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Mapping of B-Cell Epitopes on a Novel 11.5-Kilodalton *Fasciola hepatica-Schistosoma mansoni* Cross-Reactive Antigen Belonging to a Member of the *F. hepatica* Saposin-Like Protein Family

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FhSAP-2 is a novel member of the *Fasciola hepatica* saposin-like protein family that induces protection in rabbits against a challenge infection. We investigated the presence of lineal B-cell epitopes within this protein using a set of overlapping synthetic peptides. Peptides were tested in enzyme-linked immunosorbent assays against sera from rabbits infected with *F. hepatica*. Two dominant epitopes were identified, which were also highly reactive with sera from mice infected with *Schistosoma mansoni*. These peptides may be suitable to incorporate into a polyepitope-based vaccine formulation against *F. hepatica*.

Fascioliasis, caused by *Fasciola hepatica*, represents a recognized unsolved agricultural problem responsible for economic losses estimated to be around 3 billion U.S. dollars per year to rural agricultural communities and commercial producers worldwide, including the United States (5). Recent reports indicate that *F. hepatica* is also a major human pathogen and is increasingly recognized as causing significant human diseases, with 2 to 4 million people infected with this parasite (2, 5). Liver fluke infections can be highly pathogenic and can lead to severe morbidity and even death of the host. The flukicide triclabendazole is the most effective drug for controlling *Fasciola* (19, 26); however, the prohibitive cost of treatment prevents its widespread use by rural producers in developing countries. Moreover, resistance to triclabendazole has been reported in sheep infected with *F. hepatica* (19), suggesting that selection of resistant parasites may eventually compromise the use of this drug. Vaccines represent the most attractive long-term alternative to invert this scenario.

Recently, we described the molecular cloning and expression of an *F. hepatica* antigen termed FhSAP-2, which is expressed at early stages of infection and which, by its structural homology with a *F. hepatica* NK-lysin (20), three amoebapores of *Entamoeba histolytica* (14), a porcine NK-lysin (1), and several other related proteins (3, 23), falls in the saposin-like/NK-lysin protein family of *F. hepatica*. It is an 11.5-kDa polypeptide that elicits a strong antibody response during active infection and exhibits a potent lytic activity against human erythrocytes and peripheral blood mononuclear cells (7), which stimulates specific cell-mediated and humoral immune responses (6). This novel antigen is highly reactive with sera from rabbits experimentally infected with *F. hepatica*, and it is recognized by more than 83% of sera from patients with chronic *Fasciola* infection

(7). We have also found that FhSAP-2 is a potent immunogen, since rabbits vaccinated with FhSAP-2 and challenged with *F. hepatica* metacercariae had lower parasite burdens, fewer parasite eggs, and less liver damage than nonvaccinated controls (8). However, the immune mechanism that made this protection possible is not clear. Correct identification of epitopes in FhSAP-2 will greatly help in the characterization of targets for immunization-vaccination and may provide an antigen basis for designing diagnostics specific for *F. hepatica*. The aim of this study was to identify linear B-cell epitopes of FhSAP-2 by screening a synthetic peptide library based upon this protein with sera from rabbits immunized with FhSAP-2 and rabbits infected with *F. hepatica* using an enzyme-linked immunosorbent assay (ELISA) and a peptide ELISA inhibition assay. Similar studies have been carried out on other antigens associated with *Fasciola* and related parasites with success (4, 24, 30).

Recombinant protein and animal sera. Recombinant FhSAP-2 was purified from transformed *Escherichia coli* TOP10 competent cell inclusion bodies, solubilized in 6 M guanidine hydrochloride, and then purified by affinity chromatography through a HiTrap Ni^{2+} chelating column (Amersham Biosciences Corp, New Jersey) as previously described (7). Purified protein was analyzed by conventional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and identified by Western immunoblotting using a specific polyclonal antiserum (7). Eighteen peptides representing the entire 101-amino-acid sequence of FhSAP-2 were synthesized by standard 9-fluoroenyl-methoxicarbonyl polyamide solid-phase synthesis. The quality of peptides was assessed by reverse-phase chromatography on a Supelco Bio Wide Pore column and by matrix-assisted laser desorption ionization–time of flight mass spectrometry. Peptides were synthesized as 15 mers, with adjacent peptides overlapping by 10 amino acids and termed sequentially as SAP-1 to SAP-18. The last C terminus peptide (SAP-18) was synthesized as a 16 mer.

