The NTS-DBL2X Region of VAR2CSA Induces Cross-Reactive Antibodies That Inhibit Adhesion of Several Plasmodium falciparum Isolates to Chondroitin Sulfate A

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Background. Binding to chondroitin sulfate A by VAR2CSA, a parasite protein expressed on infected erythrocytes, allows placental sequestration of Plasmodium falciparum–infected erythrocytes. This leads to severe consequences such as maternal anemia, stillbirths, and intrauterine growth retardation. The latter has been clearly associated to increased morbidity and mortality of the infants. Acquired anti-VAR2CSA antibodies have been associated with improved pregnancy outcomes, suggesting a vaccine could prevent the syndrome. However, identifying functionally important regions in the large VAR2CSA protein is difficult.

Methods. Using genetic immunization, we raised polyclonal antisera against overlapping segments of VAR2CSA in mice and rabbits. The adhesion-inhibition capacities of induced antisera and of specific antibodies purified from plasma of malaria-exposed pregnant women were assessed on laboratory-adapted parasite lines and field isolates expressing VAR2CSA. Competition enzyme-linked immunosorbent assay (ELISA) was employed to analyze functional resemblance between antibodies induced in animals and those naturally acquired by immune multigravidae.

Results. Antibodies targeting the N-terminal sequence (NTS) up to DBL2X (NTS-DBL2X) efficiently blocked parasite adhesion to chondroitin sulfate A in a manner similar to that of antibodies raised against the entire VAR2CSA extracellular domain. Interestingly, naturally acquired antibodies and those induced by vaccination against NTS-DBL2X target overlapping strain-transcendent anti-adhesion epitopes.

Conclusions. This study highlights an important step achieved toward development of a protective vaccine against placental malaria.

Placental malaria (PM) is an important cause of maternal anemia, stillbirth, and low birth weight children in Africa. In this syndrome, Plasmodium falciparum infected erythrocytes (IEs) accumulate in the placenta by binding to the chondroitin sulfate A (CSA) moiety of placental intervillous chondroitin sulfate proteoglycan (CSPG). This binding is mediated by a member of the P. falciparum erythrocyte membrane protein 1 (PfEMP1) family named VAR2CSA, which is expressed on the surface of IEs [1–3]. Reduced susceptibility to placental malaria is observed in women who have acquired immunoglobulin G (IgG) that blocks adhesion of IE to the placental receptors through several malaria-exposed pregnancies [4–6]. Increased levels of those antibodies have been associated with improved pregnancy outcomes, suggesting their importance in immunity to PM [5, 7, 8]. The implication of VAR2CSA in the expression of the CSA-adhesion phenotype by IE has...
clearly been demonstrated [9], and accumulated evidence shows that naturally acquired antibodies against VAR2CSA play a major role in the protective immunity to PM [10, 11]. The var2csa gene is present in almost all P. falciparum isolate genomes as well as in the close relative Plasmodium reichinovii, and it encodes a high molecular weight, polymorphic protein of about 350 kDa consisting of 6 Duffy binding–like (DBL) domains, a cysteine-rich interdomain region (CIDR)–like domain, and 2 interdomain regions [12, 13]. The large size and the interclonal variation among var2csa alleles have been of major concern in the feasibility of an effective VAR2CSA-based vaccine. Experimental vaccination of animals with single-domain VAR2CSA recombinant proteins induced antibodies that at various degrees could inhibit CSA adhesion of erythrocytes infected by different laboratory-adapted clones of P. falciparum [14, 15].

The full-length extracellular part of the molecule has recently been expressed for the first time in eukaryotic systems [16, 17], and antibodies induced against this construct showed a very high anti-adhesion IgG titer. However technological constraints in the optimal production of such a large antigen question the use of full-length VAR2CSA in vaccine development. Defining smaller parts of the molecule with functionally important antibody epitopes shared among most VAR2CSA variants, ie, inducing antibodies that inhibit CSA binding of IEs is still of much interest in a rational way of developing a VAR2CSA-based vaccine.

In this study, we investigated the possibility of identifying such functionally important VAR2CSA regions that can induce IgG with high adhesion inhibitory capacity. Using intramuscular plasmid DNA electrotransfer, we showed that antibodies induced against a specific N-terminal region of VAR2CSA, the NTS-DBL2X, can efficiently block parasite binding to CSA at a similar level to that of IgG induced against the full-length extracellular part of the molecule. This work highlights an important achievement toward development of a protective vaccine against placental malaria.

