training (CPD-Mal), Fiocruz, Rio de Janeiro / Secretary for Health Surveillance (SVS), Ministry of Health, Brazil; and
[2]Department of Pathology, Albert Einstein College of Medicine, The Bronx, New York, USA

Several questions regarding the production and functioning of autoantibodies (AAb) during malaria infection remain open. Here we provide an overview of studies conducted in our laboratory that shed some light on the questions of whether antiphospholipid antibodies (aPL) and other AAb associated with autoimmune diseases (AID) can recognize *Plasmodia* antigens and exert anti-parasite activity; and whether anti-parasite phospholipid antibodies, produced in response to malaria, can inhibit phospholipid-induced inflammatory responses and protect against the pathogenesis of severe malaria. Our work showed that sera from patients with AID containing AAb against dsDNA, ssDNA, nuclear antigens (ANA), actin, cardiolipin (aCL) and erythrocyte membrane antigens recognize plasmodial antigens and can, similarly to monoclonal AAb of several specificities including phospholipid, inhibit the growth of *P. falciparum in vitro*. However, we did not detect a relationship between the presence of anti-glycosylphosphatidylinositol (GPI) antibodies in the serum and asymptomatic malaria infection, although we did register a relationship between these antibodies and parasitemia levels in infected individuals. Taken together, these results indicate that autoimmune responses mediated by AAb of different specificities, including phospholipid, may have anti-plasmodial activity and protect against malaria, although it is not clear whether anti-parasite phospholipid antibodies can mediate the same effect. The potential effect of anti-parasite phospholipid antibodies in malarious patients that are prone to the development of systemic lupus erythematosus or antiphospholipid syndrome, as well as the (possibly protective?) role of the (pathogenic) aPL on the malaria symptomatology and severity in these individuals, remain open questions. *Lupus* (2014) **23,** 1295–1298.

Key words: Antiphospholipid syndrome; systemic lupus erythematosus; anti-DNA antibodies; malaria

Introduction

Despite the increasing knowledge on immunology during the past decades, several questions regarding autoantibody (AAb) production and function remain open. Some of them concern the relationships between autoimmunity and infectious and parasitic diseases. The concept that AAb production and autoimmunity may follow and complicate infectious and parasitic diseases such as Group A ß hemolytical streptococcal infection and Chagas Disease is now well established.[1] However, evidence has accumulated to indicate that some parasitic diseases, such as malaria, may protect individuals against the development and severity of autoimmune diseases (AID) such as systemic lupus erythematosus (SLE).[2,3] In addition, it has been proposed that although AAb produced by individuals infected by *Plasmodia* do not seem to participate in the pathogenesis of malaria, they could even exert protective effects against the disease.[4]

Here we give an overview of studies conducted in our laboratory to approach two of the questions concerning autoimmunity and malaria: (a) Do AID-associated AAb, including those directed to negatively charged phospholipids (antiphospholipid antibodies (aPL)), recognize antigens present

*LRG and YCM contributed equally to the work

Correspondence to: Cláudio Tadeu Daniel-Ribeiro, Fundação Oswaldo Cruz Avenida Brasil, 4365 Pavillhão Leônidas Deane Rio de Janeiro, 2104-360 Brazil.
Email: ctdr@uol.com.br

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on *Plasmodia* and, could these AAb exert an anti-parasite activity? and, (b) Can similar (anti-parasite phospholipid (PL) antibodies (Ab)), produced in response to malaria, inhibit PL-induced inflammatory responses and protect against the pathogenesis of severe malaria?

Malaria remains the most prevalent parasitic disease worldwide. All five species of *Plasmodia* that naturally infect humans can cause from asymptomatic infection to severe disease, but *Plasmodium falciparum* infection is by far the most prone to cause (and is the most frequently associated with) life-threatening complications. Clinical manifestations of severe falciparum malaria vary according to transmission intensity and include hyperparasitemia, hypoglycemia, cerebral malaria, severe anemia and respiratory distress.

Many of the malaria-associated symptoms and complications result from a severe host innate immune response induced by parasite and host molecules containing pathogen-associated molecular patterns (PAMPs) released in the circulation during schizogony. *P. falciparum* glycosylphosphatidylinositol (GPI) is an abundantly expressed PL that activates leukocytes, triggers the release of pro-inflammatory cytokines and induces the expression of adhesion molecules via toll-like receptors (TLR) -2 and -4.[5]

It has been demonstrated that the administration of *P. falciparum* GPI in mice induces malaria-like symptoms,[6] and that the immunization with non-toxic moieties of GPI that elicits an anti-GPI Ab response can prevent malaria pathology and death caused by experimental *P. berghei* ANKA and *P. yoelii* 17XL infection in mice.[7,8] In addition, an association between the presence of anti-GPI Ab, age and prevalence of protected asymptomatic individuals has been shown in areas of high malaria transmission in Kenya and north-west Papua New Guinea.[9,10] Therefore, although not consensually, it has been considered that anti-GPI Ab could reflect and mediate, at least partially, the anti-disease immunity in malaria, by neutralizing the toxic effect of parasite GPI.[7]

Our group also evaluated the association between anti-GPI Ab response and development of clinical malaria[11] by comparing the incidence of these antibodies, in supposedly immune asymptomatic carriers of plasmodial infection living in an area of the Brazilian Amazon (Barcelos) with high transmission rates of malaria, with controls with no infection or previous history of malaria from the same area and Angolan (Lubango) patients with symptomatic disease. Our data indicated that the Ab response against *P. falciparum* GPI was not associated with *falciparum* asymptomatic infection in individuals chronically exposed to malaria in the Brazilian Amazon. We could not rule out that the high levels of anti-GPI Ab, correlated with degrees of parasitemia, observed in Lubango patients with symptomatic, but non-complicated, malaria could be protecting them from severe disease. However, an alternative and more tempting interpretation is that this Ab response is being boosted by the increasing quantities of *P. falciparum* GPI released in the circulation as the parasitemia level grows (Figure 1).[11]

Hence, our findings do not support the hypothesis that anti-GPI antibodies mediate clinical (anti-disease) immunity against malaria, but are in accordance with those of Boutlis et al.[12] and Cissoko et al.[13] These authors also failed to detect a relationship between anti-GPI Ab and asymptomatic infections, but, similarly to us, observed a relationship between these Ab and parasitemia levels in infected individuals from Mali and Papua New Guinea.

