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# Primary Structure of the Variable Region of Monoclonal Antibody 2B10, Capable of Inducing Anti-Idiotypic Antibodies That Recognize the C-Terminal Region of MSA-1 of *Plasmodium falciparum*

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**Previously, we reported on the properties of a monoclonal antibody, 2B10, which has the same determinant on the human erythrocyte as MSA-1 of *Plasmodium falciparum* (FCR3 strain); the binding of both ligands to erythrocyte receptors was totally sialic acid dependent. In this work, rabbit anti-2B10 idiotypic antibodies were generated. The anti-idiotypic antibodies recognized both the erythrocyte binding site of 2B10 and the C-terminal region of MSA-1 (amino acids 1047 to 1640); they were able to inhibit 2B10 and MSA-1 binding to erythrocytes and partially prevent *P. falciparum* merozoites from invading erythrocytes. The utility of 2B10 in the study of the interaction between MSA-1 and human erythrocytes prompted us to determine the nucleotide and deduced amino acid sequences of its $V_H$ and $V_L$ regions. The data show that the 2B10 $V_H$ region is part of the J558 family and is especially homologous to BALB/c anti-nitrophenyl monoclonal antibody 21.1.43; the $V_L$ region belongs to the VK1 subgroup and comes from the same genomic locus as (NZB × W)$F_1$ anti-DNA and C57BL anti-dextran monoclonal antibodies BXW-14 and 42.48.12.2, respectively. Most of the differences among the $V_H$ and $V_L$ segments are located in CDR1 and -3. The binding site of 2B10 contains both negatively and positively charged amino acid residues. The amino acid sequences of the 2B10 $V_H$ region and a region of MSA-1 from the Wellcome strain of *P. falciparum* (amino acids 1002 to 1115) share 43% similarity, and the amino acid sequences between the 2B10 $V_L$ region and another segment of the same MSA-1 (amino acids 1247 to 1394) share 48% similarity. We conclude that the interactions between erythrocyte receptors and their ligands, 2B10 and MSA-1, are related and that the C-terminal region of MSA-1 is the erythrocyte binding domain.**

In its late blood stages, the human malaria parasite, *Plasmodium falciparum*, carries a major surface antigen, Pf200, also known as MSA-1 or MSP-1 (GP195-205 [16]). Several lines of evidence suggest a functional role for MSA-1 in merozoite invasion of erythrocytes. MSA-1 binds only to erythrocytes that can be infected by *P. falciparum* (26), and antibodies specific for MSA-1 are able to inhibit invasion in vitro and in vivo (11). However, it is unclear how this protein functions in terms of merozoite recognition, attachment, and invasion of erythrocytes. The interaction sites between MSA-1 and erythrocyte receptors are still not fully determined. It has been shown that intact MSA-1 binds to erythrocytes in a sialic acid-dependent manner (25). It has also been proposed that during parasite release and the invasion of new erythrocytes, two steps of proteolytic processing occur. Initially, the high-molecular-weight precursor is cleaved into four fragments (with sizes of 30 kDa, 38 kDa, 42 kDa, and 88 kDa) which remain as a complex on the merozoite surface (17, 24). Subsequently, the antigen complex is released from the membrane when the anchoring 42-kDa fragment is cleaved. A 19-kDa carboxyl-terminal fragment remains on the merozoite membrane and is carried into the erythrocytes (2, 3). The significance of the proteolytic processing remains to be elucidated.

Monoclonal antibodies (MAbs) which recognize several regions of the MSA-1 molecule are able to inhibit parasite invasion (8, 22). A recent study demonstrated that the binding domain of EBA-175 of *P. falciparum* was at the N-terminal region, which contains a cysteine-rich motif (29). Although their binding sites on erythrocytes appear similar and both depend on sialic acid for binding, MSA-1 and EBA-175 have been shown to be unrelated on the basis of amino acid sequence and their locations on the merozoite surface (26, 30). These proteins could represent different sites on *P. falciparum* which are separately involved in the multiple-step processes of merozoite invasion.