All experiments were performed with eight 3-month-old male New Zealand White rabbits (Harland Inc., Indianapolis,

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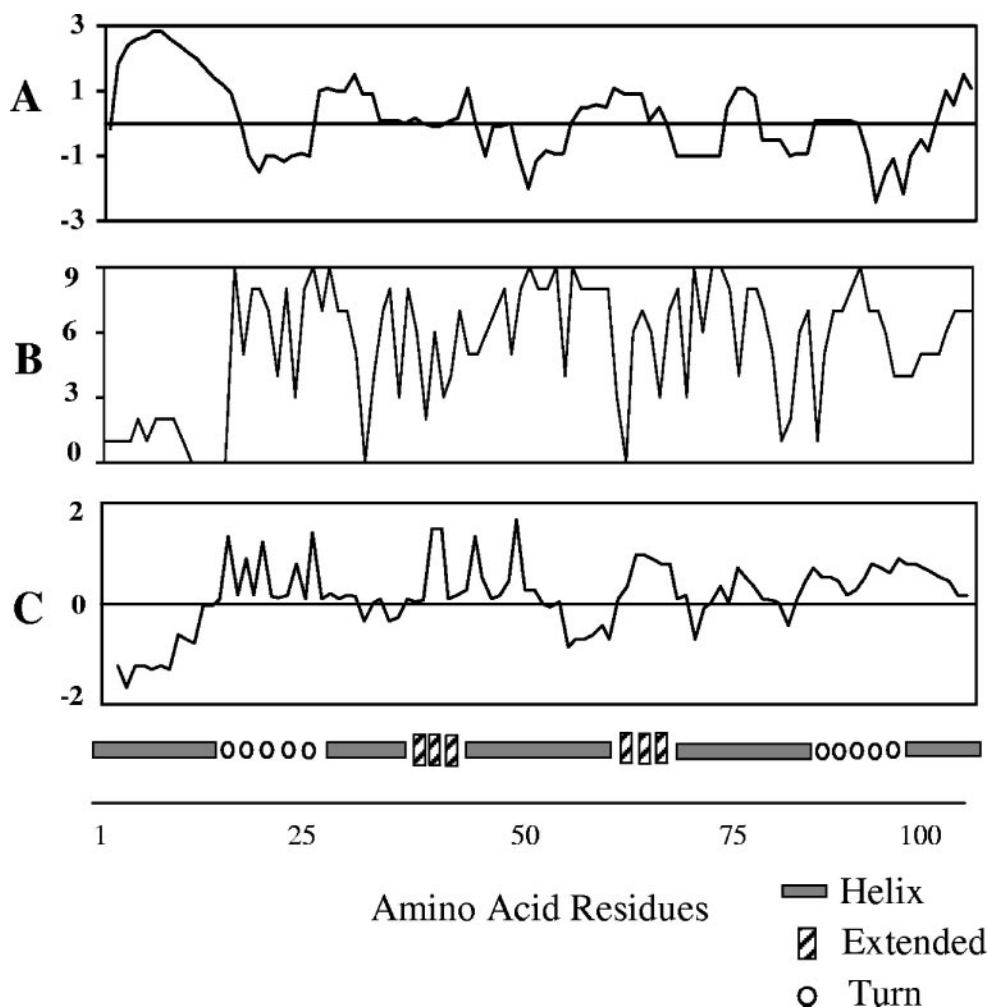
FIG. 1. (A) Prediction of hydrophobicity. >0, high hydrophobicity; <0, low hydrophobicity. (B) Solvent accessibility. 0, low accessibility; 9, high accessibility. (C) Antigenic index. Values of >0 indicate potential antigenic regions. Regions of low hydrophobicity have more solvent accessibility and a higher antigenic index. (D) Secondary structure predicted for the protein moiety of FhSAP-2 (GenBank accession no. AF286903).

Ind.) and nine Swiss male mice (Biomedical Research Institute, Maryland) which were maintained in the animal care facility at the University of Puerto Rico, Medical Sciences Campus, and treated according to international regulations for the care of laboratory animals. Rabbits were orally infected with 25 *F. hepatica* metacercariae each (Baldwin Aquatics Inc., Monmouth, Oreg.) and necropsied 12 weeks after infection. Mice were infected with 150 *S. mansoni* cercariae by skin penetration and necropsied 9 weeks after infection. Rabbits and mice were bled before infection and then biweekly until necropsy for collection of serum. Sera from rabbits and mice were tested by ELISA against FhSAP-2 following a preestablished protocol (9). FhSAP-2 reacted strongly with sera from rabbits at 4 weeks (0.23 ± 0.06) to 12 weeks (1.76 ± 0.012) postinfection with *F. hepatica*, and it was also reactive with sera from mice at 4 weeks (0.18 ± 0.06) to 9 weeks (0.76 ± 0.015) postinfection with *S. mansoni*.

