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T F Kresina, G R Olds

Research Article

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Antiidiotypic Antibody Vaccine in Murine *Schistosomiasis mansoni* Comprising the Internal Image of Antigen

Thomas F. Kresina and G. Richard Olds
Department of Medicine, The Miriam Hospital and Brown University School of Medicine, Providence, Rhode Island 02906

Abstract

This study presents the characterization of an experimental immunotherapeutic approach for schistosomiasis utilizing antiidiotypic antibodies. Antiidiotype (31-3B6) was generated in rabbits using a protective murine monoclonal antibody 31-3B6 which recognizes a 68,000-D molecular mass glycoprotein present in extracts of *Schistosomiasis mansoni* adult worm homogenetics. Immunization of mice with antiidiotype (31-3B6) before *S. mansoni* cercariae infection resulted in protection levels ranging from 16 to 41% depending on the route of administration of antiidiotypic antibody and the use of adjuvant. Levels of protection as high as 25% could be obtained with a single injection of antiidiotype (31-3B6) without the use of adjuvant. Animals noted to be resistant to infection with *S. mansoni* cercariae were also noted to exhibit a humoral immune response that bound components of *S. mansoni* adult worm homogenetics. This induced antiantigen immune response was shown to bind to the surface of *S. mansoni* schistosoma by indirect immunofluorescence. Further characterization of the induced antiantigen response showed that a portion (3–32%) of the induced humoral immune response portrayed the binding specificities of the murine monoclonal antibody 31-3B6. The data indicate that antiidiotype antibodies generated utilizing defined monoclonal antibodies can act as surrogate antigens in the protection of infection in schistosomiasis.

Introduction

The immune response to a given antigen can be regulated through a series of complementary interactions involving idiotypic and antiidiotypic determinants (1). Numerous studies have shown that idiotype-antiidiotype interactions can result in either suppression or stimulation of an immune response (2–4). When antigen is presented to the immune system, immunoglobulin reactive to antigen is produced (Ab_1). These antibody molecules have unique structural components, termed idiotypes, which can stimulate a second cascade of antibodies (Ab_2) directed at these unique structures. These anti-antiantigen molecules are termed antiidiotypic antibodies. The regulation of immune responses by these molecules is based on the complementary structural relationship between idiotype and antiidiotype, particularly when the relevant idiotype is in or near the antigen binding site. Specifically, antiidiotypic antibodies that are antigen binding site-related often portray the internal image of antigen (5–7). These internal image antiidiotypic antibodies stimulate the immune system in a similar fashion to antigen by mimicking its antigenic structure. In this capacity, antiidiotypic antibody, which portrays the internal image of antigen, can induce an immune response (Ab_3) that is anti-antigen. In effect, antiidiotypic antibodies (Ab_2) stimulate an Ab_3 response that is serologically and functionally identical to the Ab_1 response induced by antigen.

Application of these observations has resulted in antiidiotype antibody vaccine(s) against a variety of infectious pathogens and tumors in several species of experimental animals including primates (8–13). The initial report of an effective antiidiotype vaccine involved the induction of protective immunity against the protozoa, *Trypansoma rhodesiense* infection (14, 15). With other infectious diseases including *Streptococcus pneumonia* (16), *Escherichia coli* K13 infections (17), hepatitis B virus (18), Sendai virus (19), reovirus (20), and polio virus type III (21) the principle of idiotypic vaccination has been applied. In these studies varying levels of protective immunity were induced. In the present investigation an antiidiotypic antibody vaccine was developed against the multicellular helminth, *Schistosoma mansoni* and shown to induce protection against a primary challenge by infective cercariae using various vaccination protocols. Protection was noted even when the antiidiotypic antibody vaccine was given without adjuvant or after a single administration. When directly compared to purified antigen, equal or lower concentrations of antiidiotype induced equivalent in vivo protection. Analysis of the antischistosomal immune response, induced by the antiidiotype vaccine, indicated that vaccination resulted in an antibody response directed toward a defined antigen as well as the surface of schistosoma (the invasive larval form of the parasite). Taken together, these data indicate that antiidiotype vaccines in schistosomiasis induce effective protection to reinfection by acting as structural surrogates of protective antigens.

