Research News

Expanding the paradigms of placental malaria

James G. Beeson, Brian M. Cooke, J. Alexandra Rowe and Stephen J. Rogerson

Despite substantial pre-existing immunity, pregnant women typically develop placental infection and associated complications. Placental parasites have specific adhesive phenotypes, and the antibodies raised against *Plasmodium falciparum* variant and adhesive antigens, which are produced in response to placental infection, could have protective roles. New findings suggest that binding of immunoglobulins to parasite antigens can also mediate parasite accumulation in the placenta.

Recent insights into the pathogenesis of placental malaria have generated great interest in the possible development of specific vaccinations or therapies to protect against infection. Several studies in Africa have implicated the adhesion of *Plasmodium falciparum*-infected red blood cells (IRBC) to glycosaminoglycans chondroitin sulfate A (CSA) and hyaluronic acid (HA), present on syncytiotrophoblasts lining the placental blood spaces, as an important mechanism in the accumulation of IRBC in the placenta (reviewed in Ref. [1]). A significant step forward was the identification of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) as the parasite ligand mediating adhesion to CSA [2,3]. Specifically, the γ-type Duffy binding-like (DBL) domain (previously known as DBL3) of PfEMP1 has been consistently implicated in adhesion to CSA, based on studies of clonal laboratory lines and placental isolates from Cameroonian women [2–4], although in some isolates other domains also appear to be involved [1]. Furthermore, antibodies raised against the DBL-γ domain can inhibit parasite adhesion *in vitro* [2].

Role of antibodies to parasite variant and adhesive antigens

Primigravid women are highly susceptible to malaria, whereas multigravid women, who are also generally older, have reduced risk of infection and associated complications [5]. A hypothesis has been proposed to explain the partial immunity of multigravidae to placental malaria [1,6]. When a woman is infected with *P. falciparum* in her first or second pregnancy, antibodies develop against the parasite variants that adhere in the placenta and express specific forms of PfEMP1. These antibodies then contribute to protection from malaria in subsequent pregnancies. Initial studies reported some association between reduced placental infection and antibodies, probably targeting PfEMP1, that block adhesion to CSA *in vitro*; such antibodies were also more prevalent in multigravid women [6]. Recent studies, however, have not found the same associations. In Cameroonian women, there was no clear association between infection (peripheral or placental blood) and adhesion-blocking antibodies, and a difference between primigravid and multigravid women was only seen early in pregnancy [7].

An alternative approach has been to examine antibodies that bind to variant antigens, predominantly PfEMP1, on the surface of IRBC selected for high level of adhesion to CSA. Studies of pregnant women in Cameroon [8] and Malawi [9] indicate that these antibodies are significantly more common in pregnant women with active infection than in uninfected pregnant women. In Cameroon, higher antibody levels to CSA-binding parasites were inversely correlated with placental parasitaemia. Antibodies to variant antigens on IRBC have been associated with protection from clinical malaria in children and non-pregnant adults [10]. In pregnancy, it appears that such antibodies to placental isolates are frequently present concurrent with infection [8,9]. This raises questions about their proposed role in protection from or clearance of placental parasitaemia, the dynamics of development and decay of antibodies to IRBC variant antigens, and could reflect the chronic nature of infection in pregnancy. Currently, there is little data addressing these issues in populations outside Africa, where other forms of malaria, particularly *Plasmodium vivax*, are common. Studies in these populations could yield different findings from those in Africa because reports suggest that there are possible interactions between *P. vivax* and *P. falciparum* [11].

Parasite binding to immunoglobulins

Several important and unexplained observations suggest that additional processes, other than adhesion to CSA and HA, are involved in placental parasite sequestration. For example, when examining placental histology, most IRBC do not appear to adhere to placental syncytiotrophoblasts [1] and not all IRBC taken directly from infected placentas adhere to CSA and/or HA when tested *in vitro* [12,13].

