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Recombinant *Toxoplasma gondii* Surface Antigen 1 (P30) Expressed in *Escherichia coli* Is Recognized by Human *Toxoplasma*-Specific Immunoglobulin M (IgM) and IgG Antibodies

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The immunodominant surface antigen of *Toxoplasma gondii*, surface antigen 1 (SAG1), was expressed in *Escherichia coli* as a fusion protein containing a majority of the SAG1 protein supplied with six histidyl residues in the N-terminal end. The recombinant protein was purified on a Ni-chelate column and then on a fast-performance liquid chromatography column and was in a nonreduced condition. It was recognized by *T. gondii*-specific human immunoglobulin G (IgG) and IgM antibodies as well as by a mouse monoclonal antibody (S13) recognizing only nonreduced native SAG1. Antibodies induced in mice by the recombinant SAG1 recognized native SAG1 from the *T. gondii* RH isolate in culture. Recombinant SAG1 is suitable for use in diagnostic systems for detecting anti-SAG1-specific IgG and IgM antibodies.

The immunodominant surface antigen of *Toxoplasma gondii*, surface antigen 1 (SAG1; previously named P30), was expressed in *Escherichia coli* as a fusion protein containing the mature part of the SAG1 protein supplied with six histidyl residues in the N-terminal end. The construction can be produced in large amounts, is easy to purify, and is recognized by anti-SAG1 monoclonal antibodies, *Toxoplasma*-specific acute-phase immunoglobulin M (IgM) antibodies and chronic-phase IgG human antibodies. Native mature SAG1 protein is presumed to be posttranslationally modified by removal of the signal sequence and the C terminus, the latter upon addition of the GPI anchor (2, 8). We therefore cloned only the sequence coding for the mature part of the protein, residues 49 through 323 (2), into an *E. coli* expression vector, pGH433 (12), using PCR. An oligonucleotide encoding six histidyl residues and a factor Xa cleavage site (13) was cloned into the vector in frame with the SAG1-coding region (pDH26), giving rise to a recombinant SAG1 (rSAG1).

The fusion of an oligohistidine stretch to SAG1 allows purification of the fusion protein under nondenaturing conditions by immobilized metal affinity chromatography with Ni^{2+} bound to iminodiacetic acid-agarose (3, 4). rSAG1 was purified by this method on a column of iminodiacetic acid-epoxy-activated Sepharose 6B fast flow (Sigma) as described previously (15), except that a proteinase inhibitor mixture was used. This was followed by purification by fast-performance liquid chromatography (FPLC) with a Mono-S HR 5/5 cation-exchange chromatograph (Pharmacia). The FPLC chromatogram showed two peaks, of which only the first peak contained rSAG1. The purified rSAG1 was eluted in a phosphate buffer with a linear salt gradient, and the protein was eluted at 20% buffer B (0.1 M NaCl). Coomassie staining of the purified rSAG1 under nonreduced and reduced conditions shows that rSAG1 migrates differently under the two conditions (Fig. 1). On the basis of the amino acid sequence, the rSAG1 protein is calculated to have a mass of 31.3 kDa, and the size of the nonreduced protein therefore corresponds well to that of the native SAG1 protein (30 kDa). rSAG1 is recognized by human IgG-positive serum (Fig. 2A), the S13 monoclonal antibody, recognizing a conformational epitope on native SAG1 (7) (Fig. 2B), and human IgM-positive serum (data not shown). A human serum sample containing *T. gondii*-specific IgG antibodies was used to incubate the blot shown in Fig. 2A and, like the blot incubated with the S13 monoclonal antibody (Fig. 2B), showed reactivity only against the nonreduced rSAG1. The reactivities of human IgM antibodies against rSAG1 were seen in a blot incubated with serum from patients with a very early infection. The serum samples contained only *T. gondii*-specific IgM antibodies but no specific IgG antibodies (data not shown). Fifteen IgG-positive serum samples and a further nine IgM-positive serum samples were tested for their reactivities to nonreduced rSAG1 as described above (data not shown). All sera recognized rSAG1 in the nonreduced condition only.

The B- and T-cell epitopes of the SAG1 protein have been studied by several groups (9, 10, 16), and it has been found that SAG1 contains one immunodominant region in the middle of the protein, with repetitive epitopes containing all of the B-cell epitopes (16). The B-cell epitopes are conformational since human *T. gondii*-immune sera do not recognize the reduced SAG1 (16). The SAG1 gene codes for 12 cysteine residues, and the immunogenicity of the recombinant protein depends entirely on the correct folding. In *E. coli* the formation of disulfide bonds takes place within the periplasm (1), while we presume that our rSAG1 is situated in the cytoplasm, since it does not contain any secretion signal sequence. The shift in mass between the reduced rSAG1 (33 kDa) and the nonreduced rSAG1 (31 kDa) and the disappearance of all reactivity of rSAG1 under reducing conditions indicate that at least the disulfide bridges necessary for the immune reactivity with human sera and the S13 monoclonal antibody are normally formed in rSAG1. Even though the native SAG1 protein is phosphorylated (14), our rSAG1 fusion protein is not since phosphorylation modifications do not take place in *E. coli*, and phosphorylation is therefore not important for the immunogenicity of the B-cell epitopes.

The full-sized SAG1 gene has previously been expressed in

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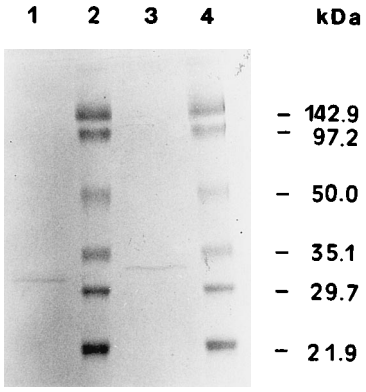

FIG. 1. Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gels showing the FPLC-purified rSAG1 under nonreduced and reduced conditions. Lane 1, nonreduced rSAG1; lane 3, reduced rSAG1; lanes 2 and 4, molecular mass markers. Molecular masses are given on the right.