B-cell epitope prediction. Because most, if not all, antigenic sites are located within surface-exposed regions of a protein, the presence of B-cell epitopes is often predicted by computer analysis based on measurement of hydrophobicity (17), hydro-

philicity and surface accessibility (21), antigenic index (13), and predicted helix or turns (11). Using the deduced amino acid sequence of the cDNA encoding FhSAP-2, these predictions revealed the presence of several putative antigenic regions randomly distributed throughout the protein (Fig. 1A to C), with the exception of the first 15 amino acid residues at the N terminus. This region exhibits the highest hydrophobic index. It has been predicted that this region contains a string of hydrophobic and hydroxylic amino acid residues that were predicted to constitute an N-terminal signal sequence with a signal cleavage site between amino acids Ala15 and Ser16 (7). Therefore, it is possible that this region may be absent in the mature protein, which could eventually explain the lack of reactivity between peptides containing residues [1]MNPLFVLM LAAVTFASFDVP[20] and sera of infected animals. A prediction of the secondary structure of FhSAP-2 is shown in Fig. 1D.

Experimental identification of linear B-cell epitopes within FhSAP-2 protein. Two rabbit anti-FhSAP-2 sera were produced as described previously (7). The ELISA was standardized for each synthetic peptide derived from FhSAP-2 using an anti-FhSAP-2 serum. Plates were coated with different peptide

concentrations (from 5 to 60 µg/ml) and tested against different antiserum dilutions (from 1:50 to 1:800) at different conjugate dilutions (from 1:2,000 to 1:10,000) to define the optimal dilution of all reagents that renders the best differentiation between a positive and a negative serum. The assay was performed essentially as previously described (7). Anti-FhSAP-2 sera, sera from rabbits infected with *F. hepatica*, and sera from mice infected with *S. mansoni* were tested in the ELISAs against each single peptide. As a result of this scanning, the presence of two areas of increased reactivity corresponding to amino acid residues 21 to 55 and 66 to 101 was demonstrated in the FhSAP-2 protein moiety. The rabbit anti-FhSAP-2 sera were capable of recognizing multiple peptides, thus indicating a complex polyclonal response to the protein moiety of FhSAP-2 (Fig. 2A). Two immunogenic domains within FhSAP-2 similar to those revealed by epitope mapping experiments using hyperimmune sera were identified when peptides were scanned using sera from rabbits with 12 weeks of *Fasciola* infection (Fig. 2B). Three peptides without reactivity with the anti-FhSAP-2 sera or infection sera were identified. The nonreactive peptides covered amino acid residues 1 to 20 (peptides SAP-1 and SAP-2) and residues 51 to 65 (SAP-11). When sera from mice infected with *S. mansoni* were individually tested against peptides, reactivity was observed only with peptides SAP-4 and SAP-16 (Fig. 2C).

To define the fine localization of dominant immunoreactive regions, peptides were tested for their ability to inhibit the binding of rabbit antibody to a pool of peptides and FhSAP-2. A peptide inhibition assay was performed essentially as previously described (9). Peptides that inhibited the ELISA by more than 50% were considered as containing a dominant B-cell epitope. Comparison of the amino acid sequences of immunoreactive peptides revealed that FhSAP-2 possessed three main antigenic sites. The first antigenic site (site 1) was considered to be present in residues [21]SKQPTIDIDLCDICT[35] of peptides SAP-3, SAP-4, SAP-5, and SAP-6. All sera gave relatively high optical density (OD) values (between 0.6 to 1.2), with peptides SAP-4 and SAP-5 containing [21]SKQPTIDIDL[30] as part of their sequence, and these peptides inhibited the antibody binding to a peptide-coated plate by more than 50% (Fig. 3). Since peptide SAP-4 shared residues [21]SKQPT[25] with poorly immunoreactive peptide SAP-3 and since SAP-5 shared residues [26]IDIDLCDICT[35] with the less immunoreactive peptide SAP-6, we concluded that antigenic site 1 contained a dominant epitope present in residues [21]SKQPTIDIDL[30].

