Isolation from Phage Display Libraries of Single Chain Variable Fragment Antibodies That Recognize Conformational Epitopes in the Malaria Vaccine Candidate, Apical Membrane Antigen-1* 

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Phage display of single chain variable fragment (scFv) antibodies is a powerful tool for the selection of important and useful antibody specificities. We have constructed such a library from mice protected from malaria challenge by immunization with recombinant Plasmodium chabaudi DS apical membrane antigen (AMA-1). Panning on refolded AMA-1 enriched a population of scFv's which specifically bound the antigen. The single chain antibodies recognize conformational epitopes on AMA-1 from the P. chabaudi DS strain but not on AMA-1 of the 556KA strain of P. chabaudi. A subset of the antibody fragments recognized AMA-1 from the human malaria parasite Plasmodium falciparum. Nucleotide sequencing revealed that at least four unique scFv genes were selected by the panning procedure. These scFv antibodies are valuable reagents for probing the structure and function of AMA-1 and will be used to test the feasibility of using recombinant antibodies in a passive immunization therapy against malaria.

The emergence of strains of Plasmodium falciparum which are resistant to many anti-malarial drugs has made the development of a malaria vaccine an urgent priority. Considerable effort has been directed toward the development of a subunit vaccine incorporating recombinant forms of the corresponding AMA-1 (11, 12). Protection against P. chabaudi was obtained only if mice were immunized with AMA-1 that had been refolded in vitro, under conditions that allowed correct disulfide bond pairing, and not if the mice were immunized with a preparation of reduced and alkylated AMA-1 (13). The humoral arm of the immune system has been shown to play a critical role in protection. In the trials conducted to date, a strong correlation has been established between anti-AMA-1 antibody titers and levels of protection (10, 11, 13). A monoclonal antibody against PK66 was shown to block the invasion in vitro of rhesus erythrocytes by P. knowlesi (14); moreover, Fab fragments were found to be more effective in this blocking than the intact antibody (15). In addition, passive transfer of purified IgG from rabbits immunized with recombinant P. chabaudi AMA-1 effected a rapid elimination of parasites from the circulation of mice infected with P. chabaudi (12).

The success of passive immunization strategies using anti-AMA-1 antibodies raises the possibility that life-threatening cases of malaria in humans could be treated with anti-AMA-1 antibodies. Such a strategy would require a readily available source of anti-AMA-1 antibodies with defined specificities. Large scale production of antibody reagents is now possible because of recent advances in recombinant antibody technology (16, 17), which overcome many of the problems associated with the production of monoclonal antibodies by conventional hybridoma technology. We have therefore undertaken the production of a library of single chain variable fragment (scFv) anti-
bodies against *P. chabaudi* AMA-1. This work represents an important advance toward the development of a recombinant antibody-based therapy for malaria.

scFv antibodies consist of the variable regions of the light and heavy chains of antibodies linked via a short peptide spacer (18). scFv molecules derived from hybridomas have been shown to retain the binding specificities of the parent monoclonal antibody and, where measured, have similar binding affinities (18, 19). Specific scFvs can also be produced by selection from a diverse library displayed on the surface of phage (20, 21). There are several important advantages associated with the production of recombinant antibodies. First, the phage display technology includes a powerful enrichment strategy that allows rapid and simple selection of reagents with desired properties. Second, the scFv fragments can be produced in large quantities in bacteria. Third, the genes for the scFvs can be manipulated to introduce mutations that enhance the binding characteristics of the antibody reagents (22).

In this work we have used spleens from mice protected against malaria challenge by previous injection with recombinant *P. chabaudi* AMA-1 (12) as the starting material for the production of a library of phage-displayed scFv. Selective binding strategies were used to isolate four independent scFvs that specifically recognize AMA-1 from *P. chabaudi* DS strain. Perhaps surprisingly, none of the scFvs tested recognized AMA-1 from the 556KA strain of *P. chabaudi*, which differs from AMA-1 (DS) by only 36 nucleotide substitutions (12). Interestingly, a subset of scFvs recognized AMA-1 from *P. falciparum*. The epitopes recognized by the scFv antibodies were found to be disulfide bond-dependent, thus mimicking the conformational constraint of AMA-1 which is necessary for the induction of a protective immune response. This work provides a defined set of recombinant antibodies against AMA-1 which can be used to probe the structure of AMA-1 to identify “protective” epitopes. This analysis will be valuable in the development of both passive and active immunization strategies based on AMA-1.