In order to study the interaction between the parasite ligand and the erythrocyte receptor previously identified as glycophorin (25), we prepared several anti-glycophorin MAbs, among which 2B10 was highly effective in inhibiting the invasion of the erythrocyte by the merozoite. We showed that 2B10 bound to the N-terminal region of glycophorin A, that the binding of 2B10 and MSA-1 to erythrocytes could be blocked reciprocally, and that 2B10 was a very effective inhibitor of *P. falciparum* merozoite invasion of erythrocytes. In this report, we describe the properties of polyclonal idiotypic antibodies (anti-Id) prepared by immunization of a rabbit with 2B10. The anti-Id mimicked glycophorin A and bound to the binding site of 2B10, inhibited 2B10 and MSA-1 binding to erythrocytes, and partially blocked the invasion of erythrocytes by *P. falciparum* merozoites. Interestingly, the same antigen-mimicking anti-Id also bound to a C-terminal fragment of MSA-1 (amino acids 1047 to 1640). Further, we analyzed the nucleotide sequences of the variable regions of the 2B10 heavy and light chains. The $V_H$ region is confined to the first gene family, the J558 family; the $V_L$ region belongs to the VK1 subgroup. Although 2B10 binding to erythrocytes is totally dependent on sialic acid (31), its binding site contains both negatively and

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positively charged amino acid residues. Comparison of the amino acid sequences of the $V_H$ and $V_L$ regions of 2B10 and the amino acid sequences of two fragments of MSA-1 from the Wellcome strain of *P. falciparum* shows that the $V_H$ and $V_L$ regions of 2B10 and the two fragments of MSA-1 (amino acids 1002 to 1115 and 1247 to 1394) show similarities of 43 and 48%, respectively.

## MATERIALS AND METHODS

**Anti-Id preparation.** A New Zealand White rabbit (West Seneca Lab, West Seneca, N.Y.), received two subcutaneous immunizations of 1 mg of 2B10 conjugated to hemocyanin by glutaraldehyde mediated cross-linking 1 month apart. Briefly, antibody at a concentration of 1 mg/ml was mixed with an equal mass of hemocyanin, and the mixture was made 0.5% in glutaraldehyde. After being stirred for 1 h, the solution was dialyzed against 0.1 M ammonium bicarbonate for 4 h and overnight against saline. The resulting product was suspended in Titer Max (Vaxcel Inc., Atlanta, Ga.) adjuvant according to the instructions of the manufacturer. Two weeks after the second injection, the animal was bled and serum was tested for anti-2B10 titers by enzyme-linked immunosorbent assay (ELISA) as described below. The serum was clarified by centrifugation (8,000 × *g* for 20 min), and the supernatant was adjusted to 50% saturation with ammonium sulfate. The precipitate of immunoglobulin was redissolved in a minimum amount of water and dialyzed against phosphate-buffered saline. Antiisotypic and antiallotypic antibodies were removed by adsorption with a specificity-unrelated MAb P3NP [immunoglobulin G1(κ)]-Sepharose column. The antibody was derived from a BALB/c P3NP hybridoma (American Type Culture Collection, Rockville, Md.). The anti-Id preparation was then affinity purified on a 2B10-Sepharose column.

**Inhibition ELISA.** An ELISA was used to determine the fine specificity of the rabbit anti-Id, as previously reported (31). For the testing of anti-2B10 specificity, 0.5 μg of 2B10 per ml was applied to 96-well plates and 0.1 μg of rabbit anti-Id per ml was used as the binding agent. The inhibitors, MAbs 2B10, 3H2, 3H12, 1E4 (31), and P3NP, were diluted in series. For the testing of antigen-binding-site specificity, 0.5 μg of glycophorin A per ml was applied to plates, 0.1 to 0.5 μg of MAbs 2B10, 3H2, 3H12, and 1E4 per ml (each) was used as the binding agent, and anti-Id were used as the inhibitor. In general, for this and other analyses (agglutination and binding studies), assays were carried out in triplicate.

**Agglutination assay.** An assay to test the ability of rabbit anti-Id to inhibit MAb binding to erythrocytes was performed (31). Fifty microliters of a 0.02% suspension of erythrocytes in 5% fetal calf serum–saline and 50 μl of a dilution series of anti-Id were added to wells of a 96-well plate. After incubation for 1 h at 37°C, 50 μl of a suitable dilution of the 2B10 MAb was added; the plate was incubated for an additional hour at 37°C and then overnight at 4°C. Agglutination was determined by visual inspection.

**Inhibition of binding assay.** An assay was carried out to assess the ability of anti-Id to interfere with the binding of the major merozoite surface protein to the erythrocyte (31); inhibitors used were 0.5 mg of glycophorin A (the normal receptor and a positive control), 5 mg of rabbit anti-Id, and 5 mg of rabbit anti-cytochrome antibodies (as a negative control for nonspecific antibody effects).