The second antigenic site (site 2) appeared to be in residues [46]ADQTVEEHIG[55] of peptides SAP-8, SAP-9, and SAP-10 but not in the nonreactive peptide SAP-7 and only partially present in the nonreactive peptide SAP-11. However, because the OD values of the sera tested with these peptides were low (between 0.3 to 0.37) and none of these peptides significantly inhibited antibody binding to the peptide-coated plate, we concluded that this B-cell epitope is not dominant.

The last antigenic site (site 3) was considered to be in residues [66]RSQDACIEFVQQEVDYIIDHVDQHNATEICRLIK LC[101] of peptides SAP-12, SAP-13, SAP-14, SAP-15, SAP-16, SAP-17, and SAP-18. All sera gave low OD values (between 0.26 to 0.35) with peptides SAP-12, SAP-13, SAP-14, SAP-17, and SAP-18 but gave moderate to high OD values (between 0.43 to 0.8) with peptides SAP-15 and SAP-16, which contain

the amino acid residues [76]QQEVDYIIDH[85] as part of their sequence. Peptide SAP-15 shared the residues [71]CIEFV[75] with the less immunoreactive peptide SAP-14, and peptide SAP-16 shared the residues [81]YIIDHVDQHN[90] and [86]VDQHN[90] with the poorly immunoreactive peptides SAP-17 and SAP-18, respectively. Because peptides SAP-15 and SAP-16 were the only peptides from this group that inhibited antibody binding to a peptide-coated plate by more than 50%, we concluded that the region spanned by amino acid residues [76]QQEVDYIIDH[85] is a dominant B-cell epitope (Fig. 3).

Quantitative comparison between both dominant B-cell epitopes demonstrates that sera were significantly ($P < 0.05$) more reactive with the dominant B-cell epitope of site 1 (N-terminal portion of protein) than with the dominant B-cell epitope of site 3 (C-terminal portion of protein). It is necessary to note that, unlike peptides SAP-4, SAP-5, (from the N-terminal region), and SAP-16 (from the C-terminal region) that were the only peptides that reacted with most sera, the other antigenic peptides from the antigenic site 1, site 2, and site 3 were not recognized by most of the positive sera tested (Fig. 2B). This suggested that it will not be possible to further define or shorten the epitopes SKQPTIDIDL, ADQTVEE HIG, and QQEVDYIIDH, since shortening of the peptide in these regions will probably result in decreasing the OD reading or elimination of the reactivity with some sera. Similar results have been reported by other investigators (27) and may occur because some antibodies in a particular serum react more avidly with certain amino acid residues than with others within the epitope. This would mean that a shortened peptide may react only with a portion of FhSAP-2 antibodies present in a particular serum, resulting in a weak-positive or false-negative reaction. Previous studies relating to the epitope mapping of *Mycoplasma bovis* surface lipoproteins have shown B-cell epitopes to be around three to seven amino acid residues long (22). However, other studies have shown reactive B-cell epitopes relating to fungal, bacterial, or parasitical antigens to be as long as 10 or even 15 residues (15, 27, 28, 29, 30), similar to the size of the 10-mer SKQPTIDIDL, ADQTVEEHIG, or QQEVDYIIDH described here.