E. coli, but the protein product was poorly recognized by anti-SAG1 antibodies, indicating that this recombinant SAG1 protein was not properly folded (2). This lack of folding could very well be due to the presence of the signal sequence at the N terminus of the SAG1 product, since this sequence is not cleaved off by *E. coli*. In addition, the C-terminal end may be cleaved in *T. gondii* but not in *E. coli*, and the presence of this stretch of hydrophobic amino acids may also contribute to the incorrect folding, and hence the lack of recognition of the full-sized rSAG1 by anti-SAG1 antibodies (2). Other groups have successfully expressed the SAG1 gene product in heterologous systems. Correctly folded and immunogenic SAG1 has been produced in CHO cells (5). Immunization of mice with a fusion protein consisting of glutathione *S*-transferase and SAG1 produced in *E. coli* could decrease *T. gondii* infection of macrophages, but the immunogenicity of rSAG1 was not reported (6). In other studies this fusion protein was expressed in *E. coli* as well as in animal cells by using a recombinant Sindbis virus (11, 17). The fusion products showed the same immunological phenotypes, since rabbits immunized with either of the two fusion proteins elicited significant antibody responses against rSAG1, but only rabbits immunized with rSAG1 expressed in eukaryotic cells displayed serological reactivity

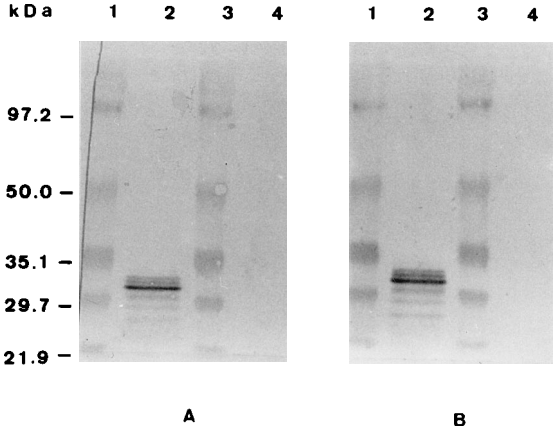

FIG. 2. Immunoblot profiles of FPLC-purified rSAG1 detected with human *T. gondii* hyperimmune serum (A) and anti-SAG1 monoclonal antibody (S13) (B). Lanes 1 and 3, molecular mass markers; lanes 2, nonreduced rSAG1; lanes 4, reduced rSAG1. Molecular masses are given on the left.

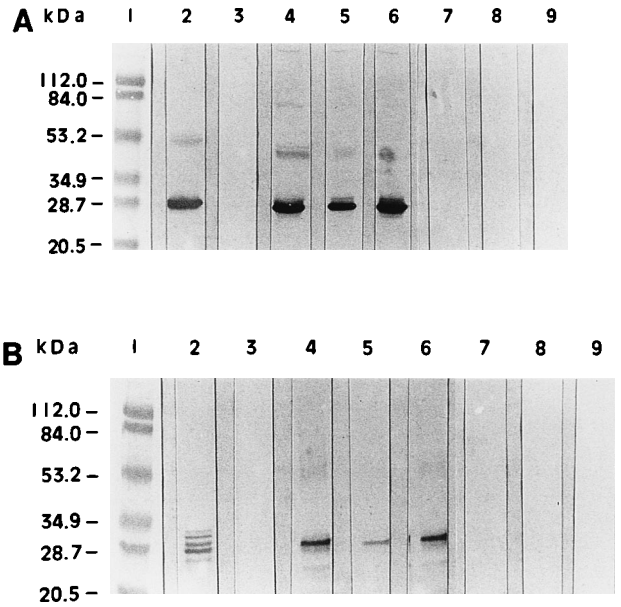

FIG. 3. Immunoblot profiles of sera from three mice immunized with rSAG1. Immune sera from the three mice recognize both the native SAG1 (A) and rSAG1 (B), while preimmune sera from the same mice do not recognize either the native SAG1 (A) or rSAG1 (B). Lanes 1, molecular mass markers; lanes 2, positive controls; lanes 3, negative controls; lanes 4 to 6, immune sera from mice 1, 2, and 3, respectively; lanes 7 to 9, preimmune sera from mice 1, 2, and 3, respectively. Molecular masses are given on the left.

against a tachyzoite lysate (17). Three 7-week-old CF-1 female mice were immunized intraperitoneally four times at 14-day intervals. Each immunization dose consisted of 250 μl of rSAG1 from the strongest FPLC fraction mixed with Al $(OH)_3$ (final concentration, 1 mg/ml). Mice were bled before the first immunization and 10 days after the last immunization. Both the preimmune and the immune sera from the immunized mice and sera from nonimmunized control mice were tested in an immunoblot. S13 monoclonal antibody was used as a positive control. Sera from all three immunized mice recognized both rSAG1 and native SAG1 from in vitro-cultured *T. gondii* (RH strain) (Fig. 3A and B). The S13 monoclonal antibody was used as the positive control.

The recombinant SAG1 produced in *E. coli* is a promising new antigen that can be used in diagnostic assays for specific antibodies against *T. gondii*. Assays based on the recombinant protein are expected to be easier to standardize and to be more reproducible because only a single protein with a few immunodominant epitopes is used.

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