**Invasion assay.** An assay to determine blocking of the invasion of the human erythrocyte by the merozoite was performed with stained parasite DNA and by fluorescence-activated cell sorting (FACS) as described previously (31); inhibitors used were 0.2 mg of glycophorin A, 2 mg of rabbit anti-Id, 2 mg of rabbit anti-cytochrome antibodies, and 2 mg of rabbit preimmune serum proteins.

**SDS-PAGE and Western blot (immunoblot) analysis.** Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on an SDS–7.5% polyacrylamide gel. Anti-Id specificity was determined by Western blot analysis (31).

**MSA-1 fragment expression.** Recombinant expression plasmids pME-1 and pME-2 were kindly provided by A. A. Holder (National Institute for Medical Research, London, United Kingdom). pME-1 and pME-2 were derived from pWRL507, in which ME-1 and ME-2 were inserted at the 5′ end of a truncated *trpE* gene. Expression was under the control of the *trp* promoter. The product was insoluble and was partially purified by differential solubility and extraction. Bands were cut from gels after SDS-PAGE and eluted with an electroeluter (model 422; Bio-Rad, Hercules, Calif.).

**Preparation of cDNA libraries.** Total cellular RNA was obtained from the 2B10 hybridoma with an RNA isolation kit (Stratagene, La Jolla, Calif.). mRNA was further isolated by biotin-oligo(dT) priming (PolyATRact mRNA isolation systems; Promega, Madison, Wis.). cDNA was generated with reverse transcriptase and by oligo(dT) priming (SuperScript plasmid system for cDNA synthesis and plasmid cloning; GIBCO BRL, Gaithersburg, Md.). The largest fragments of cDNA, which were selected by column chromatography, were ligated into plasmid pSORT1 (GIBCO BRL). The ligated cDNA was then transformed into *Escherichia coli* DH10B (GIBCO BRL) and cloned on Luria-Bertani plates containing 100 μg of ampicillin per ml.

**Screening and sequencing of $V_H$ and $V_L$ regions.** Each cDNA clone was expanded in 1 ml of Luria-Bertani medium, and half of the cell suspension was boiled in distilled water to extract plasmids. Screening was carried out by PCR amplification, with the $V_H$ region being screened twice with two 3′ end primers used separately. The sequences of the primers used for screening were as follows: 5′ end for both the $V_H$ region and the $V_L$ region, 5′d(CCCAGTCACGACGT TGTAAAACG)3′, which matches the vector, pSPORT1; 3′ end for the $V_H$ region, 5′d(ACTGTTGACCCTGCATTTGAACTCCT)3′ and 5′d(AGGGAAA TAGCCCTTGACCAGGCAT)3′, which match the CH2 and CH1 regions, respectively; and 3′ end for the $V_L$ region, 5′d(GGTGGGAAGATGGATACAGT TGGTGCAGC)3′, which matches the K constant region. PCR was performed for 30 cycles with a thermal cycler according to the following temperature and time schedule: denaturation, 94°C for 1 min; annealing, 60°C for 1 min; and extension, 72°C for 2 min. All PCRs were conducted with a GeneAMP PCR reagent kit (Perkin-Elmer Cetus, Norwalk, Conn.). PCR bands were resolved by electrophoresis in a 1% agarose gel.

One of 108 clones from the $V_L$ region screening was positive, and one of 1,500 clones from the $V_H$ region screening was positive. The positive clones in stock suspension were expanded, and the plasmids were extracted and purified with a QIAGEN plasmid kit (QIAGEN Inc., Chatsworth, Calif.).

DNA sequencing was performed by the dideoxy nucleotide termination method (10) with TAQuence version 2.0 (United States Biochemical Corp., Cleveland, Ohio).

**Computer-aided sequence comparison.** Nucleic acid and deduced amino acid similarities between sequences in the GenBank and EMBL data banks and our sequences were determined with the FASTA program. Amino acid similarities between the sequence of MSA-1 of the Wellcome strain of *P. falciparum* and the sequences of the 2B10 $V_H$ and $V_L$ regions were determined with the BESTFIT program.

**Nucleotide sequence accession number.** The sequences reported in this paper have been deposited in the GenBank database (accession no. L38706).

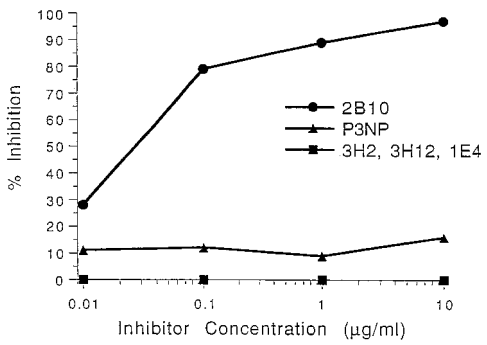


FIG. 1. Specificity of rabbit anti-2B10 antibodies for the 2B10 idiotype. The ability of 2B10, 3H2, 3H12, 1E4, and an allotype-matched control, P3NP, to inhibit the binding of rabbit anti-2B10 antibodies to plate-bound 2B10 was tested. The amount of inhibitor is plotted against the percent inhibition.