METHODS

Ethics Statement
The study was approved by the Comité Consultatif de Déontologie et d’Ethique of the Research Institute for Development (France); the ethical committee of the Ministry of Health, Senegal; and the ethics committee of Health Science Faculty, University of Abomey-Calavi (Benin). All procedures complied with European and national regulations.

All procedures regarding animal immunizations complied with European and national regulations. Ethics statement details are given Supplementary material.

Parasites and Human Plasma
In this study, we used in vitro–propagated P. falciparum parasites FCR3 and HB3 grown in O+ erythrocytes without human serum as described [18]. We tested antibody reactivity with IEs on unselected cultures and cultures selected for IE adhesion to CSA. We selected cultures following several panning on the choriocarcinoma cell line BeWo as described [19].

Primary field P. falciparum isolates and plasma samples were collected from a cohort of pregnant women enrolled in the STOPPAM project in the district of Comé at the Mono province, located 70 km West from the economical capital of Benin, Cotonou [20]. The isolates were obtained either from the peripheral blood of children below the age of 5 years (n = 5) and pregnant women (n = 24), or from placental blood at delivery (n = 6). Peripheral blood isolates were maintained in vitro for ≤48 hours before testing.

Plasma samples from a previous study conducted in Senegal were also used [8].

Animal Immunization and Antibody Screening
DNA sequences encoding single and multiple domains of VAR2CSA protein were cloned into a pVax1 vector derivative and fused to the mEPO signal sequence as already described [21]. We anesthetized 6-week-old Swiss female mice by intraperitoneal injection of 0.3 mL of a mix of ketamine (8.66 mg/mL) and xylazine (0.27 mg/mL) in 150 mM sodium chloride. 40 μg of plasmid DNA was injected into the tibial cranial muscle. Transcutaneous electric pulses (200 V/cm, 20 ms, 5Hz) were then applied at each side of the leg [21]. New Zealand rabbits were anesthetized by intramuscular injection of a ketamine–xylazine mix, 300 μg of plasmid was injected in 5 sites of each longissimus dorsi muscle, and electrical pulses (120V/cm, 20 ms, 5Hz) were applied. We performed mice protein immunizations by intraperitoneal injection of a recombinant protein–Algel mix. Animals were immunized at days 0, 30, and 60, and antisera were collected at days 45 and 75. IgG serum titres were determined by enzyme-linked immunosorbent assay (ELISA) tests using each recombinant DBL domain. Animal immunization and antibody screening details are given as SI text.

IgG Preparation
We manually purified total IgG from final bleed mouse or rabbit sera on a Hi-Trap Protein G High Pressure (HP) column according to manufacturer’s recommendations (GE Healthcare). We affinity-purified construct-specific IgG from plasma pools of women exposed to placental malaria and exposed males using HiTrap N-Hydroxy-Succinimide (NHS)-activated HP columns (GE Healthcare, http://www.gehealthcare.com) on which the corresponding recombinant protein was coupled following the manufacturer’s instructions.

Antibody Reactivity With Plasmodium falciparum Laboratory Lines and Field Isolates
In vitro propagated P. falciparum parasites FCR3 and HB3 were repeatedly panned on the human choriocarcinoma cell line BeWo as described [19]. We analyzed the derived CSA-adhering
IIEs (FCR3-BeWo, HB3-BeWo) and 35 primary field P. falciparum isolates collected at Comé, southwestern Benin [22] to determine reactivity of all generated antibodies. We used flow cytometry (FACS Calibur) to test the serum reactivity of vaccinated animals to the surface of the IE as previously described [23].

**Protein Expression, Purification, and Evaluation**

The NTS-DBL2X region of the var2csa gene from FCR3 parasite line (synthetic gene) was cloned from amino acid N9 to A864 into the baculovirus vector pAcGP67-A (BD Biosciences) upstream of a histidine tag in the C-terminal end of the construct. This construct was expressed and purified. Protein expression, purification, and evaluation details are given as SI text.

Specific recognition of the purified protein was evaluated in ELISA using plasma samples from pregnant women of Benin and Senegal, unexposed pregnant French women, and malaria-exposed children (Senegal) and men (Benin and Senegal).