On the other hand, it is becoming progressively clear that autoimmune responses generated during malaria infection are not harmful, but might present protective properties against the disease. Evidence exists for AAb against erythrocytes,[14–16] PL,[17–20] brain proteins,[21,22] and nuclear antigens.[20,23–25]

For the last decade, our team has been collecting evidence that autoimmunity is involved in the protection against malaria.[26] Zanini et al.[23] investigated the presence of AAb in the sera of malarious patients, the reactivity of AAb from Brazilian autoimmune patients against plasmodial antigens and the effect of such AAb on the *in vitro* growth of *P. falciparum*. The results showed that the frequency of sera from malarious patients reacting with actin and the cardiolipin PL was higher ($p<0.001$) than in sera from SLE patients and, conversely, that antinuclear antibodies (ANA) were more frequent in SLE than in malaria sera ($p=0.004$). In addition, AAb (directed against dsDNA and ssDNA, nuclear Ag, actin, cardiolipin and erythrocyte membrane) from patients with autoimmune processes recognized plasmodial antigens in the absence of malaria, (44% of the SLE – and 81% of the malaria – sera reacted with plasmodial Ag by the immunofluorescent antibody test (IFAT)); and 47.3% of the SLE patients' (and 87.5% of the malaria patients') sera inhibited plasmodial growth *in vitro*. The presence of anti-DNA and ANA was important in such anti-plasmodial activity, but no relationship was found between the *P. falciparum* inhibitory growth of

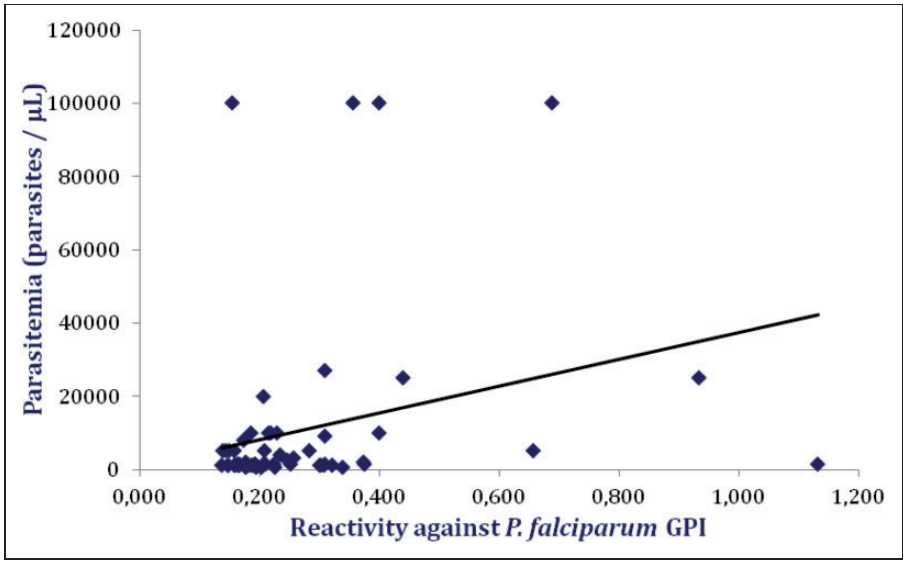

Figure 1 Correlation between the intensity of the immune response directed against the *Plasmodium falciparum* glycosylphosphatidylinositol (GPI) phospholipid (as assessed by ELISA) and the parasitemia (parasites / microliter) in peripheral blood of acute falciparum malaria patients from Lubango (Angola). Spearman's rank correlation $r = 0.3198$ and $p = 0.0106$.

immunoglobulins from patients with autoimmune disease and the presence of anti-actin or anti-cardiolipin AAb.

We also studied the relationship between autoimmunity and antimalarial responses by determining – in addition to the presence and the pattern of reactivity to plasmodial antigens of sera from 93 French patients with 14 different AID and not previously exposed to malaria – the effect of 21 monoclonal AAb with different specificities on the *in vitro* growth to *P.* falciparum;[20] 56% of sera from AID patients and none of healthy individuals reacted with plasmodial antigens. The frequency of sera reacting with at least one of the three strains and two species of *Plasmodia* depended on the specificity of the AAb the sera contained, but the differences were not statistically significant. In addition, they reacted more frequently with the young (less differentiated?) rather than with the mature blood stages of *Plasmodium*. Finally, 85% of the 13 murine monoclonal AAb reacted with plasmodial antigens and 72%, including three out of four anticardiolipin AAb, inhibited the *in vitro* growth of *P. falciparum*, although this inhibition did not correlate with the intensity of reactivity against the parasite, as assessed by the IFAT.

Taken together, these results and data from other groups indicate that autoimmune responses may have anti-plasmodial activity, mediated by AAb of different specificities in the presence or not of other serum factors, and protect against malaria. The potential effect of the theoretically malaria-protective anti-parasite PL in (SLE or anti-PL syndrome-prone) malarious patients as well as the (possibly protective?) role of the (pathogenic) anti-PL AAb in these individuals on the symptomatology and severity of malaria remain open questions.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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