**MATERIALS AND METHODS**

**mRNA Isolation**—Spleens from four hyperimmune mice were homogenized in 4 mL guanidine thiocyanate, and total RNA was precipitated after extraction with phenol/chloroform. mRNA was isolated from the total RNA using the poly(AT)tract system according to protocols outlined after extraction with phenol/chloroform. mRNA was isolated from spleens from four hyperimmune mice that had been boosted with AMA-1 (12) as the starting material for the production of recombinant antibodies. First, the phage display technology includes a powerful enrichment strategy that allows rapid and simple selection of reagents with desired properties. Second, the scFv fragments can be produced in large quantities in bacteria. Third, the genes for the scFvs can be manipulated to introduce mutations that enhance the binding characteristics of the antibody reagents (22).

**Production of Soluble scFv Antibodies**—The phage produced after four rounds of infection were used to infect *E. coli* TG1 cells and the phagemid DNA was recovered (Wizard miniprep, Promega). To produce soluble scFv antibodies, the DNA was used to transform *E. coli* HB2151 cells. This strain of *E. coli* recognizes the amber stop codon between the scFv and the gene III fragment. Overnight induction of 50 mL of *E. coli* HB2151 cells containing phagemid DNA with 1 mM isopropyl β-D-thiogalactopyranoside led to the production of soluble forms of the scFv antibody fragment, which is secreted into the periplasm. Cells were pelleted by centrifugation, and supernatants (containing any extracellular soluble scFvs) were filtered through a 0.45-μm filter and stored at −20 °C.

**Enzyme-linked Immunosorbent Assay (ELISA)**—Recombinant phage were rescued from individual clones and screened for AMA-1 binding by ELISA. Microtiter wells were coated with AMA-1 as described previously and blocked with 5% BLOTTO for 1 h at 37 °C. Phage were incubated in the wells for 1 h at 37 °C and then subjected to three washes with TBS, 0.05% Tween 20. Bound phage were detected by incubation with a 1:1,000 dilution of rabbit anti-M13 antibody (Stratagene) incubated in the wells for 1 h at 37 °C, then incubation with a 1:1,000 dilution of rabbit anti-M13 antibody (Stratagene) for 30 min at 37 °C. Detection was achieved by the addition of ABTS substrate. When soluble scFvs were transferred to a clean tube and stored at −20 °C, the remaining cell pellet was also stored at −20 °C for further analysis.

**Antibody Sequencing**—Sequences of all scFv fragments were determined by DNA sequencing. Nucleotide sequences of expressed genes and assess V, D, and J segment usage. The sequence analysis workshop (SAW) was used to propose assignment of scFvs to potential germline genes and assess V, D, and J segment usage. The IMGT tool DNAPLOT was used to analyze the sequence data available through the Web (URL: http://www.ncbi.nlm.nih.gov/BLAST/) to compare them with previously sequenced VH and VL chains. The sequence analysis workshop (SAW) program (23) and the international immunogenetics (IMGT) data base (24) were used to propose assignment of scFvs to potential germline genes and assess V, D, and J segment usage. The IMGT tool DNA
Anti-malaria scFv Antibodies from a Phage Display Library

RESULTS

cDNA was generated by reverse transcription from mRNA isolated from the spleens of four mice that had been protected against malaria by prior immunization with the refolded ectodomain of AMA-1 from *P. chabaudi* (12). The gene fragments corresponding to the variable regions of the heavy chain \((V_\text{H})\) and light chains \((V_\text{L})\) of the repertoire of antibody genes were amplified by PCR. The fragments were joined to form a series of scFv gene sequences that were ligated into the phagemid vector pCANTAB5E (Pharmacia). To examine the integrity of the library, 20 colonies were picked at random, and all were found to contain inserts of the appropriate size. Recombinant phage, expressing a library of scFv polypeptides on their surface, were produced by helper phage rescue and selectively enriched by panning against immobilized refolded *P. chabaudi* AMA-1 ectodomain. The phage were pooled after each successive round of panning and tested for their ability to bind to *P. chabaudi* AMA-1 in an ELISA. The pool of phage rescued from the library before panning showed no binding to *P. chabaudi* AMA-1; however, after four rounds of selection, phage showed greatly enhanced specific binding to *P. chabaudi* AMA-1 (Fig. 1). The specificity of the interaction with AMA-1 was indicated by the absence of binding of the phage population to BSA (data not shown).

