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Short communication

Binding affinity of *Plasmodium falciparum*-infected erythrocytes from infected placentas and laboratory selected strains to chondroitin 4-sulfate

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Abstract

The adherence of *Plasmodium falciparum*-infected red blood cells (IRBCs) in human placenta is mediated by chondroitin 4-sulfate (C4S). The C4S-adherent parasites selected from laboratory strains have been widely used for determining the C4S structural elements involved in IRBC binding and for the identification of parasite adhesive protein(s). However, as far as we know, the relative binding strength of the placental versus laboratory-selected parasites has not been reported. In this study, we show that IRBCs from the infected placentas bind to C4S about 3-fold higher than those selected for C4S adherence from laboratory strains. Although adherent parasites selected from several laboratory strains have comparable binding strengths, the one obtained from 3D7 parasites designated as 3D7N61 used for malaria genome sequencing, exhibits markedly lower binding strength. Furthermore, 3D7N61-CSA parasites lose most of the binding capacity by tenth generation in continuous culture.

Keywords: *Plasmodium falciparum*; Infected erythrocytes; Adherence; Chondroitin 4-sulfate; Laboratory parasite strains; Placental parasite isolates; Binding strength

A unique feature of *Plasmodium falciparum* infection in pregnant women is that parasite-infected red blood cells (IRBCs) sequester extensively in the placenta, causing placental malaria that is associated with poor pregnancy outcomes and severe maternal anemia and death [1–3]. While CD36 on the endothelial cell surface is the major receptor for IRBC sequestration in the microvascular capillaries [2,4], chondroitin 4-sulfate (C4S) chains of uniquely low sulfated chondroitin sulfate proteoglycans (CSPGs) mediate placental IRBC accumulation [3,5–7]. Developing a vaccine for placental malaria based on disrupting C4S–IRBC interaction, thereby preventing placental IRBC accumulation using the parasite adhesive protein as a candidate has been extensively pursued [1,8]. However, a critical requirement for such efforts is the definitive identification of parasite adhesive protein(s) expressed on the IRBC surface. Although a number of studies have reported DBL-γ domain of *var1* subfamily *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) as the ligand for IRBC binding to C4S [2,9,10], recent studies suggest that DBL-like domains of *var2* subfamily PfEMP1 mediates the cytoadherence [8,11,12]. However, in both cases, involvement of PfEMP1 in IRBC adhesion has not been conclusively established. A scenario wherein novel proteins unrelated to PfEMP1 playing roles in the adhesive process cannot be ruled out. Consistent with these predictions, recent studies implicate novel proteins in IRBC adherence [13–16]. Thus, the development of a vaccine for placental malaria remains challenging.

A promising alternative approach for the treatment of placental malaria would be to develop inhibitors that efficiently disrupt C4S–IRBC adherence. In this regard, either structurally defined C4S oligosaccharides or rationally designed peptide mimetics might prove to be effective therapeutic agents. Such efforts require a comprehensive understanding of the structural interactions involved in C4S–IRBC binding. Although recent studies have provided a considerable amount of information on the critical structural elements involved in C4S–IRBC adhesion [7,17–19], further understanding of the precise sulfate group distribution required for maximal IRBC binding and determination of binding strength are also critically needed. Studies aimed at

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delineating the structural elements involved in C4S–IRBC binding have used C4S-adherent parasites selected from different strains of laboratory-cultured parasites. The adherent parasites were obtained by panning laboratory strains either on immobilized bovine tracheal C4S or CSPG purified from human placenta. Recently, a human choriocarcinoma trophoblast cell line, BeWo, has been used for selecting C4S-adherenrt parasites [20–22] and found that the selected parasites bind predominantly to CSA, although minor populations exhibited binding specificity to other ligands. However, it should be noted that CSPG synthesized by cultured BeWo cells have not been characterized and it is possible that CSPGs synthesized by these cells are markedly different from those present in human placenta. Some studies have noted that the adherent parasites selected from various strains exhibit different binding capacity [6,23]. However, the reasons for the observed differences in binding ability of selected parasites from various laboratory strains remain unclear. Possible reasons are: (i) the selected parasites might be phenotypically different, expressing polymorphic adhesive protein(s) or they could express different levels of adherent protein(s) on the IRBC surface. (ii) The differences could be simply a reflection of variations in the binding strengths with which the parasites were selected on structurally distinct receptors used for panning; for example, the bovine tracheal CSA, an unnatural receptor, is structurally different from C4S chains of the placental intervillous CSPG, a natural receptor [24]. (iii) The binding studies might have been performed with parasites cultured for different duration after panning; it is important to assess the C4S–IRBC interactions soon after panning because their binding strengths drastically decreases after prolonged culturing [25]. To rationalize the results obtained using C4S-adherent parasites from various laboratory strains for therapeutic applications, it is essential to understand the differences and similarities in binding strength of these parasites as compared to those of placental isolates. Therefore, relative evaluation of variously selected parasites and those isolated from infected placenta is required. In this study, we focused to comparatively assess the binding strengths of a panel of C4S-adherent laboratory strains by panning on CSPG purified from human placenta and two parasite isolates obtained from infected placentas. All IRBC binding and inhibition studies were performed using the placental CSPG, the receptor that mediates *in vivo* IRBC adherence.