**Inhibition of IE Binding to CSPG by Specific IgG**

The static assays employed to evaluate the capacity of the antibodies to interfere with CSA-specific adhesion of IEs was described in detail elsewhere [24]. In this assay, plates were coated overnight at 4°C with 20 μL of ligand: 1% bovine serum albumin (BSA), 5 μg.mL⁻¹ decorin: CSPG (Sigma) or 50 μg.mL⁻¹ bovine CSA (Sigma) diluted in phosphate-buffered saline (PBS). Each spot was subsequently blocked with 3% BSA in PBS for 30 minutes at room temperature (RT). Late stage–infected IEs were also blocked in BSA/RPMI for 30 minutes at RT. Parasite suspensions adjusted to 20% parasite density were incubated with serum (1:5 final dilution), purified IgG (0.01 mg.mL⁻¹ to 1 mg/mL final concentration), or 500 μg.mL⁻¹ soluble CSA for 30 minutes at RT before they were allowed 15 minutes at RT to bind to ligand. Nonadhering cells were removed by an automated washing system. Spots were fixed with 1.5% glutaraldehyde in PBS and adhering IEs were quantified by microscopy.

**Competition ELISA**

Prior to competition of ELISA, the anti–NTS-DBL2X IgG titre was determined in plasma pools composed of samples from exposed multigravid women from Benin, DNA-vaccinated rabbits, and protein-immunized mice (plasma pools from D75). Microtiter plates were coated with recombinant NTS-DBL2X (0.5 μg.mL⁻¹ in PBS). Competition ELISA details are given as SI text.

**RESULTS**

**Plasmid DNA Immunization Induced High Titre Surface Reactive Antibodies**

A total of 13 plasmids representing single and overlapping multiple domains of VAR2CSA from the FCR3 (Figure 1A; Table 1) parasite line were constructed and used for immunization. All immunizations with single- to triple-domain constructs of VAR2CSA induced polyclonal antibodies with a high ELISA titre (>1 E⁻⁰⁵) following intramuscular plasmid electrottransfer. However, for plasmids containing more than 3000 base pairs of coding sequence, effective humoral immune response in all vaccinated animals, both mice and rabbits required the use of a codon-optimized sequence (GenBank accession no. GU249598).

Although all single domains and multidomains of VAR2CSA could induce antibodies reacting with native VAR2CSA on the surface of the CSA-adhering erythrocytes infected with the homologous FCR3, constructs containing DBL1x, DBL2x, DBL5e, and DBL6e were the most efficient in inducing surface reactive antibodies (Figure 1B). None of the polyclonal anti-VAR2CSA antisera recognized the erythrocytes infected with the non-CSA adherent FCR3 parasite line.

**Antibodies Induced Against VAR2CSA Abrogate Binding of Infected Erythrocytes to Chondroitin Sulphate Proteoglycan**

The petri dish–based static binding assay was used to screen sera for the ability to inhibit parasite binding to CSA. Of all the FCR3 VAR2CSA regions tested, only sequences located between the NTS and the DBL3X appeared to induce inhibitory antibodies (Figure 1B). Highly inhibitory antibodies were obtained with the full-length extracellular VAR2CSA construct, which totally abrogated binding. Interestingly, similar inhibition was seen with sera from animals (both mice and rabbits) vaccinated with the NTS-DBL2X construct. In addition, we investigated the inhibitory activity of sera from NTS-DBL2X vaccinated animals on a heterologous parasite line; the CSA adherent HB3 line. The same pattern of inhibition was observed (Figure 2A).

To confirm that the inhibition observed with NTS-DBL2X antisera was mediated by IgG, we purified IgG and tested for binding inhibition activity. The purified IgG recognized the surface of BeWo-selected FCR3 IEs (Figure 2B). The IgG inhibited 100% of the binding of infected erythrocytes to CSA at a concentration of 0.5 mg.mL⁻¹ (Figure 2D).

**Antibodies Induced Against NTS-DBL2X Specifically Recognized Isolates From Pregnant Women**

Flow cytometry analysis clearly demonstrated that murine anti–NTS-DBL2X antibodies specifically recognize the surface of PM parasites among the field isolates. In this study, we used flow cytometry to analyze 35 isolates, which comprised 24 peripheral blood isolates from pregnant women, 6 placental isolates, and 5 peripheral isolates from children. Twenty-one of the 24 peripheral blood isolates from pregnant women were recognized by polyclonal murine antibodies, whereas none of the 5 children isolates tested were labeled.