Methods

31-3B6 is an IgM monoclonal antibody produced from the fusion of splenocytes of a Balb/C mouse hyperimmunized with adult schistosome antigens (SWAP) with the Balb/C myeloma line P_3NS1 (22). Supernatants from the resultant hybrid clones were screened for reactivity with SWAP by ELISA and binding to living schistosomula by indirect immunofluorescence and shown to be a candidate vaccine (22). Large quantities of monoclonal antibodies were produced in Balb/C mice as ascites fluid.

For the present studies, 31-3B6 antibody was purified from ascites fluid by ammonium sulfate precipitation and molecular sieve chromatography using a CL6B column (Pharmacia Fine Chemicals, Piscataway, NJ). The IgM fraction was recovered and dialyzed extensively versus PBS, pH 7.6. Assessment of purity was performed by SDS electrophoresis and silver staining.

Xenogeneic antiidiotypic antisera was generated in the following manner. 5 mg of chromatographically purified 31-3B6 antibody was emulsified in complete Freund's adjuvant and injected subcutaneously into numerous spots on the back, axilla, and groin of three 3–5-kg New Zealand white rabbits.

Immunization was repeated 2 wk later with purified 31-3B6 antibody and incomplete Freund's adjuvant. All rabbits were then boosted with 31-3B6 antibody (5 mg/rabbit) subcutaneously every other week for a total of eight doses. 1 wk after the last immunization, animals were bled via an ear artery and serum was collected. Balb/C immunoglobulin was prepared from ascites fluid of pristane-treated mice by a 33% ammonium sulfate precipitation. All three rabbit sera produced a dense precipitation band with purified 31-3B6 as well as purified Balb/C immunoglobulin by Ouchterlony analysis.

Purified Balb/C immunoglobulin was covalently coupled to Sepharose. Mouse antiisotypic and antiallotypic antibodies present in these rabbit sera were then absorbed by chromatography. Reactivity for 31-3B6 antibody versus normal mouse IgM was determined by Ouchterlony analysis and subsequently by an assay for idiotype reactivity (23, 24) as described below. Only one rabbit produced an absorbed anti-sera that precipitated on Ouchterlony analysis as a dense band with 31-3B6 antibody but not with Balb/C immunoglobulin. This rabbit was repeatedly boosted and bled and various serum pools created. One pool of 200 cm³ of serum was absorbed, tested, and used for the experiments outlined.

Quantification of anti-id activity. Rabbit antiidiotype activity was quantified by an indirect method of precipitation as described previously (23, 24). In brief, purified 31-3B6 was labeled with ^{125}I using chloramine T (25). 10 ng of this labeled ligand was then incubated with serial dilutions (1:10 to 1:20,480) of absorbed rabbit antisera. This reaction mixture also contains 10 μg of normal Balb/C immunoglobulin to absorb any residual antibody activity directed toward isotypic or allotypic determinants. The mixture was incubated for 1 h at 37°C and immune complexes precipitation facilitated by the formation of a carrier precipitate comprised of absorbed goat antirabbit ovalbumin and goat antirabbit IgG (23, 24). Data are presented as ^{125}I-precipitable counts per minute binding 31-3B6 above background counts per minute, which ranged from 1 to 3% of total counts per minute.

Characterization of antiidiotype 31-3B6. A solid-phase radioimmune assay (23, 24) and ELISA were used to determine the characteristic binding patterns of rabbit anti-idiotype 31-3B6. ELISA was performed as described previously (22) utilizing absorbed rabbit antiidiotype 31-3B6 as the well coat. The solid-phase radioimmune assay used purified 31-3B6 antibody (kindly provided by Dr. Charles King, Case Western Reserve University, Cleveland, OH) as the well coat. Inhibition of binding of the (31-3B6-antiidiotype) binding was determined for the following reagents: sera from mice administered antiidiotype 31-3B6 and shown to be protected from infection; purified 31-3B6 antigen (68 kD glycoprotein kindly provided by Dr. Charles King) (22); and pooled sera from Fisher rats exposed to two infections with *S. mansoni* cercariae and portraying a 50–60% level of protection rom reinfection (kindly provided by Dr. Paul Knopf, Brown University, Providence, RI). In this inhibition assays, binding levels of rabbit antiidiotype 31-3B6 were determined using ^{125}I specifically purified goat antirabbit IgG (Cappel Laboratories, Cochranville, PA).