As with all proteins belonging to the saposin-like/NK-lysin protein family, FhSAP-2 possesses six conserved cysteine residues as well as seven conserved hydrophobic residues, which are within five amphipathic α-helix domains (1, 18, 20, 23, 25). The number and the positioning of cysteine residues in this family strongly suggest a common structural organization among these proteins and indicate that a favorable tertiary structure that combines amphipathic α-helices with folding of a disulfide bond is conserved (7). An important observation of this study is that the immunoreactivity of the B-cell epitopes mapped on FhSAP-2 was found to be inversely related to the number of hydrophobic residues that include its sequence. That is, peptide SAP-7 (residues [32]DICTNT_MD_V_IKKML_[45]), SAP-11 (residues [51]EEHIGY_LV_KY_L_CKIA[65]), SAP-17 ([81]Y_II_ DH_V_DQHNATE_I_C[95]) and SAP-18 ([86]_V_DQHNATE_I_CRL_I_K LC101), which are the less immunoreactive peptides, span each one, three, or four conserved hydrophobic residues (underlined). Similarly, peptides SAP-8, SAP-9, and SAP-10 ([36]N TMD_V_IKK_ML_ADQTV[50], [41]IKK_ML_ADQTVEEHIG[55], and [46]ADQTVEEHIGY_LV_KY[60], respectively), which cover the central part of the protein moiety and exhibit a moderate
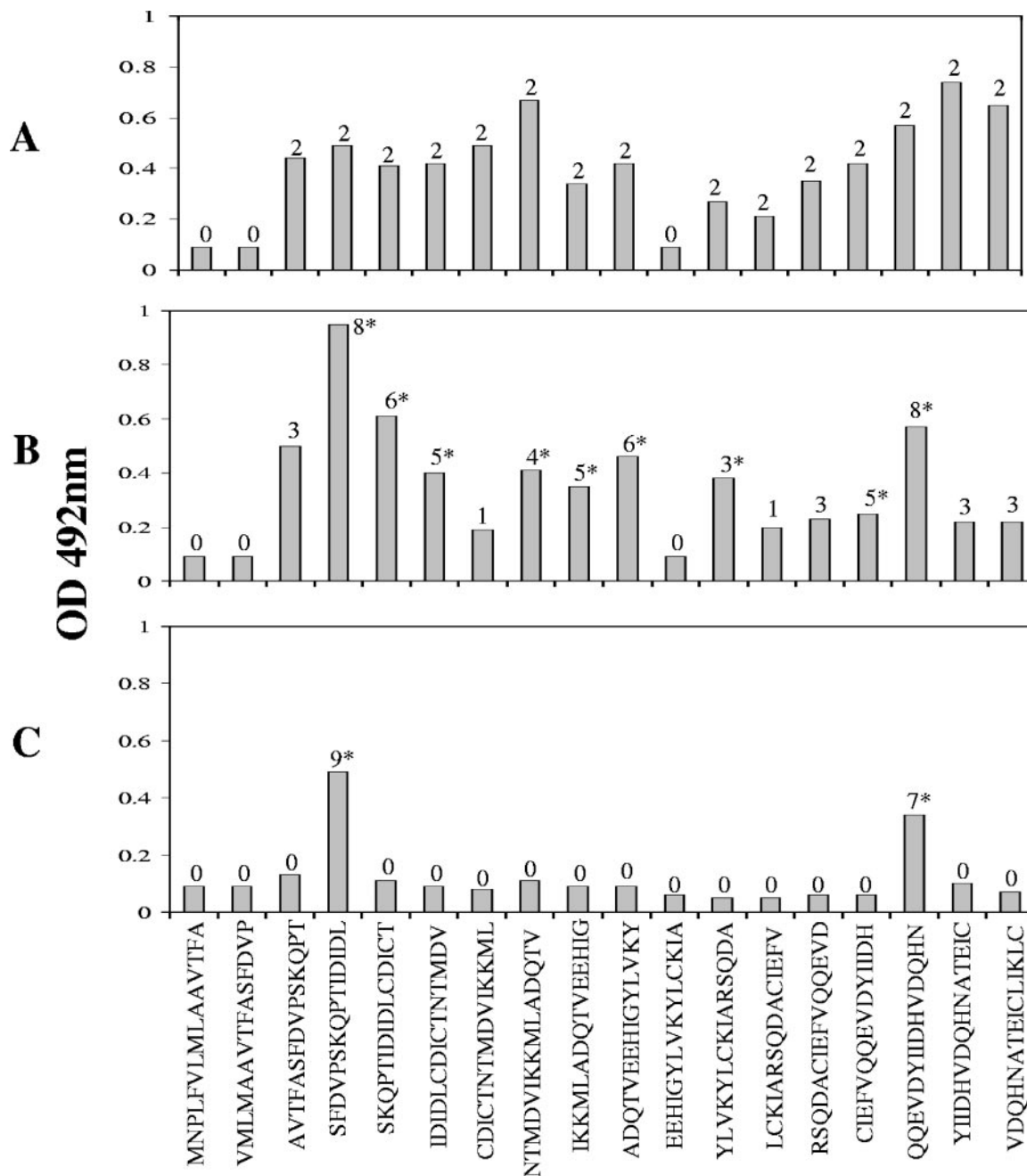
FIG. 2. Mapping analysis using peptides spanning the entire sequence of the protein the FhSAP-2. For this experiment ELISA plates were coated with 20 µg/ml per well. Rabbit and mouse sera were tested at dilutions of 1:200. To visualize specific peptide-antibody reactions, peroxidase-labeled anti-species immunoglobulin G (Bio-Rad Laboratories, Hercules, CA) conjugate, diluted 1:5,000, was used. Individual peptides were tested with two specific anti-FhSAP-2 sera (A), eight sera from rabbits at 12 weeks postinfection with *Fasciola* (B), and nine sera from mice at 9 weeks postinfection with *S. mansoni* (C). Serum was considered positive when its OD at 492 nm exceeded the previously established cutoff value (cutoff, 0.19), which was calculated as the mean absorbance plus two standard deviations of the negative rabbit or mouse sera. Numbers above columns indicate the number of sera reacting with a given peptide. An asterisk over the bar indicates statistical difference ($P > 0.05$) between mean absorbance values with infected sera and negative sera for each specific peptide.