The pool of phage selected by four rounds of panning was used to infect an *E. coli* (TG1) culture, and subsequent plating on agar containing ampicillin allowed individual colonies harboring phagemid to grow. To verify that individual phage clones could bind to *P. chabaudi* AMA-1, phage were rescued from 24 individual colonies (selected at random) and tested for binding to *P. chabaudi* AMA-1 by ELISA. All but two clones exhibited substantial binding to immobilized *P. chabaudi* AMA-1. This is in contrast to 24 random clones selected from the unpanned library, none of which showed significant binding activity (Fig. 2).

To produce soluble scFv, *E. coli* HB2151 cells were infected with phage obtained after four rounds of panning (R4 phage). Recognition of the amber stop codon between the scFv and the M13 gene III protein resulted in the production of soluble scFv by this strain of *E. coli*. The soluble scFvs are directed to the periplasmic space by an NH\textsubscript{2}-terminal gene III protein leader sequence. Incorporation of a small peptide epitope (an E-tag) at the COOH-terminal end of the scFv allows detection with an anti E-tag antibody. Upon induction with isopropyl \(\beta\)-D-thiogalactopyranoside, an immunoreactive band with an apparent molecular mass of about 34 kDa was detected in the periplasmic extracts from *E. coli* infected with R4 phage (Fig. 3). This represents the pool of scFvs enriched by panning. A band of similar size was also observed in the bacterial growth medium, indicating that there was some leakage of the scFvs from the periplasm (data not shown).

The periplasmic extract prepared from HB2151 cells infected with R4 phage contained functional scFv polypeptides that bound to *P. chabaudi* AMA-1 and could discriminate between *P. chabaudi* AMA-1 and BSA even at a 1:1,000 dilution of the periplasmic extract (Fig. 4A). This specificity was also obvious when *P. chabaudi* AMA-1 and BSA were probed with scFvs in immunoblotting experiments (Fig. 4B). Periplasmic extracts prepared from bacteria infected with R3 phage (i.e. isolated after three rounds of panning) showed less binding to *P. chabaudi* AMA-1. Soluble scFvs prepared from the unpanned phage library did not bind to *P. chabaudi* AMA-1 (Fig. 4B). The culture supernatant also contain functional scFvs that specifically recognize *P. chabaudi* AMA-1 (Fig. 5A, lane 3).

The binding characteristics of the scFvs obtained from four rounds of panning (R4 scFv) were examined in further detail by immunoblot analysis. The pool of R4 scFv antibodies bound...
filters containing \textit{P. chabaudi} reducing conditions (\textit{P. chabaudi} antibody. bound scFv was detected by anti E-tag antibody. Incubation with nitrocellulose filters containing immobilized recombinant \textit{P. chabaudi} AMA-1 or BSA (panel A), or culture supernatant diluted 1:1 with BLOTTO was incubated with nitrocellulose filters containing \textit{P. chabaudi} AMA-1 transferred after SDS-polyacrylamide gel electrophoresis run under both nonreducing conditions (lane 1) and reducing conditions (lane 2). Detection of bound scFv was achieved by using an anti-E-tag antibody as described under “Materials and Methods.”)

FIG. 4. Soluble R4 scFv from periplasmic extracts and bacterial supernatants bind to \textit{P. chabaudi} AMA-1. Periplasmic extracts without further purification were added to microtiter wells precoated with \textit{P. chabaudi} AMA-1 (\textbullet{}) or BSA (\textbullet{}), or culture supernatant diluted 1:1 with BLOTTO was incubated with nitrocellulose filters containing immobilized recombinant \textit{P. chabaudi} AMA-1 or BSA (panel B), and bound scFv was detected by anti E-tag antibody.