## RESULTS

**Production and characterization of rabbit anti-Id against 2B10.** Polyclonal anti-Id were generated from a New Zealand White rabbit immunized with purified MAb 2B10; these antibodies were purified by chromatography on an allotype-matched, MAb P3NP-Sepharose column and finally by affinity chromatography on a MAb 2B10-Sepharose column.

The specificity of the polyclonal anti-2B10 antibodies was tested by an inhibition ELISA. As can be seen from Fig. 1, the binding of plate-bound 2B10 to rabbit antibodies was inhibited specifically by 2B10 but not by allotype-matched control MAb P3NP or by three other glycophorin A-specific MAbs, 3H2, 3H12, and 1E4. The purified anti-Id bound only to 2B10 (data not shown). The results indicated that the purified polyclonal antibodies were 2B10 specific within the panel of MAbs tested. It appeared that the anti-Id were not able to detect a cross-reactivity present on the group of anti-glycophorin MAbs—2B10, 3H2, 3H12, and 1E4—tested.

The antigen-binding-site specificity of anti-Id was analyzed. A putative anti-Id beta-antibody, which mimics an epitope on the antigen, may inhibit antigen-antibody binding (19). This anti-Id would thus be a useful tool for the purpose of studying

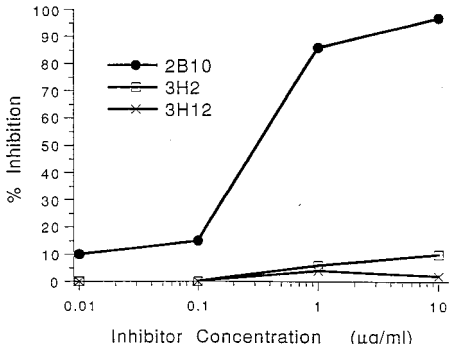


FIG. 2. Specificity of rabbit anti-Id for the glycophorin A binding site of 2B10. The ability of anti-Id to inhibit the binding of 2B10, 3H2, and 3H12 to plate-bound glycophorin A was tested. The amount of inhibitor is plotted against the percent inhibition.

the interaction between ligand and receptor. Therefore, the ability of anti-Id to inhibit glycophorin A binding to 2B10 was specifically measured. We have shown previously that 2B10, 3H2, 3H12, and 1E4 all recognized glycophorin A but had different specificities (31). Accordingly, an inhibition ELISA was performed (Fig. 2), with rabbit anti-2B10 antibodies being used as the inhibitor. Anti-Id completely inhibited 2B10 binding to glycophorin A but had no effect on the binding of three other MAbs to glycophorin A. This assay indicated that anti-Id recognized the binding site-related epitope of 2B10.

**Inhibition of 2B10 or MSA-1 binding to erythrocytes.** Previously, we have shown that anti-glycophorin MAbs 2B10, 3H2, 3H12, and 1E4 bound to En-a+ but not En-a− (glycophorin-negative) erythrocytes (31). To test the ability of anti-Id to inhibit the binding of 2B10 to erythrocytes, an indirect agglutination assay was performed. The results show that the binding of 2B10 but not of 3H2, 3H12, or 1E4 to erythrocytes was inhibited by the anti-Id (data not shown). This is additional evidence that the anti-Id exhibit an internal image reflective of the interactive site between glycophorin and 2B10.

We have also shown that MAb 2B10 and MSA-1 competed with each other for binding to erythrocytes (31). Accordingly, the ability of anti-Id to inhibit MSA-1 binding to erythrocytes was examined by an inhibition of binding assay as previously described (13); data were quantitated by scanning densitometry. As can be seen from Fig. 3, the binding of MSA-1 to erythrocytes was inhibited 54% by anti-Id (lane c) but not by

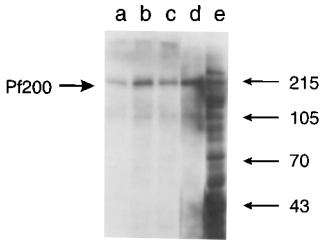


FIG. 3. Blocking the binding of MSA-1 to erythrocytes by glycophorin A and anti-Id. The erythrocyte-binding proteins from *P. falciparum* were eluted from erythrocytes with 1 M NaCl and visualized after SDS–7.5% PAGE by autoradiography in the absence (lane d) or presence of inhibitors glycophorin A (lane a), anti-cytochrome antibodies (lane b), and anti-Id (lane c). Lane e, total [$^{35}$S]methionine-labeled parasite proteins added to the erythrocyte suspension. Anti-dopamine antibodies were used as a control. The numbers on the right are kilodaltons.

