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Letter to the Editor

Characteristics of *Plasmodium falciparum*-Infected-Erythrocyte Adhesion to Chondroitin Sulfate

In a recent article in *Infection and Immunity*, Madhunapantula et al. stated that the characteristics of adhesion of *Plasmodium falciparum*-infected erythrocytes (IEs) to chondroitin-4-sulfate (C4S) at different stages of parasite development were not known (6), and they presented findings that examined these properties. However, those authors have overlooked earlier reports that documented the effects of parasite development on IE adhesion to C4S.

Previously, we studied *P. falciparum* over its full intraerythrocytic life cycle and reported that adhesion to C4S (also known as chondroitin sulfate A) varies with the developmental stage of the parasite (3). Adhesion to immobilized C4S occurred at low levels when parasites were at the immature ring stage and increased with parasite age until adhesion was maximal at the mature pigmented trophozoite stage (24 to 30 h). The levels of adhesion then declined as parasite age increased, and the adhesion of mature schizont-stage parasites was 38% of the maximal level of adhesion observed (3). Madhunapantula et al. now report similar findings, also demonstrating that adhesion declined as parasites matured into schizonts (6). Others reported that C4S-mediated adhesion of IEs to *Saimiri* brain endothelial cells did not significantly change from 24 to 44 h postinvasion (7), suggesting that adhesion to intact cells may differ from adhesion to purified receptors. The levels of adhesion to C4S across developmental stages are broadly consistent with the developmental stages of parasites that appear to be sequestered in the placenta (1). Of further relevance is the fact that others have reported that the adhesion of IEs to uninfected erythrocytes (rosette formation) and the binding of specific immunoglobulin G to IE surface antigens were lower among schizonts (8).

Madhunapantula et al. also incorrectly stated that we found that the adhesion of the parasite line 3D7-CSA to C4S was "completely unaffected" by trypsin treatment (100 μg/ml) of the IE surface (6). Instead, we found that the adhesion of 3D7-CSA was inhibited by 57% at 10 μg/ml and >90% at 100 μg/ml (4) after a 15-min incubation, which is not dissimilar to results now reported by Madhunapantula et al. (although different concentrations of trypsin and incubation conditions were used). We did find that the parasite line CS2 was largely resistant to trypsin treatment at 100 μg/ml, even though the surface *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) was cleaved (4). Although the sensitivity of IE adhesion to trypsin varied between parasite lines, trypsin cleaved PfEMP1 in all lines we studied (4). Differences between parasite lines in the levels of sensitivity of C4S adhesion to trypsin might be explained by polymorphisms in *var2csa* that affect the sites at which cleavage occurs (4). Interestingly, polymorphisms in *var2csa* also correspond with differences between isolates in the fine specificity of interactions with structural motifs of C4S for IE adhesion (2). It should be noted that the conditions used for the trypsin treatment of IEs has varied among different reports. We used short incubation times (10 to 15 min), consistent with conditions used in the original characterization of PfEMP1 (5). An awareness of these differences may be important when comparing results of studies.

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James G. Beeson
The Walter and Eliza Hall Institute of Medical Research
1G Royal Parade
Parkville 3050, Victoria, Australia

Phone: 61-3-9345-2555
Fax: 61-3-9347-0852
E-mail: beeson@wehi.edu.au

Authors' Reply

In the abstract of our paper, we stated that "the adherence characteristics of IRBCs at different stages of parasite development and through successive parasite generations after selection for C4S adherence are not known" (3). While it is true that the binding strength of *P. falciparum*-infected red blood cell (IRBC) adhesion to placental chondroitin sulfate proteoglycan during different stages of parasite development and the binding characteristics of C4S-adherent IRBCs through successive generations in continuous culture were not known before our study, it was brought to our attention after the publication of the paper that previously two groups had reported briefly on the stage-dependent binding of IRBCs to chondroitin sulfate A (CSA) in their papers that dealt with the dual specificity of IRBC binding to CSA and hyaluronic acid (1) and the biochemical characterization of IRBC adherence to C4S (4). Therefore, the above-quoted sentence should be read as "the adherence characteristics of IRBCs at different stages of parasite development and through successive parasite generations after selection for C4S adherence remain poorly understood." Further, previously only the binding of CS2-CSA IRBCs was found to be resistant to trypsin treatment (2), and

not that of both CS2-CSA and 3D7-CSA IRBCs, as stated on page 4413 of our paper.

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SubbaRao V. Madhunapantula
Rajeshwara N. Achur
D. Channe Gowda*
Department of Biochemistry and Molecular Biology
Pennsylvania State University College of Medicine
500 University Drive
Hershey, Pennsylvania 17033

*E-mail: gowda@psu.edu

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