Specifying scFv antibodies specifically to AMA-1 from \textit{P. chabaudi} but, under the conditions of this experiment, was unable to recognize \textit{P. falciparum} AMA-1 (Fig. 5A) even though the ectodomains of these molecules share 48% homology at the amino acid level. Importantly, the scFvs also discriminate between the nonreduced and reduced forms of \textit{P. chabaudi} AMA-1 (Fig. 5B, lanes 1 and 2, respectively), indicating that the selected scFvs recognize conformational epitopes stabilized by disulfide bonds in the refolded \textit{P. chabaudi} AMA-1. It is interesting to note that the serum of the mice that were used in the construction of the library contained antibodies that recognize reduced AMA-1, as well as antibodies that recognize folded AMA-1 (data not shown). The enrichment strategy used in the production of the scFvs involves panning on the refolded antigen, which appears to select scFvs recognizing reduction-sensitive epitopes. Because the above studies were performed on the enriched pool of scFv antibodies it was important to examine the binding characteristics of individual scFvs. Periplasmic extracts, containing soluble scFvs, were prepared from individual colonies after four rounds of panning. Each cloned scFv showed specific binding to \textit{P. chabaudi} AMA-1 (Fig. 6). None of the scFv clones showed any binding to BSA (not shown).

The scFv inserts from 50 clones (R4) were amplified by PCR using oligonucleotide primers to the flanking vector sequence. Digestion of the products with BstNI, which cuts mammalian DNA frequently, enabled the assignment of clones into various groups defined by the pattern of restriction fragments. The 50 clones from the R4-selected phage gave a restricted set of digestion patterns, and each clone could be assigned to one of four groups designated scAMA-A, scAMA-B, scAMA-C, and scAMA-D (for single chain antibody to AMA-1). Ten clones selected at random from the unpanned library each showed unique BstNI restriction patterns (data not shown). The majority of clones (43/50) were assigned to the scAMA-A group, possibly indicating that this group of scFvs has the highest binding affinity.

To determine the fine specificity of individual recombinant antibodies, we performed dot-blot analysis of single clones from scAMA-A, -B, and -C group antibodies using three different AMA-1 molecules. As expected, all three antibodies recognized AMA-1 from the \textit{P. chabaudi} DS strain used to pan the phage display library (Fig. 7). There was, however, no detectable binding of any of the antibodies to AMA-1 from the heterologous 556KA strain of \textit{P. chabaudi} (Fig. 7, middle row). Interestingly, the clone representing the scAMA-B group showed strong binding to \textit{P. falciparum} AMA-1. The scFvs from the scAMA-C group recognized this heterologous antigen to a lesser extent, whereas the scAMA-A scFvs, the major contributor to the R4 pool of scFv antibodies, only recognized \textit{P. chabaudi} DS AMA-1. These data indicate that the four rounds of panning have enriched for a subset of scFv which recognizes \textit{P. chabaudi} AMA-1 and that scFvs with distinct antibody specificities have been obtained.

The nucleotide sequences encoding the V$_H$ and V$_L$ chains were determined from at least two representative clones from each group, with the exception of scAMA-D, for which only one clone was obtained. The deduced amino acid sequences are shown in Fig. 8. Sequencing of different clones within the scAMA-B group revealed two sequences that differed by six nucleotides (the alternative sequence is designated scAMA-B$^*$). Five of these nucleotide substitutions would cause amino acid substitutions (Fig. 8). Thus, five unique heavy chains have been selected which can participate in binding to \textit{P. chabaudi} AMA-1. As expected, the majority of the diversity is contained...
FIG. 6. Individual scFv antibodies bind to P. chabaudi AMA-1. 22 individual clones from R4 E. coli HB2151 colonies were induced to express their soluble scFv. Periplasmic extracts were added to microtiter wells precoated with P. chabaudi AMA-1, and binding of scFv was detected by anti E-tag antibodies. Clones representing scAMA-A, -B, and -C classes of scFv are indicated. The last two bars represent the ELISA signals from R4 periplasmic extracts binding to wells without immobilized antigen (second last), and the signal obtained when TBS was used in place of R4 periplasmic extracts (last bar).

FIG. 7. Specificity of binding of three scFv antibodies to AMA-1. Recombinant AMA-1 from P. chabaudi DS, P. chabaudi 556KA, and P. falciparum was immobilized onto nitrocellulose and incubated with periplasmic extracts containing soluble scFv molecules from clones of scAMA-A, -B, and -C. Binding of scFv was detected by anti E-tag antibodies.

within the complementarity determining region 3 (CDR3) of the VH chain, which varies both in sequence and in length. The DNA sequences from both the VH and VL were searched against the Kabat database using the BLAST algorithm and were shown to be novel sequences. The VH gene, D segment, and J segment usage for the four scFv groups were analyzed by IMGT data base searching via the World Wide Web, and the results are shown in Table I. All the VH gene segments code for V kappa fragments of the 4/5 group, V1 subgroup. Thus each scFv from groups scAMA-A, -C, and -D consisted of a unique VH-V kappa Pairing. However, the same V kappa chain was associated with the two distinct but related VH sequences of scAMA-B and scAMA-B*.