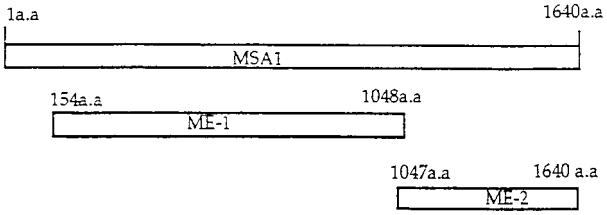


FIG. 4. Schematic presentation of MSA-1 fusion proteins. a.a., amino acid.

control antibodies (lane b); binding was also inhibited by glycophorin A by 90% (lane a), consistent with previous data (31).

**Inhibition of invasion by *P. falciparum* merozoites into erythrocytes.** Anti-Id were evaluated for the ability to inhibit *P. falciparum* merozoite invasion of human erythrocytes. Smears of erythrocytes infected in the presence or absence of inhibitors at 24 and 36 h after initiation were made, and cell morphology was examined. The inhibitors did not influence parasite development (data not shown). After rupture and reinvasion (48 h), erythrocytes were again collected. More than 95% of the parasite was found to be at the ring stage; overall parasitemia (14 to 16% in the absence of inhibitors) was determined by FACS analysis. Anti-Id (5 mg) inhibited the invasion of *P. falciparum* merozoites into erythrocytes by a mean ± standard deviation of 31.4% ± 5.2% ($P < 0.0001$ by Student's *t* test), somewhat less than the inhibition by 0.2 mg of glycophorin A (48.0% ± 4.3% [$P < 0.0001$ by Student's *t* test]). On the other hand, preimmune serum proteins (5 mg) and rabbit anti-cytochrome antibodies (5 mg), used as negative controls, had effects not measurably different from zero (4.0% ± 1.0% [$P < 0.01$ by Student's *t* test] and 9.1% ± 1.1% [$P < 0.01$ by Student's *t* test], respectively). Experiments were performed twice in triplicate. The results were consistent with the fact that anti-Id partially inhibited MSA-1 binding to erythrocytes. Both of these inhibition experiments are indirect but important evidence that anti-Id contain an image analogous to the glycophorin site.

**Expression of fusion protein and anti-Id binding specificity.** Since anti-Id inhibited MSA-1 binding to erythrocytes, it was of interest to determine which part of MSA-1 was recognized by the anti-Id.

Two fusion proteins containing the tryptophan P gene, ME-1 (135 kDa) and ME-2 (105 kDa) (Fig. 4), were expressed in *E. coli* and purified. The molecular mass of the *tryP* sequence was 37 kDa; the purity of the fusion products was shown by SDS-PAGE (Fig. 5).

To test the anti-Id binding specificity, Western blot analysis was performed. Identical amounts (15 μg) of ME-1, ME-2, and 2B10 were loaded on the gel, and anti-Id were used as a probe; detection was by indirect immunofluorescence with peroxidase-conjugated goat anti-rabbit immunoglobulin G followed by incubation with substrate. As can be seen from Fig. 5, anti-Id bound to the 2B10 heavy chain and to ME-2 but not to ME-1. Anti-Id thus recognized the MSA-1 C-terminal region encompassing amino acids 1047 to 1640.

**Sequences of 2B10 $V_H$ and $V_L$ regions.** On the basis of the data obtained, 2B10 appears to be an important MAb in terms of defining the interaction between the merozoite and the erythrocyte; accordingly, sequencing of the variable regions of its heavy and light chains was performed (Fig. 6).