Isolation of the 68-kD antigen recognized by 31-3B6. A purified 68-kD glycoprotein antigen was purified by immunoaffinity column chromatography using a 31-3B6-coupled Sepharose column. The eluted material was suspended in PBS. Protein concentration was determined by the method of Lowry et al. (26). Optimal concentrations were used for protection comparative studies as previously determined (22).

Immunization protocols and infection. CF1 outbred mice (Charles River Breeding Laboratories, Wilmington, MA) were immunized with antiidiotypic antibody with or without complete Freund's adjuvant (CFA; Gibco Laboratories, Grand Island, NY). Unless otherwise indicated, 500 μg of protein in 200 μl was used. Adjuvant injections comprised a mixed 1:1 (vol/vol) tight emulsion. Nonadjuvant containing

preparations were suspended in physiologic saline. Animals were injected with antiidiotypic antibody and CFA either intraperitoneally or subcutaneously, while nonadjuvant injections were made intradermally, subcutaneously, or intravenously via the tail vein.

10 d after the last immunization, animals were bled retroorbitally and individual serum samples collected. 2–4 wk after the last immunization, animals were challenged transdermally with cercariae of a Puerto Rican strain of *S. mansoni* (27).

Resistance to infection was determined by either recovery of migrating larvae from the lungs (28) or recovery of adult worms from the mesenteric vasculature of infected mice (27). In the former assay, mice were anesthesized and infected with 500 cercariae. Animals were killed 7 d later, their lungs were removed, and the number of migrating larvae were enumerated microscopically (28). Adult worm recoveries were performed in animals infected with 100 cercariae 6 wk postinfection (27). Anesthesized mice have their portal systems perfused and the number of adult worms/mouse enumerated.

To determine percent protection the following formula was used: Worms recovered from control mice − Worms recovered from × 100 experimental mice/Worms recovered from control mice. Statistical analysis of data was performed using the Student's t test.

Antibody response of vaccinated animals. Serum from control animals or mice vaccinated with antiidiotypic antibody was examined for its ability to bind soluble worm antigens (SWAP) by ELISA as previously described (29). In brief, SWAP is produced from adult *S. mansoni* worms by emulsification with a tissue grinder and ultracentrifugation (30). SWAP is diluted in PBS, pH 7.2, to a final concentration of 100 ng/ml and 100 μl/well added to a 96-well flexible microtiter plate (Microtest III; Falcon Labware, Oxnard, CA). After overnight incubation, BSA (Sigma Chemical Co., St. Louis, MO) is used to block unused protein binding sites on the plate. Serum to be tested is diluted from 1/10 to 1/100,000 along with a normal mouse serum control. After incubation, the plate is vigorously washed and the binding of mouse immunoglobulin detected by a rabbit anti-mouse-alkaline phosphatase conjugate (New England Nuclear, Boston, MA). Binding of this second reagent is detected by adding 1 mg/ml p-nitrophenyl phosphate to the washed plate and determining absorbance over time at 405 nm.

Binding to living schistosomula was determined by indirect immunofluorescence. Schistosomula were prepared mechanically and used 4 h later (31). 50 schistosomula were incubated with 1/50 and 1/100 dilutions of mouse sera for 1 h at 37°C and then 1 h at 4°C. Schistosomula were then washed four times with Hanks' balanced salt solution (K.C. Biologicals, Kankakee, IL) and antibody binding detected using a fluorescence (FITC) conjugated goat anti-mouse Ig (Cooper Biomedical, Malvern, PA) according to the method of Zodda and Phillips (32).