New findings have implicated IRBC binding to non-immune immunoglobulins (Ig) in placental malaria [14]. A cloned parasite line, selected for a high level of binding to Ig, adhered to placental sections *in vitro* in a manner dependent on Ig, but independent of CSA and HA. Furthermore, parasites isolated from four infected placentas of Cameroonian women appeared to bind IgG *in vitro*. These placental isolates also bound CSA. However, it is not clear how much the binding phenotypes overlapped. The laboratory isolate did not bind to CSA, suggesting that parasites binding Ig in the placenta constitute a different population from those that bind CSA or HA. The isolate used and other isolates that were shown to bind Ig previously also have a strong tendency to form rosettes (the adhesion of IRBC to uninfected RBC), which is not a common feature of placental parasite isolates [15]. Flick *et al.* [14] propose that IgG could act as a bridging molecule between IRBC and Fc receptors present on syncytiotrophoblasts, thereby mediating parasite sequestration in the placenta, but this remains to be demonstrated. IgG-binding was found to be a property of PfEMP1, expressed on the surface of IRBC and mapped to the DBL2β domain [14]. IgM binding has previously been

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Box 1. Priorities for further research on malaria during pregnancy

Parasite factors

- Identification of mechanisms (other than parasite adhesion to syncytiotrophoblasts) that contribute to placental sequestration.
- Determine whether infected red blood cell (IRBC) adhesion to leukocytes or the formation of IRBC aggregates contribute to sequestration.
- Investigate possible immunoglobulin (Ig) -mediated bridging between IRBC and Fc receptors on syncytiotrophoblasts.
- Establish the extent of sequence, structural and functional conservation, or diversity of *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) domains implicated in placental infection.

Host factors

- Determine the nature of immune modulation in pregnancy as it relates to increased susceptibility to malaria.
- Examine the immune consequences of Ig binding to IRBC in the placenta.
- Assess the ability of placental monocytes or macrophages to phagocytose IRBC effectively.
- Examine associations between antibodies to particular PfEMP1 types or domains, and protection from placental infection in prospective studies.
- Extend observations from African studies of antimalarial immune responses in pregnancy to populations where *Plasmodium vivax* is prevalent.

attributed to the cysteine-rich interdomain region 1α associated with rosette formation [16].

Examination of infected placental tissue from six individuals by immunohistochemistry revealed that IgG was associated with the surface of ~50% of the sequestered IRBC. IgM was also detected to a lesser extent [14]. Only IgG was detected on IRBC apparently adherent to syncytiotrophoblasts. Reports indicate that many women with placental infection have antibodies to placental type isolates [8,9], but it is not clear whether the Ig detected on the parasites sequestered in the placenta is non-immune Ig as suggested [14], or is acquired antibody. This could have important implications for vaccine developments targeting PfEMP1. In addition, there are naturally occurring antibodies that can bind to antigens, such as the RBC anion transporter Band 3, which is exposed on the surface of IRBC [17].

The usual consequence of Ig binding to cells is opsonic phagocytosis mediated by Fc receptors on monocytes or macrophages. Given the presence of monocyte infiltrates in some infected placentas [18], interactions between these cells and IRBC binding of specific or non-immune Ig need investigation. Cellular immune responses are modified in pregnancy [19] and this could account for impaired clearance of parasites sequestered in the placenta, despite the presence of specific antibodies. Aggregates formed by IRBC and monocytes are frequently observed in the placenta by histology and could also contribute to parasite sequestration [20].

Future directions

We believe that there are two processes that need to be clearly differentiated to further our understanding of maternal malaria in general (Box 1). First, there are several factors that contribute to the higher susceptibility of pregnant women to malaria, including altered immune function in pregnancy [19] and the selection of specific variants that can evade existing immunity [1]. Animal studies will provide valuable information in this regard. However, women living in endemic areas have acquired substantial malarial immunity before becoming pregnant and it will be difficult to determine the effect of such immunity in animal models of maternal malaria . Prospective studies are needed to confirm the reported associations between immune responses and apparent protection [1]. Second, the mechanisms of placental parasite sequestration closely linked to adverse clinical outcomes need to be carefully assessed in the broader context of maternal malaria. The emerging understanding of placental infection suggests a complex process involving multiple events, which could pose many challenges to the development of effective interventions.