Comparison of the amino acid sequence of the 2B10 heavy chain with previously reported sequences (12) shows that the $V_H$ segment of 2B10 belongs to the first $V_H$ group, the J558 family. Within this group, the sequence of the 2B10 $V_H$ seg-

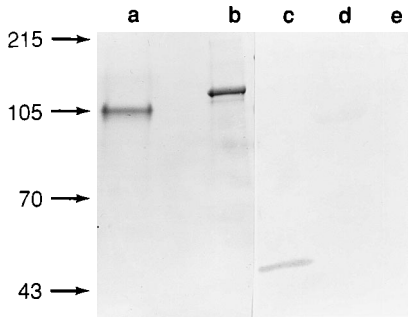


FIG. 5. Binding of anti-Id to 2B10 and MSA-1 fragments. Lanes a and b, Coomassie brilliant blue staining showing the expressed ME-2 (a) and ME-1 (b) protein bands by SDS–7.5% PAGE; lanes c to e, Western blot testing of the binding of anti-Id to 2B10 (c), ME-2 (d), and ME-1 (e). All samples were subjected to SDS-PAGE under reducing conditions. The numbers at the left are kilodaltons.

ment is most homologous (85%) with that of BALB/c anti-nitrophenyl MAb 20.1.43 (4), as shown in Fig. 7A. However, the CDR3s of these two antibodies are strikingly different. Within this region, the 20.1.43 sequence is longer than the 2B10 sequence by 4 amino acid residues. This difference imparts the apparent variety at the D region and the $V_H$-D and D-$J_H$ junctions.

A similar comparison of the sequence of the 2B10 kappa

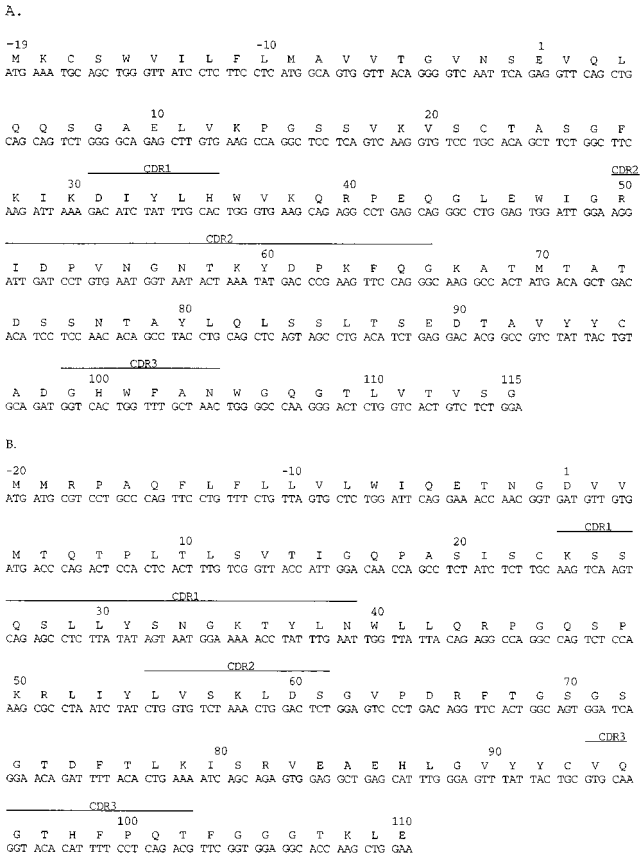


FIG. 6. Alignment of nucleotide and deduced amino acid sequences (one-letter code) of the variable regions of the 2B10 heavy chain (A) and light chain (B), including the leading sequence. CDRs are shown by solid lines above the appropriate amino acid residues.

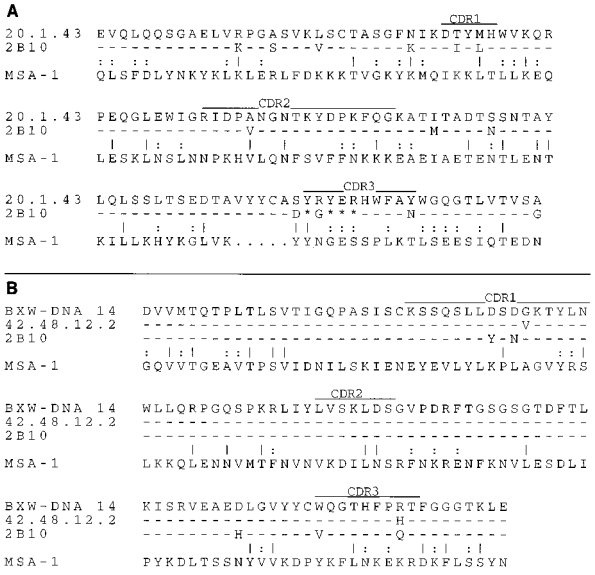


FIG. 7. (A) Comparison of the amino acid sequences of the variable region of the 2B10 heavy chain, MAb 20.1.43 belonging to the J558 family, and an MSA-1 fragment encompassing amino acids 1002 to 1115. CDRs are shown by solid lines above the appropriate amino acid residues. (B) Comparison of the amino acid sequences of the variable region of the 2B10 light chain, MAbs BXW-DNA 14 and 42.48.2 belonging to the VK1 subgroup, and an MSA-1 fragment encompassing amino acids 1247 to 1394. CDRs are shown by solid lines above the appropriate amino acid residues. The vertical line indicates identity between two amino acid residues, and the colon indicates similarity (conservative change) between two amino acid residues.