immunoreactivity, span two conserved hydrophobic regions. By contrast, peptides that were highly immunoreactive, specifically SAP-4 and SAP-5 (residues [16]SFDVPSKQPTIDIDL[30] and [21]SKQPIDIDLCDICT[35]) do not span any hydrophobic conserved residues in their sequences. Nevertheless, the highly immunoreactive peptide SAP-16 (residues [76]QQEVDYIIDH

VDQHN[90]) includes a conserved hydrophobic residue ([86]V), but this residue is not included within the dominant epitope ([76]QQEVDYIIDH[85]). Another interesting observation is that, while the highly reactive N-terminal (residues [21]SKQPTIDID L[30]) dominant B-cell epitope is totally located in an extended region, the C-terminal dominant B-cell epitope (residues [76]Q

PEPTIDE	SEQUENCE	INHIBITION

FhSAP-2 MNPLFVLMLAAVTFASFDVPSKQPTIDIDLCDICTNTMDVIKKMLADQTVEEHIG

Peptide	Sequence	Inhibition
SAP-1	MNPLFVLMLAAVTFA	0
SAP-2	VLMLAAVTFASFDVP	0
SAP-3	AVTFASFDVP SKQPT	30%
SAP-4	SFDVP **SKQPTIDIDL**	70%
SAP-5	**SKQPTIDIDL**CDICT	53%
SAP-6	IDIDLCDICTNTMDV	42%
SAP-7	DICTNTMDVIKKML	17%
SAP-8	NTMDVIKKML ADQTV	18%
SAP-9	IKKML ADQTVEEHIG	37%

FhSAP-2 YLVKYLCKIARSQDACIEFVQQEVDYIIDHVDQHNATEICRLIKLC

| Peptide	Sequence	Inhibition
SAP-10	ADQTVEEHIG YLVKY	31%
SAP-11	EEHIG YLVKYLCKIA	0
SAP-12	YLVKYLCKIA RSQDA	35%
SAP-13	LCKIA RSQDACIEFV	45%
SAP-14	RSQDACIEFVQQEVD	45%
SAP-15	CIEFV **QQEVDYIIDH**	50%
SAP-16	**QQEVDYIIDH** VDQHN	74%
SAP-17	YIIDH VDQHNATEIC	18%
SAP-18	VDQHNATEICRLIKLC	0

FIG. 3. Inhibition percentage obtained when peptides were individually added at 20 μg/ml to sera from rabbits at 12 weeks postinfection with *F. hepatica*. For this assay two pools of peptides were prepared. Pool 1 included the N terminus peptides (SAP-1 to SAP-9), and pool 2 included the C terminus peptides (SAP-10 to SAP-18). Microtiter plates were coated with 20 μg/ml of each pool of peptides. Serum diluted 1:200 was mixed with 20 μg/ml of a single peptide and incubated for 1 h at 37°C to favor peptide-antibody complex formation. After incubation, a serum-peptide mixture was added to the plates and incubated for 1 h at 37°C. Conjugate was then added, followed by the substrate solution as previously described (9). Percent inhibition (*I*) was calculated as follows: $I = [1 - (\text{OD at 492 nm of serum-peptide mixture}/\text{OD at 492 nm of serum-PBS-Tween mixture})] \times 100$. Peptides that inhibited the binding capacity of antibodies to antigen-coated plates by >50% were considered distinctive of dominant B-cell epitopes. The three antigenic regions identified within the FhSAP-2 protein are enclosed in a box. The amino acid residues that were considered to contain dominant B-cell epitopes are in bold type.

QEVDYIIDH[85]), which is less reactive, is partially located in an α-helical region. These observations are consistent with the concept that extended regions have a stronger influence than helical regions on the outcome of the antibody recognition (28).