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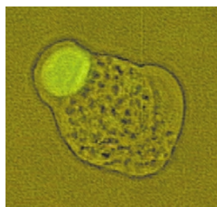
Meeting Report

Entamoeba histolytica Genome Project: an update

Barbara J. Mann

A round table discussion for scientists participating in the *Entamoeba histolytica* Genome Project was held 13 November 2001, at the American Society of Tropical Medicine and Hygiene meeting, Atlanta, GA, USA.



This was the second meeting of the *Entamoeba histolytica* Genome Project. The first meeting was held at The Institute for Genomic Research (TIGR) in Rockville, MD, USA in October 1999. Both meetings were made possible by support from the Burroughs Wellcome Fund, which was instrumental in helping to establish this genome project. The project is supported by a National Institute of Allergy and Infectious Diseases (NIAID) and National Institutes of Health (NIH) grant and by the Wellcome Trust (London, UK).

TIGR and Sanger sequencing projects

Brendan Loftus (TIGR, Rockville, MD, USA) has completed an estimated twofold coverage of the 20 MB *E. histolytica* genome. The current genome assembly appears to be accurate, based on the analysis concerning the orientation of forward and reverse sequencing reads of the same DNA fragment on an assembled fragment. The presence of multiple copies of an episome encoding the ribosomal DNA (rDNA) genes has complicated the sequencing process because ~15% of the resulting sequence corresponds to this episome. The plans for future sequencing and assembly of the genome at TIGR have been modified as a result of the participation of the Wellcome Trust Sanger Centre project led by Neil Hall. The two centers will work in collaboration to increase coverage and assembly of the *E. histolytica* genome. Sequences will be organized into FTP files, which will be transferred between the Sanger Centre and TIGR. A library, which is relatively free of rDNA circles, has been derived at the Sanger Centre by linearizing the rDNA circles with a restriction digest by *Ppo*I, as suggested by Alok Bhattacharya (Jawaharlal Nehru University, New Delhi, India) and then physically removing the linearized DNA from a pulse field gel. The small-insert library, free of rDNA circles, has now been prepared and will be sequenced to a fivefold coverage of the genome (200 000 sequence reads) by May 2002. This library will be compared with the sequences obtained at TIGR to ensure that nothing (apart from the rDNA circle) has been lost.

Assembly of the sequence will probably require large insert (10–45 kb) libraries partly because of the repetitive nature of the *E. histolytica* genome. To enable assembly of contigs and finishing of the genome, the plan is to prepare a bacterial artificial chromosome (BAC) library with 30–60 kb inserts and to sequence the ends of the cloned inserts. As soon as an eightfold or more sequence coverage of the genome is achieved from the combination of small-insert and large-insert libraries (goal for May 2002), the intent is to 'freeze' the data, construct draft chromosomes, assign gene names and annotate the genome. The GlimmerM software from TIGR, which was used to annotate the *Plasmodium* spp. genomes, will be adapted to *E. histolytica* genes.

Complete vs partial genome sequences

The scientific importance of having a complete sequence vs 99% partial sequence (expected to lack 60 of the 6000 estimated genes) was discussed. The consensus was to move forward with plans to 'freeze' the data when 99% coverage of the genome has been obtained. Annotation could then begin in May 2002 instead of waiting for two years (the expected time to complete the genome). A compromise might be to completely sequence one chromosome, so that issues concerning gene organization and structure can be addressed. Final decisions will be made when a large-insert library has been produced.

The proposed naming of genes is based on the gene ontology program, currently in widespread use in genome projects (see http://www.geneontology.org). The advantages of the gene ontology program include: (1) it is not organism specific and (2) it allows simplified querying of genome databases. Genes are assigned names that reflect their molecular function (protein or gene activity), biological process (e.g. mitosis and purine metabolism) and subcellular location (e.g. nucleus, telomere and membrane). The gene ontology program will allow easy-to-perform searches and enable gene clustering for microarray analysis.

Other *Entamoeba*?

Partial sequencing of a different *Entamoeba* species in the final year of NIH support (2003) at TIGR was debated. Possibilities include partial sequencing of *Entamoeba dispar* (a non-invasive human parasite closely related to *E. histolytica*) or *Entamoeba invadens* which is more distantly related to *E. histolytica* than *E. dispar*, but offers the advantage of a

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