Competitive inhibition of binding of the antischistosomal immune response. Sera of vaccinated mice noted to contain both anti-SWAP antibody (by ELISA) and antischistosomula antibody (by fluorescence) were then analyzed further by two other methods to determine the specificity of the induced anti-worm antigen antibody (Ab₃) response. Initially, murine serum samples (Ab₃ containing serum) and control sera were analyzed for the presence of IgG antibodies which bound antigen (SWAP:) but could be inhibited from binding to antigen by monoclonal antibody 31-3B6. Therefore, this RIA determines the percentages of the Ab₃ response that is sterically hindered from binding antigen (i.e., antibodies which bind to epitope(s) on SWAP which are also recognized by 31-3B6). In this assay, SWAP was solubilized and diluted and used to coat 96-well microtiter plates as described previously (29). After incubation, BSA was utilized to block the remaining reactive sites of the microtiter wells. 31-3B6 monoclonal IgM antibody was incubated overnight in the wells. After incubation, the wells were washed thoroughly and a nonsaturating dilution of murine sera, containing IgG (Ab₃ molecules), was incubated in the wells at room temperature for 1 h and then washed. Binding of IgG Ab₃ molecules was detected by an absorbed isotype-specific rabbit anti-mouse IgG

(Cooper Biomedical). Data are presented as percent inhibition of binding by 31-3B6 of Ab₃ antibody binding to antigen.

A second assay performed to determine the specificity of induced Ab₃ antibody in vaccinated mice utilized the purified 68-kD antigen as a competitive inhibitor. The 68-kD antigen, recognized by monoclonal antibody 31-3B6 was used to inhibit the binding of induced Ab₃ to antigen (SWAP). Therefore, this RIA would substantiate the initial observation that Ab₃ antibodies induced by vaccination with antiidiotypic antibody (Ab₃) could recognize a specific SWAP antigen related to the molecule used to induce the Ab₃ population. In these studies increasing amounts of 68 kD antigen (10–5,000 ng) were preincubated with murine antisera at 37°C for 1 h before incubation in SWAP-coated polyvinyl microtiter plates. Binding was detected using a ^{125}I-goat anti-mouse IgG. Data is presented as percent inhibition of binding to antigen by nanogram quantities of 68 kD antigen.

Results

Antiidiotypic binding of rabbit serum. Serum from outbred rabbits hyperimmunized with monoclonal antibody 31-3B6 was absorbed by passage through normal mouse immunoglobulin coupled-Sepharose columns. Absorbed sera that was negative for binding to normal mouse immunoglobulin but which precipitated 31-3B6 antibody was then analyzed quantitatively for binding to radiolabeled ligand (31-3B6) in an assay for idiotype. As shown in Fig. 1 antiidiotype antibody containing absorbed rabbit sera bound > 80% of radiolabeled 31-3B6 monoclonal antibody in the presence of a two-log excess of normal mouse immunoglobulin. These data indicate that the absorbed rabbit sera recognized epitopes specific to variable regions found on monoclonal antibody 31-3B6. Background binding levels in this assay, using either BSA or an irrelevant monoclonal antibody, were < 5% of the labeled ligand. Analysis of the binding curve also demonstrated that 500 μg of ab-
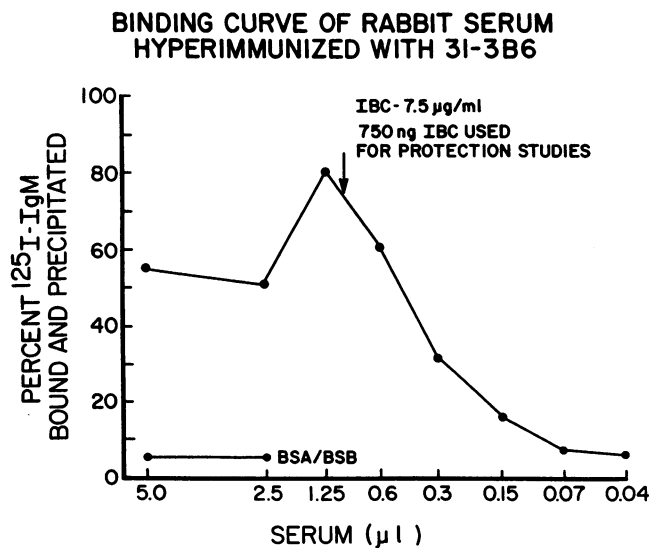
sorbed rabbit serum contained 750 ng idiotype binding capacity (IBC) of activity.