Of the 21 isolates from pregnant women that reacted with anti–NTS-DBL2X antibodies by flow cytometry, 16 showed specific adhesion to CSPG, whereas 5 isolates did not bind. Among the 3 peripheral blood isolates that were not labeled in flow cytometry, 2 bound to CSPG but their interaction could not be abrogated by soluble CSA, and 1 isolate did not bind.
We further processed in binding inhibition assay 15 samples containing sufficient amounts of parasites. These comprised 14 isolates from peripheral blood samples and 1 placental isolate. The binding to CSA of 12 of the 15 pregnant women isolates tested was highly inhibited by specific anti–NTS-DBL2X sera (Table 2).

Animals Immunized With Recombinant NTS-DBL2X or DNA Electrotransfer Produced Antibodies of Similar Specificity

Murine polyclonal antibodies induced either by recombinant protein or plasmid DNA of NTS-DBL2X showed similar reactivity. The reactivity to the erythrocyte surface and inhibitory activity on binding to CSA were similar on BeWo-selected FCR3 infected erythrocytes. The inhibitory activity was compared in dilution series of sera from mice immunized with either the full-length construct or NTS-DBL2X (both DNA and protein vaccination). Down to the dilution 1:100, sera from mice vaccinated with full-length DNA construct or recombinant NTS-DBL2X totally inhibited binding of infected erythrocytes (Figure 3A). The inhibition capacity of the serum samples following plasmid DNA immunization with the full-length construct or by protein vaccination with NTS-DBL2X was seen at subsequent dilutions, these sera were diluted 1:5000 before inhibition vanished (Figure 3A). This observation clearly strengthens the importance of the NTS-DBL2X part of VAR2CSA in eliciting adhesion-inhibitory antibodies by vaccination.

Antibodies Induced in Animals by Vaccination With NTS-DBL2X Target the Same Epitopes as Naturally Acquired Antibodies

The recombinant NTS-DBL2X produced in insect cells was recognized by plasma from malaria-exposed pregnant women from Benin and Senegal in a parity-dependent manner (Supplementary Figure 1). This NTS-DBL2X was used in competition ELISA to analyze the target epitopes among
antibodies induced in animals by plasmid DNA immunization and protein immunization, as well as the naturally acquired antibodies against the NTS-DBL2X part of VAR2CSA in pregnant women. A mutual inhibition pattern was observed in the ability of all 3 antisera to recognize the recombinant NTS-DBL2X protein. The inhibition pattern between sera from DNA immunizations and protein immunizations was concentration-dependent (Figure 3B). A similar inhibition was observed when antibodies in a human plasma pool from exposed multigravidae competed with specific antisera from rabbits (Figure 3C).

The Naturally Acquired Human IgG Against VAR2CSA NTS-DBL2X Inhibit Adhesion of Infected Erythrocytes to CSA

Plasma samples from women included in the STOPPAM project are routinely analyzed for anti-adhesion capacity on the FCR3-BeWo and HB3-BeWo parasite lines. The recombinant NTS-DBL2X protein was used to affinity-purify IgG from plasma of malaria-exposed Beninese pregnant women (selected for having a high anti-adhesion activity on CSA-binding parasite lines). Interestingly, naturally acquired antibodies targeting the NTS-DBL2X of VAR2CSA demonstrated anti-adhesion activity. This activity was shown both on FCR3-BeWo and HB3-BeWo parasite lines, with a clear concentration-dependent effect of purified IgG (Figure 4). This is the first time that naturally acquired antibodies to a specific VAR2CSA region have been shown to inhibit P. falciparum–infected erythrocytes binding to CSA.

DISCUSSION

Molecular details of the interaction of the P. falciparum ligand VAR2CSA with the placental receptor CSA are currently not well delineated, but recent studies suggest that the binding site depends on a higher-order architecture in which DBL domains and the interdomain regions of VAR2CSA fold together to form a ligand-binding pocket [16, 25]. However, polyclonal antibodies induced by immunization with the recombinant extracellular part of VAR2CSA highly inhibit binding of IEs to CSA in vitro [16]. This suggest that protective immunity to placental malaria acquired over a few pregnancies in areas of intense P. falciparum transmission that correlates with levels of anti-adhesion antibodies [7] is mostly mediated by anti-VAR2CSA IgG. Nevertheless, a recent work reports that immunization with full-length VAR2CSA did not induce potent cross-inhibitory antibodies [26]. This is surprising, but, as indicated by the authors, it could relate to the recombinant protein preparation (FCR3 allele in baculovirus insect cells versus 3d7 allele in mammalian cells), the animal species immunized, the adjuvant employed (Freund complete/incomplete adjuvant vs titer-max), or simply the size and complexity of full-length VAR2CSA immunogens.