DISCUSSION

The antibody phage display system has been used to select specific scFv against one of the leading candidate antigens for inclusion in a vaccine against malaria. AMA-1 is considered to have potential as a component in a recombinant vaccine because immunization with the antigen has been shown to protect against malaria challenge in monkey and other rodent models (10–13). The results of recent vaccine trials demonstrate that immunization with the ectodomain of P. chabaudi AMA-1 protects mice against challenge with P. chabaudi (12, 13). Several lines of evidence serve to highlight the crucial role of antibodies in the protection generated by immunization with AMA-1. First, protection in both monkey (11) and murine (13) trials correlates with antibody titer to AMA-1. Second, antibodies against AMA-1, and their Fab fragments, inhibit invasion of erythrocytes by merozoites (10, 12). Last, IgG from rabbits immunized with P. chabaudi AMA-1 protected mice challenged with P. chabaudi (12, 13). The demonstration that Fab fragments are more effective than whole IgG at inhibiting the invasion of rhesus erythrocytes by P. chabaudi merozoites indicates that the Fc portion of the antibody is not necessary for inhibition. Consequently, scFv molecules (consisting of linked VH and VL regions) might be expected to inhibit malarial growth at least as well as intact IgG molecules. Indeed, given that their smaller size may allow them to reach their target more rapidly, the scFvs may even be more efficient provided that they remain for a sufficient period within the bloodstream. This is consistent with the kinetic model proposed by Saul (25), which suggests that Fabs against AMA-1 would have a faster binding rate than the intact antibody. According to this model a high rate of binding is proposed to be a more important parameter in vaccine design than high avidity.

To generate monoclonal antibodies that might be protective, we constructed a library of phage displaying on their surface scFv antibodies from the spleens of mice that were protected against malaria by immunization with P. chabaudi AMA-1. Such a library should contain phage expressing antibodies against the vaccinating antigen, along with many other specificities. We have shown that even from a library as small as 4 x 10^6 clones, one can isolate scFvs specific for the immunizing protein. It may be significant, in this regard, that the mice from which the spleens were obtained had very high titers (>1:10^6) toward P. chabaudi AMA-1 (13).

Western blot analysis of the sera from the mice whose spleens were utilized to construct the phage display library revealed the presence of antibodies against both reduced and nonreduced epitopes on P. chabaudi AMA-1. Thus we would expect both types of scFvs to be present in our library. Because there is a good correlation between protection from malaria and the presence of antibodies recognizing nonreduced, conformational epitopes we decided to pan our library against the refolded P. chabaudi AMA-1. This strategy was successful in enriching for scFv that strongly discriminate between reduced and nonreduced antigen.

Although we isolated four different scFvs by panning, one (scAMA-A) predominated, suggesting that it had high affinity for folded P. chabaudi AMA-1; however, all scFv (except D, which was not tested) were shown to bind specifically to P. chabaudi AMA-1. A pool of isolated scFv antibodies was able to bind to native AMA-1 present in Triton X-114 extracts of P. chabaudi DS parasites in dot-blotting experiments (data not shown). Experiments are under way to identify the molecular location of the structural epitopes within the antigen for each of these scFvs. P. chabaudi AMA-1 has been delineated into three distinct domains based on the disulfide bonding pattern (9), and production of these domains as recombinant proteins in bacteria is under way. In addition, future passive transfer experiments should delineate those scFv that are protective from those that do not confer protection.

The specificity of three of the four antibodies that were isolated from the phage display library from immunized mice were examined for binding to AMA-1 molecules from various sources. It was shown that all three antibody clones recognized AMA-1 from the P. chabaudi strain DS. This was expected because this antigen was used to immunize the donor mice (13) and also for the panning procedure. None of the three antibodies reacted with AMA-1 from the related P. chabaudi strain 556KA. The gene for AMA-1 from 556KA has been sequenced, and the deduced 556KA polypeptide sequence differs from the
DS sequence at only 36 amino acid residues, all in the ectodomain (12). The amino acid substitutions were found to be clustered primarily in domain 1 between the first and third cysteines, with a second cluster in domain 2. Thus, the scFvs are recognizing conformational epitopes that include sites of amino acid substitutions that have presumably been selected for by protective immune responses.