The relative binding strength of laboratory C4S-selected parasites versus placental isolates was assessed by measuring the amount of a partially sulfated C4S polysaccharide required for inhibition of IRBC binding to human placental CSPG. The proteoglycan was isolated from the isotonic buffer extracts of freshly delivered placentas, collected from the Medical Center Maternity Ward, by DEAE-Sephacel column chromatography using NaCl gradient elution as described previously [5]. The CSPG was purified by CsBr density gradient centrifugation followed by gel filtration on Sepharose CL-6B [5]. The CS chains of the purified CSPG consists of ∼8% 4-sulfated and ∼92% non-sulfated disaccharides. This CSPG is comparable to the previously reported low sulfated CSPG present in the intervillous space and could specifically and efficiently bind IRBCs from both placental isolates and C4S-selected laboratory parasites [5]. Laboratory parasite strains were cultured using O-positive human erythrocytes and O-positive human plasma and the C4S-adherent IRBCs were selected by panning on plastic plates coated with 250 ng/ml CSPG in PBS [17]. The IRBC binding assays were performed by allowing IRBCs to bind to the purified human placental CSPG coated on plastic plates at different concentrations and counting the IRBCs bound to unit area of the plates under microscopy. Inhibition assays were performed by allowing IRBCs pretreated with different concentrations of C4S to bind to plastic plates coated with 250 ng/ml of the purified placental CSPG [17]. The extent of inhibition was assessed, using light microscopy, by measuring the IRBCs bound to CSPG at each C4S inhibitory concentration. The partially sulfated C4S polysaccharide used for inhibition assays consisted of 36% 4-sulfated and 64% non-sulfated disaccharides and was prepared by regioselective 6-*O*-desulfation of bovine tracheal CSA (Sigma) followed by fractionation on DEAE-Sephacel column as reported previously [17,24]. We have previously shown that this partially sulfated C4S fraction effectively inhibits IRBC binding to the human placental CSPG [17,24]. All inhibition assays were performed three times each in duplicates. Statistical analysis of data was performed using GraphPad Prism 4.0. Variations between two groups were determined by Student '*t*' test. Two-way ANOVA was used to analyze differences among group means. p values of ≤ 0.05 was considered significant.

The laboratory *P. falciparum* strains assessed in this study were those previously used by various investigators for studying C4S–IRBC interactions. These include, C4S-selected parasites from a 3D7 clone derived from NF54, FCR3, CS2 parasite strains [10,17], and the 3D7N61 clone that was used for the parasite genome sequencing. The C4S-adherent parasites were selected by 5–6 rounds of panning, each time after culturing for 4–5 generations, on plastic Petri dishes coated with the placental CSPG; further rounds of panning did not enhance the binding capacity (not shown). The C4S-selected laboratory parasites were designated as 3D7-CSA, FCR3-CSA, CS2-CSA, and 3D7N61-CSA. To determine the IRBC binding ability and binding strengths by adherence and adherence inhibition assays, the selected laboratory parasites were analyzed at the early trophozoite stage because parasites at this stage exhibit maximum adherence capacity [25]. Since we have previously shown that the binding capacity of 3D7-CSA and FCR3-CSA decreases gradually over successive generations after selection [25], the second-generation parasites after selection were used in this study. To determine the binding characteristics of 3D7N61-CSA parasites at various generations after C4S-selection, IRBCs from continuous culture were used. The placental parasite isolates were obtained from two *P. falciparum*-infected Cameroonian women placentas. The parasites from infected placentas were cultured for one generation (∼10% parasitemia), harvested at the early trophozoite stage as described previously [26], and stored frozen in glycerolyte solution at −80 °C until used. The two placental isolates were not genotyped and therefore, it is not known whether these isolates are genetically identical or diverse.

The C4S-adherent laboratory parasites and placental isolates, adjusted to ∼10% parasitemia, were assessed for their binding capacity. In binding assays, both laboratory selected