Although antibody response may directly inhibit IE adhesion placenta, it also might be implicated in opsonization [27, 28].

VAR2CSA thus appears as an important candidate for vaccine development, however sequence analyses among parasites have shown that it is a polymorphic protein composed of alternating areas of substantial interclonal polymorphism [12, 13]. The rationale for developing an effective VAR2CSA-based vaccine against placental malaria thus requires definition of VAR2CSA areas containing functionally important epitopes that transcend this interclonal diversity. In this study, full-length and truncated VAR2CSA constructs were studied for their ability to induce adhesion-inhibitory antibodies.

The DNA vaccine technology that has proven efficient on various pathogens and tumor antigens [29] was successfully achieved here with the P. falciparum var2csa gene. The resurgence in interest for such concept observed in the last few years is due to several technical improvements, such as codon optimization strategies, novel formulations, and more-effective delivery approaches. The delivery of electrical pulses after intramuscular plasmid DNA injection particularly enhanced DNA uptake and resulted in a stronger and more specific humoral response when the antigen was fused to a leader sequence [21]. Several clinical trials based on this approach are currently being conducted in the fields of cancer and infectious diseases (http://clinicaltrials.gov/ct2/results?term=electroporation); 1 of the trials that started in July 2010 targets Plasmodium falciparum malaria.

In this study, a strong immune response was obtained both in mice and rabbits vaccinated with VAR2CSA genetic fragments that were fused to the mEPO leader sequence. Interestingly, all antibodies induced were able to recognize the native protein expressed on the surface of erythrocytes infected with the homologous FCR3 parasite line. In line with data previously reported by Khunrae et al [16], the plasmid encoding the full-length extracellular part of the protein induced a robust humoral response.

Table 1. VAR2CSA Constructs of Plasmodium falciparum Parasite FCR3

<table>
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<tr>
<th>VAR2CSA construct</th>
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<tr>
<td>NTS-DBL2X</td>
<td>9–866</td>
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<tr>
<td>DBL2X-Id2</td>
<td>446–1208</td>
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<tr>
<td>Id2-DBL3X</td>
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<tr>
<td>DBL4a(a)</td>
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<td>DBL4a(b)</td>
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The antigens cloned from FCR3 VAR2CSA used for immunizations are described. Antigen boundaries are indicated as start and stop amino acids from the coding sequence.
that completely blocked IE binding to CSPG. However, the major finding of this study is that a shorter construct of the N-terminal moiety of VAR2CSA corresponding to NTS-DBL2X was able to induce high-potency antibodies with similar inhibitory capacity as those elicited against the full-length VAR2CSA. Moreover, competition ELISA analysis revealed that antibodies raised by experimental immunization (plasmid DNA or purified recombinant protein) or those naturally acquired by pregnant women to this particular region of VAR2CSA predominantly target similar epitopes. This result is in line with others that reported that pregnant women do acquire cross-reactive antibodies [30, 31]. This suggests that vaccination may reproduce, at least partially, natural acquired immunity against placental malaria.

Recombinant NTS-DBL2X expressed in insect cells was specifically recognized by sera from malaria-exposed women in a parity-dependent manner, supporting the fact that this recombinant protein exhibits important targets of the immune response against VAR2CSA. Murine polyclonal antibodies raised against this construct from the FCR3 parasite strain stained the surface of most isolates from pregnant women. Remarkably, antibodies raised against a single variant of NTS-DBL2X showed consistent inhibitory activity against several isolates originating from pregnant women. The binding of IEs to CSPG/CSA in isolates from 12 of the 15 pregnant women tested was inhibited by more than 50%. This highlights the existence of functionally important
epitopes within this region of VAR2CSA that are shared by most placenta-sequestering *P. falciparum* isolates. However, all isolates were not inhibited as a probable consequence of antigenic polymorphism. Possible mechanisms of action include that anti–NTS-DBL2X antibodies inhibit IE adhesion to CSPG/CSA by blocking a single, unique CSA-binding site or by modifying the assembly of such high-ordered structure mediating the binding of native VAR2CSA to CSA [15].