light chain with the known sequences of various kappa subgroups reveals that the 2B10 light chain is confined to the VK1 subgroup (27). The amino acid sequence comparison indicates that the light chain of 2B10 is very similar to that of (NZB × W)$F_1$ anti-DNA and C57BL anti-dextran MAbs named BXW-14 (21) and 42.48.12.2 (1), respectively (Fig. 7B). The major differences are located in CDR1 and CDR3.

**Comparison of the primary structures of the 2B10 variable region and MSA-1.** Since 2B10 and MSA-1 appear to bind to the same determinant on the erythrocyte, the primary structures of the 2B10 variable region and the MSA-1 molecule were compared, with one goal being to define better the erythrocyte binding domain of MSA-1. The similarities between the amino acid sequences of the 2B10 $V_H$ and $V_L$ regions and MSA-1 from the Wellcome strain were examined with a computer algorithm. Figure 7 shows that there are some similarities between the 2B10 $V_H$ region and the fragment of MSA-1 for amino acids 1002 to 1115; a second homology region for the 2B10 $V_L$ domain and the MSA-1 fragment (amino acids 1247 to 1394) is also apparent. These two fragments of MSA-1 are within the portion of MSA-1 (the ME-2 construct, amino acids 1047 to 1640) which was recognized by the anti-Id.

A summary of the similarities between these amino acid sequences is shown in Table 1. A comparison of the 2B10 $V_H$ region and MSA-1 shows that 17% of the amino acids are identical and an additional 26% of them are homologous, for an overall similarity of 43%. A comparison of the B10 $V_L$ region and MSA-1 shows that 25% of the amino acids are identical and 23% of them are homologous, for a similarity of 48%. The similarities of the hypervariable 2B10 $V_H$ and $V_L$ regions and MSA-1 are 39 and 47%, respectively; all of these are well beyond what might be expected for nonrelated proteins. Amino acids 1002 to 1115 are confined to block 11

TABLE 1. Summary of the similarities of the amino acid sequences of the 2B10 $V_H$ and $V_L$ regions and MSA-1 fragments

| Sequence | % Identity | % Homology | % Similarity |
|---|---|---|---|
| Heavy chain[a] | | | |
| $V_H$ | 17 | 26 | 43 |
| CDR | 14 | 25 | 39 |
| Light chain[b] | | | |
| $V_L$ | 25 | 23 | 48 |
| CDR | 25 | 22 | 47 |

[a] Similarity of the amino acid sequences of the 2B10 $V_H$ region and CDR and the MSA-1 fragment (amino acids 1002 to 1115).
[b] Similarity of the amino acid sequences of the 2B10 $V_L$ region and CDR and the MSA-1 fragment (amino acids 1247 to 1394).

(semiconserved sequence) and block 12 (conserved sequence), and amino acids 1247 to 1394 are confined to block 15 (semiconserved sequence) and block 16 (variable sequence) (33). These two amino acid sequences match sequences present in processed fragments with sizes of 38 and 42 kDa as determined from MSA-1 on merozoite surfaces, respectively (2, 17, 24).

## DISCUSSION

The idiotype network concept (18) has been studied for more than two decades. One postulate of this theory is that a specific anti-Id, classified as an internal image (19), can sterically mimic an external antigen and recognize the binding site epitope of its antigen-specific antibody. Some strong evidence of internal images of antigens in immunoglobulin repertories was obtained by comparing the amino acid sequences of antigens and anti-Id. Mazza et al. (23) found the GAT motif in the primary structure of $Ab_2$ MAbs ($V_H$ region of the antibody containing the internal image) which induced a GAT-specific $Ab_3$ response (i.e., an antibody to the internal image structure). Bruck et al. (6) identified an area of sequence similarity shared by the reovirus type 3 cell attachment protein and an anti-Id that could bind to the same cell surface receptor.

This internal image concept has been used as an effective approach in the development of immune protection against parasitic (15), bacterial (32), and viral (20) pathogens. The mimicry of the topography of biological structure by the internal image provided by the variability of immunoglobulin molecules has also provided a useful way to study receptor-ligand interactions for hormones (13), vitamins (28), neurotransmitters (34), and drugs (14).