Antiidiotypic antibody as a vaccine in S. mansoni. In initial studies, anti-idiotypic antibody was used to hyperimmunize CF1 outbred mice. As shown in Table I, mice hyperimmunized with rabbit anti-id (31-3B6) showed a 41% reduction in migrating larvae recovered from the lung when compared to mice hyperimmunized with rabbit serum ($P < 0.001$). Lung recoveries of migrating larvae was used as an initial screen since this assay has been shown to have a good correlation with adult worm recovery and can be performed in far shorter time intervals. The results of these initial studies were confirmed by measuring adult worms recovered from the mesenteric vasculature 6 wk postinfection in additional studies utilizing identical treatment protocols. As shown in Table I, a level of 43% protection was noted.

Protection from S. mansoni infection varies with immunization protocol. The previous observation noted that mice hyperimmunized with antiidiotype (31-3B6) were resistant to primary infection with S. mansoni cercariae. Subsequent studies used varying immunization protocols, with and without adjuvant, with resistance to infection assessed by lung recoveries. Table II summarizes this series of experiments. Immunization with antiidiotypic (31-3B6) induced protection from a challenge with S. mansoni cercariae in all experiments tested. Most notably, antiidiotypic (31-3B6) was effective when administered without adjuvant, as a single intravenous, intraperitoneal, or subcutaneous immunization, or when as little as 75 ng IBC was used. The induction of resistance was confirmed for critical protocols by adult worm recoveries as before. These data indicate that vaccination protocols which can induce resistance to S. mansoni infection need not require the use of adjuvant or multiple immunizations.

BINDING CURVE OF RABBIT SERUM HYPERIMMUNIZED WITH 31-3B6

Figure 1. Binding curve of rabbit serum derived from animals hyperimmunized with 100 μg of soluble egg antigen preparation emulsified in complete Freund's adjuvant. Data reflect binding of serial dilutions of rabbit serum determined in the assay for idiotype described in Methods. Idiotype binding capacity is determined by the pentultimate value of the linear portion of the binding curve as described previously (23, 24).

*Table I. Resistance to Infection with S. mansoni Cercariae of Mice Hyperimmunized with Antiidiotypic (31-3B6) or Normal Rabbit Serum (NRS)**

Animal no.	Larvae		Adult worms	
	NRS	Anti-id (31-3B6)	NRS	Anti-id (31-3B6)
1	191	99	32	42
2	221	104	46	14
3	182	107	42	18
4	198	112	34	26
5	161	74	46	34
6	170	133	40	28
7	188	132	46	16
8	169	87	44	26
9	192	135	50	14
	186±6[‡]	109±7	42±2	24±3
	41% protection $P < 0.001$		43% protection $P < 0.001$	

* Groups of nine mice were given four intraperitoneal injections of antiidiotype (31-3B6) emulsified in CFA or NRS every 2 wk. 4 wk later two groups of mice were challenged with 500 S. mansoni cercariae and then migrating larvae extracted from their lungs 7 d later. Two other groups were challenged with 100 S. mansoni cercariae and adult worms found in their mesenteric vasculature enumerated 6 wk postinfection.
‡ Data represent mean±SEM.

Table II. Summary of Vaccine Protocols and Resultant Resistance to Infection Induced by Anti-ID (31-3B6)*

Immunization protocol	Use of adjuvant	Dosage (IBC)[‡]	Number of injections	Protection
I.P.	Yes	750 ng	4	41%[§]
SubQ and I.P.	Yes	750 ng	2	31%
SubQ	No	750 ng	2	27%[§]
SubQ	No	750 ng	1	25%[§]
I.P.	No	750 ng	1	24%
I.V.	No	750 ng	1	28%
SubQ	No	75 ng	1	16%[§]

* Groups of 8–10 mice were immunized with either antiidiotype 31-3B6 or normal rabbit serum by various protocols. All animals were then challenged 2–4 wk after the last immunization with 500 *S. mansoni* cercariae and killed 7 d later. Percent protection was determined by counting the average number of migrating larvae found in the lungs of experimental versus control groups of mice. In all cases, protection was significant at $P < 0.05$.