Despite the failure of any of these scFv antibodies to react with the heterologous

\textit{P. chabaudi} AMA-1, two of the three antibodies examined reacted with AMA-1 of the heterologous species, \textit{P. falciparum}. This is a surprising result as the two \textit{P. chabaudi} AMA-1 polypeptides are 95% identical, whereas there is only about 51% identity between \textit{P. chabaudi} DS and \textit{P. falciparum} AMA-1 used in these studies.

When the 36 sites of amino acid substitutions between DS and 556KA are examined there is identity between DS and \textit{P. falciparum} (3D7) at five residues, four of which are clustered between the first and third cysteine residues of domain 1 of the protein. This region of AMA-1 contains most of the sequence diversity when \textit{P. chabaudi} DS and 556KA strains are compared. The cross-reacting antibodies presumably recognize epitopes that include some of these residues. It is intriguing that of the three scFv antibodies whose fine specificities were examined, none recognized the closely related 556KA strain, whereas two showed some binding to the more distantly related \textit{P. falciparum} AMA-1. The explanation for this is not known; however, it is interesting to note that the mice whose spleens were used in the construction of the library while protected against the DS strain were not protected against challenge with 556KA parasites. Further analysis of the fine specificity of further scFvs from this library, in combination with mutational analysis from field and experimental isolates, may illuminate the differences in immune pressure that is exerted during \textit{P. chabaudi} and \textit{P. falciparum} infections.

The use of the phage display system to generate these anti-

\textit{AMA-1} antibodies facilitates the rapid sequencing of the corresponding genes. Analysis using the SAW data base (23) and a search of the IMGT data base suggest that each heavy chain originates from a different germline sequence and not by somatic hypermutation of the same germline sequence. Interestingly, within the scAMA-B class of scFv, there exists two heavy chains that differ only in six nucleotides, five of which lead to changes in their corresponding amino acid residues. The silent mutation is in framework two, and four of these substitutions are either in the CDRs or adjacent (within three residues) to CDR1 and CDR3. Only one mutation resulting in an amino acid substitution is located within a framework region, and this is a conservative substitution of a lysine for an arginine. Hence it is unlikely that these substitutions have been introduced as a result of the infidelity of \textit{Taq} polymerase at the amplification step in the construction of the library. scAMA-B and scAMA-B* may have arisen by somatic mutation of a germline sequence during the affinity maturation response in the mouse. It will be of interest to examine other scFv with a spectrum of affinities for \textit{P. chabaudi} AMA-1 to examine the role of somatic mutation in generating high affinity antibodies to this antigen.

In conclusion we have demonstrated the validity of the ap-

\textbf{Table I}

\textit{Analysis of V\textsubscript{H} gene usage in anti-\textit{P. chabaudi} AMA-1 scFvs}

| V segment | D segment | J segment |
|-----------|-----------|-----------|
| scAMA-A   | VH14      | DSP2.9(2) | JH2       |
| scAMA-B   | VH14      | DQ52(2)  | JH2       |
| scAMA-C   | VH1       | DSP2.3(3)| JH2       |
| scAMA-D   | VH1       | DSP2.7(3)| JH4       |

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\textbf{FIG. 8. Amino acid sequence analysis of four anti-\textit{P. chabaudi} AMA-1 scFvs.} A comparison of the \textit{V\textsubscript{H}} and the \textit{V\textsubscript{L}} chains is shown. The \textit{V\textsubscript{H}} chain of one clone from the scAMA-B scFv class (designated \textit{B}*) was found to have five residue differences in the predicted amino acid sequence from the other scAMA-B clones sequenced (dots represent amino acid identities; letters represent the differences). CDRs and framework regions (Fr) are indicated.
proach of constructing phage display libraries directly from the spleens of mice used in a vaccine trial. Mice that were protected from malaria by immunization with the recombinant malarial antigen \textit{P. chabaudi} AMA-1 that had been refolded \textit{in vitro}, provided a source of antibodies that were selected by panning the resultant phage display library on \textit{P. chabaudi} AMA-1. These antibodies specifically recognize the antigen used for immunization and panning and recognize reduction-specific epitopes. The selected antibodies differed in the fine specificity of their interaction with AMA-1 molecules from several sources. The ability of these scFv antibodies to inhibit merozoite invasion and to protect mice from \textit{P. chabaudi} challenge passively is currently under investigation.

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