In this report, a new internal image model is developed for the study of a specific receptor-ligand interaction. The rabbit polyclonal anti-Id were generated from MAb 2B10, and it was demonstrated that anti-Id could mimic glycophorin A in recognizing the antigen binding site of 2B10. Anti-Id also bound to the C-terminal region (amino acids 1047 to 1640) of MSA-1. This experiment was based on the assumption that since 2B10 and MSA-1 share the same determinant on the erythrocyte, the internal image which is specific for the binding site of 2B10 should bind to MSA-1. The inhibition of binding assay and the inhibition of invasion assay revealed that anti-Id partially inhibited MSA-1 binding to erythrocytes and blocked parasites from invading the erythrocytes. It is possible that 2B10 and MSA-1 bind to tightly overlapping but not identical epitopes on glycophorin A. Alternatively, MSA-1 could bind to the same epitope as 2B10 but with lower affinity. The anti-Id data indicate that the MSA-1 C-terminal region functions as a ligand for interaction with the receptor on an erythrocyte.

Previously, it was demonstrated that MAb 2B10 binding to erythrocytes fully depended on sialic acid residues (31). Since the hypervariable regions of immunoglobulin have a major role in the binding of antigen, the ionic character of the antigen binding site was analyzed. As can be seen from Fig. 7A, in CDR1 and -2 of the heavy chain, there are three amino acids with a positively charged polar side chain (two lysines and one arginine) and three aspartic acids with a negatively charged side chain. The rest of the amino acids carry either nonpolar or neutral polar side chains. CDR3, which is believed to be critical in the constitution of the binding site, has an overall neutral charge.

In CDR1 and -2 of the light chain (Fig. 7B), there are three lysines and one aspartic acid. The rest of the amino acids have hydrophobic or neutral side chains.

Usually, a minimum of four out of six complementarity determining regions (CDRs) in the Fab fragment make contact with an antigen epitope. Therefore, at least one CDR in the 2B10 molecule with a negatively charged aspartyl residue contacts the antigen.

The amino acid sequence analysis shows that the binding site of 2B10, which is largely derived from hypervariable regions (7), contains both negatively and positively charged residues. It seems that the stereochemical and ionic interactions between the binding site of the antibody and the erythrocyte receptor are complex. The sialic acid residues may be necessary to maintain but not directly participate in the conformational structure of the binding site. Alternatively, since usually more than 10 amino acids from the antibody and antigen are directly involved in potential contacts (35), the negatively charged aspartic residues on the binding site of 2B10 may be close to the positively charged residues in the binding site cavity or surface area of the antigen.

Since MSA-1 and 2B10 bind to the same determinant region on the erythrocyte, a similar situation may exist for the interaction between MSA-1 and the erythrocyte receptor.

Determination of the amino acid sequence of 2B10 was undertaken because this MAb binds to the same erythrocyte receptor as does the parasite, it effectively inhibits the invasion of erythrocytes by *P. falciparum* merozoites, and it induces an internal image antibody which recognizes the C-terminal region of MSA-1.

The $V_H$ segment of 2B10 belongs to the J558 family. This assignment is not surprising, since this family is the largest, containing more than half of all $V_H$ genes (5), and both 2B10 and J558 come from BALB/c mice. Within this group, the 2B10 $V_H$ sequence is most homologous with that of a BALB/c anti-nitrophenyl MAb. On the other hand, the $V_L$ segment of 2B10 is confined to the VK1 subgroup and is very similar to those of the $(NZB \times W)F_1$ anti-DNA and C57BL anti-dextran MAbs. Consistent with a previous report (9), the major differences among these $V_H$ or $V_L$ segments are localized to CDR1 to -3. The sequence homology among the different MAbs stresses the importance of random events in the generation of antibody specificity-combinational events or somatic mutations.

The unexpected similarity of peptide sequences from the 2B10 $V_H$ and $V_L$ regions and MSA-1 fragments might indicate that the secondary and tertiary folding patterns could be comparable. It should be emphasized that these two fragments, amino acids 1002 to 1115 and 1247 to 1394, are on the C-terminal region of MSA-1, which is recognized by anti-Id. Reports from other laboratories have indicated that fragments of the merozoite surface complex with sizes of 38 and 42 kDa are important; these are contained within the recognized MSA-1 sequence. If they are part of the binding domain, this region must consist of, on the gene level, conserved, semiconserved, and variable sequences.

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