In our previous studies with FhSAP-2, we demonstrated that reduction of disulfide bonds with dithiothreitol (DTT) notably reduced the reactivity of protein with serum from infected animals (7). To determine if the antigenic regions described in the present study are reduction sensitive, DTT was added to single peptides following an established protocol (7). Then, sera from *Fasciola*- or *Schistosoma*-infected animals were tested by ELISA against peptides treated with DTT and untreated peptides. Interestingly, no diminution of reactivity against sera was observed even on high DTT concentrations (data not shown). This suggests that, in addition to the lineal epitopes described in this study, other conformational epitopes are necessarily formed in the native protein. Also, since FhSAP-2 is potentially a glycoprotein, as predicted from the deduced amino acid sequence (7), the carbohydrate portion of the molecule is likely to contain antigenic determinants that

cannot be identified using this methodology. In our previously published study, we demonstrated that the lytic activity of FhSAP-2 is not a property ascribed to the conserved cysteine residues (7). Here, we found that neither of the hydrophobic conserved regions is immunoreactive. This experimental evidence supports the hypothesis that either the immunoreactivity with sera or the lytic activity of FhSAP-2 could be functions associated with the nonconserved residues present in the α-helix bundles themselves or, alternatively, with the unrelated regions connecting each helix rather than associated with the conserved regions.

Computer algorithms (10) applied to the primary structure of FhSAP-2 have predicted the existence of at least five potential major histocompatibility complex class II binding regions with their corresponding P1 anchor region contained in the α-helix regions, as well as regions that might form T-cell epitopes. Antigenic sites described in this study may interact with such helper T-cell epitopes, influencing the repertoire and specificity of antibody-producing cells. If this is the case in the antibody response to FhSAP-2, differences in the response elicited by the antigenic site may affect properties of other
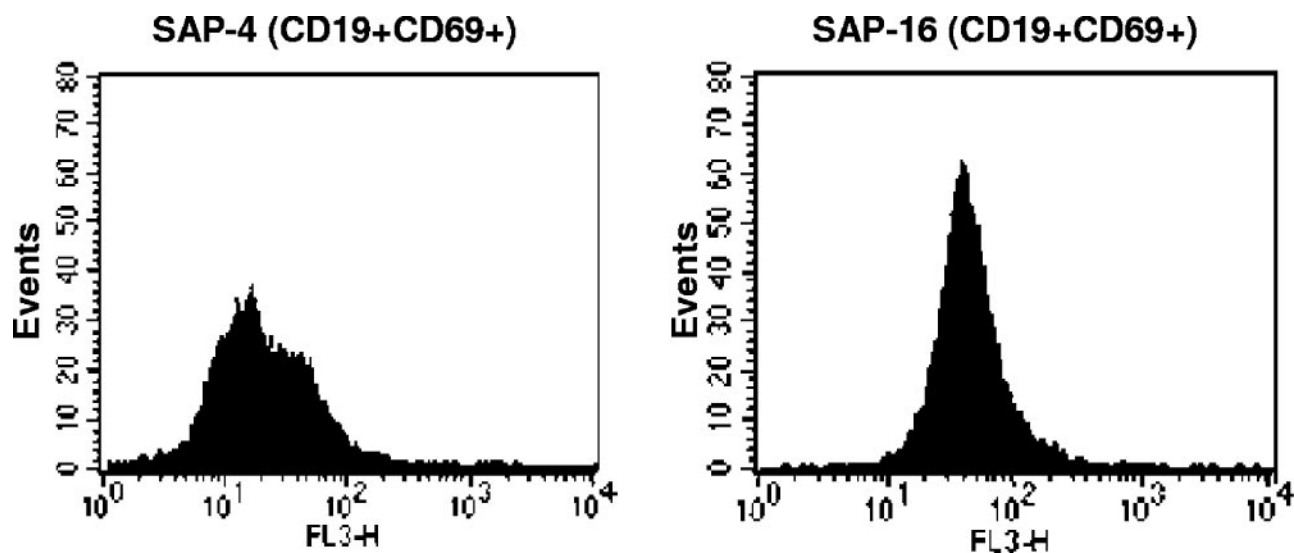
FIG. 4. Peptides SAP-4 and SAP-16 contain, respectively, the immunodominant B-cell epitopes [21]SKQPTIDIDL[30] and [76]QQEVDYIIDH[85]. They were conjugated with keyhole limpet hemocyanin, emulsified in Freund's adjuvant, and injected separately into two mice. Splenocytes were isolated from each immunized animal and cultured in triplicate at 10^6 cells/well in 200 μl of RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin and stimulated with 5 μg/ml of each peptide. Cells were incubated for 48 h at 37°C under 5% CO_2 atmosphere. After incubation, cells were centrifuged, washed with PBS, stained with anti-CD19-FITC or anti-CD69-PE-Cy7 (BD Pharmigen, San Diego, CA) monoclonal antibodies, and analyzed by fluorescence-activated cell sorting using a FACSort (Becton and Dickinson, San Jose, CA) instrument. Splenocytes were identified by their characteristic appearance on a dot plot of forward scatter versus side scatter. Splenocytes stained with FITC- or PE-Cy7-labeled antibodies were detected by FL1 or FL3, respectively. B-cells were gated on the basis of CD19 staining. The results were reported as the percentage of positive cells within this gate that appear activated.