A recent work has indicated that antibodies directed against a DBL1X-DBL3X VAR2CSA construct induced inhibitory antibodies [32]. In our system, a lower inhibitory activity was seen with antibodies against the larger fragment NTS-DBL3X, suggesting that this construct, with our defined boundaries, does not accurately reproduce the critical antibody epitopes present in the native molecule or that the focus of the response is predominately directed toward epitopes that are distant from the functional site. The results presented here clearly indicate that antibody recognition of just a few VAR2CSA variants containing key epitopes might be sufficient to markedly affect the binding of VAR2CSA-expressing IEs to CSA.

Of particular interest, maternal antibodies purified with the recombinant NTS-DBL2X reacted with both -BeWo-selected FCR3 and HB3 strains and showed high inhibitory activity on these 2 distinct parasite lines. This indicates that the inhibitory properties of anti-VAR2CSA antibodies observed in the current study are of biological significance in the acquired immune protection to placental malaria. It was recently shown that some VAR2CSA-specific human monoclonal IgG from *P. falciparum*-exposed women can exhibit some moderate degree of adhesion inhibition that increases with their combination [23]. To the best of our knowledge, this study clearly shows functional evidence on a specific area of VAR2CSA that is a target of significantly naturally acquired anti-adhesion antibodies.

Antibodies induced in rats against the VAR2CSA DBL4e have previously been shown to possess adhesion-inhibitory activity [15]. Those antibodies were able to inhibit CSA-binding of *P. falciparum* IE from pregnant women [33]. No inhibitory activity with antibodies against DBL4e was found in the current study. It is possible that such antibodies cannot be obtained in mice and that acquisition of adhesion-inhibitory antibodies to this area of var2csa is host dependent. Our data nevertheless still support the hypothesis that simultaneous targeting of epitopes in different VAR2CSA regions is optimal. Demonstration that those identified VAR2CSA constructs are the target of naturally acquired cross-reactive and anti-adhesion antibodies should however be of major consideration in the effort to develop an effective VAR2CSA-based vaccine.

In conclusion, genetic immunization by intramuscular plasmid electrotransfer represents a general technology for fast and efficient screening of immunogenic domains within large proteins of which optimal production as recombinant proteins are technically demanding. This work showed that a truncated N-terminal region of VAR2CSA was a major target of anti-adhesion immune response in placental malaria and therefore

**Table 2. Adhesion Inhibitory Capacity of Specific Antibodies Induced Against NTS-DBL2X on Plasmodium falciparum-Infected Erythrocytes From Naturally Infected Pregnant Women in Benin**

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Bound IE/mm² on BSA</th>
<th>Bound IE/mm² on CSPG</th>
<th>MFI (Ratio to negative control)</th>
<th>% Prebleed inhibition</th>
<th>% anti-NTS-DBL2X inhibition</th>
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Antibody interference with the binding of 15 *P. falciparum*-infected erythrocytes collected from pregnant women to CSPG is shown. Control binding to BSA and competition of binding to CSPG with soluble CSA are shown for each parasite. The surface reactivity of induced antibodies is shown on each IE. The binding inhibitory activity of prebleed (pool of mice sera collected before immunization at day 0) is shown beside that of the corresponding immune serum (pool of sera collected at day 75 after the first immunization). The idenification numbers of the parasites tested are shown. Abbreviations: BSA, bovine serum albumin; CSA, chondroitin sulfate A; CSPG, chondroitin sulfate proteoglycan; IE, infected erythrocyte; MFI, median fluorescence intensity.
an attractive vaccine target. Further studies are required to ascertain the impact of sequence variation within this particular VAR2CSA region to its potential for cross-reactivity.

**Supplementary Data**

Supplementary material is available at *The Journal of Infectious Diseases* online.

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Figure 4. Naturally acquired immunoglobulin G (IgG) against VAR2CSA NTS-DBL2X target strain-transcendent anti-adhesion epitopes. Human IgG specific to NTS-DBL2X was affinity-purified from a pool of plasma from 10 multigravid women that previously showed high anti-adhesion capacities. The FCR3-BeWo and HB3-BeWo infected erythrocytes were incubated with different concentrations (12.5, 25, or 50 μg/mL) of the purified human anti-NTS-DBL2X IgG and the activity was compared with binding without competitor (blank) or soluble competing chondroitin sulfate A (CSA). None of the infected erythrocytes bound to BSA.

References