sites, which remain to be further investigated. It must be noted that the epitope mapping techniques used in the present study are limited to the identification of linear (continuous) epitopes defined by contiguous amino acid residues in the primary structure of the protein. In addition to the linear epitopes described herein, other discontinuous epitopes, defined by the interaction between amino acid residues that are distant in the protein sequence but brought together as a result of the natural folding of the protein, may be found on FhSAP-2 in its native conformation.

Immunogenicity of dominant B-cell epitopes. To ascertain whether dominant epitopes are able to induce specific antibodies, peptide SAP-4, which contains the dominant epitope at residues [21]SKQPTIDIDL[30], and peptide SAP-16, which contains the dominant epitope at residues [76]QQEVDYIIDH[85], were coupled to keyhole limpet hemocyanin (Pierce, Rockford, Ill.) and used to immunize two BALB/c mice. The immunization schedule consisted of three subcutaneous injections at biweekly intervals of 70 μg each in phosphate-buffered saline (PBS), mixed with an equal volume of Freund's adjuvant (complete for initial

dose; incomplete for booster injections). Two weeks after the last injection, animals were anesthetized, bled, and sacrificed to remove the spleen. Analysis of the resulting antisera revealed that both peptides were highly immunogenic and elicited titers of anti-peptide antibodies higher than 1:25,000. Anti-SAP-4 and anti-SAP-16 sera recognized the free peptides bound to the wells of microtiter plates in either a dose-dependent or saturable manner. The specificity of the reaction was demonstrated by the fact that each soluble peptide was an effective competitor of binding of the anti-peptide antiserum to the same peptide immobilized in the wells of a microtiter plate and was able to abolish binding at high concentrations (data not shown).

Specific B-cell receptor identification. The spleens from immunized mice were washed and minced in PBS. After centrifugation for 10 min at $1,000 \times g$, red blood cells were removed by hypotonic lysis for 30 s, and the resulting suspensions were washed, centrifuged, and cultured for 2 days with optimum concentrations of each free peptide. Using standard protocols, stimulated cells were stained for fluorescence-activated cell sorter analysis with fluorescein isothiocyanate (FITC)-conju-

gated anti-CD19 or with phycoerythrin-Cy7 (PE-Cy7)-conjugated anti-CD69 (Pharmigen, San Diego, CA). As was expected, the vast majority of cells derived from animals immunized with peptides SAP-4 and SAP-16 stained specifically with anti-CD19, from which 93.2% and 99.41%, respectively, stained also with anti-CD69 (Fig. 4), which demonstrated that cells marked for CD19 were activated. Since CD19 is a characteristic B-cell marker expressed from the early stages of commitment to B-cell lineage and on all mature B cells, the large number of activated cells expressing this marker indicates that the sequences [21]SKQPTIDIDL[30] and [76]QQEVDYIIDH[85] from FhSAP-2 are actually B-cell epitopes.

It is interesting to recall that peptides SAP-4 and SAP-16 showed a strong reactivity with sera from mice infected with *S. mansoni*. Cross-reactivity between *F. hepatica* and *S. mansoni* antigens has been repeatedly demonstrated in serological assays (12, 16), suggesting the existence of ancestral genes conserved in the phylogeny of both species. The identification of these cross-reactive epitopes in the FhSAP-2 protein strongly suggests that the regions [21]SKQPTIDIDL[30] and [76]QQEVDYIIDH[85] of FhSAP-2 could be regions conserved between both species and could suggest the existence of a homolog of protein FhSAP-2 in *S. mansoni*. From a practical point of view, the design of diagnostic assays for *F. hepatica* based on synthetic peptides containing such sequences will have serious limitations; synthetic peptides should be used with caution, particularly in areas where fascioliasis and schistosomiasis are endemic. However, these polypeptides may be suitable for incorporation into a polyepitope-based vaccine formulation against these parasites.

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