‡ IBC, idiotype binding capacity, a relative measure of 1 ml of serum to binding 31-3B6 monoclonal antibody (idiotype).

§ Protection confirmed by adult worm recoveries.

Mechanism of antiidiotypic induced resistance. Animals immunized with antiidiotypic 31-3B6 vaccine were bled ten days after their final immunization but prior to challenge with *S. mansoni* cercariae. Sera were then assayed for antibody reactivity with soluble worm antigens (SWAP), a neutral salt soluble extract of *S. mansoni* adult worms which contains the 68-kD antigen bound by the monoclonal antibody 31-3B6. As shown in Table III, mice hyperimmunized with antiidiotype (31-3B6) produced an antibody population that bound SWAP (Ab₃ or anti-anti-anti-antigen).

Table III. Anti-worm Antibody Activity of Mice Hyperimmunized with Antiidiotype (31-3B6) or Normal Rabbit Serum*

Animal no.	NRS		Anti-id (31-3B6)	
	O.D.	End-titer	O.D.	End-titer
1	0.02±0.01	0	0.32±0.04	1/6,400
2	0.01±0.01	0	0.84±0.07	1/100,000
3	0.02±0.01	−1/25	0.71±0.09	1/75,000
4	0.02±0.01	0	0.52±0.02	1/25,000
5	0.03±0.01	0	0.41±0.01	1/6,400
6	0.01±0.01	0	0.96±0.06	1/100,000
7	0.04±0.01	1/25	1.12±0.10	1/400,000
8	0.07±0.01	1/100	0.19±0.01	1/6,400
9	0.03±0.01	1/100	0.63±0.03	1/25,000
10	0.02±0.01	1/10	0.20±0.02	1/10,000

* Animals were individually bled 10 d after their last immunization (as outlined in Table I) but before challenge with cercariae. Serum was prepared and tested for antisoluble worm antigen antibody activity by solid-phase ELISA. Data is expressed as the mean optical density of triplicate wells at a 1/1,600 dilution or as an end-titer defined as the last dilution statistically higher than the same dilution of control serum ($P < 0.05$). In the case of the single negative titer, control serum was significantly higher than the NRS immunized animals both at a 1/25 dilution.

Further studies were performed to confirm that these antiidiotype induced Ab₃ antibodies portrayed similar binding characteristics as 31-3B6. As shown in Table III, serum from antiidiotype immunized mice contained Ab₃ molecules that bound to SWAP by ELISA and as shown in Fig. 2, also bound to schistosomula by indirect immunofluorescence. Both of these characteristics are noted as well for 31-3B6 monoclonal antibody.

Molecular mimicry of antigen by anti-idiotype 31-3B6. Further studies were performed to detail the specificity of these Ab₃ antibody molecules induced by vaccination with antiidiotype 31-3B6. Competitive inhibition studies were performed using both monoclonal antibody 31-3B6 and affinity purified 31-3B6 antigen, a 68,000-D glycoprotein constituent of SWAP, as inhibitors. Initially, monoclonal antibody 31-3B6 (IgM isotype) was used to inhibit the binding of IgG Ab₃ to SWAP. As shown in Table IV, inhibition of binding was noted in all of the samples tested and ranged from 3 to 32% of total Ab₃ binding. The mean±SE inhibition of binding of Ab₃ to antigen by monoclonal antibody 31-3B6 was 13%±6. Such an assay would not detect IgM Ab₃ molecules but this data demonstrates that a portion of the induced Ab₃ antibody population and Ab₁ (31-3B6) portray similar antigenic binding. Further studies using affinity purified 31-3B6 antigen revealed similar findings. Inhibition of binding of Ab₃ to SWAP by 31-3B6 antigen (5,000 ng) ranged from 4 to 20% with a mean±SE value of 13%±3. These data indicate that a proportion of anti-schistosomal antibodies induced by immunization with antiidiotype 31-3B6 bind identical antigens as monoclonal antibody 31-3B6. These data strongly suggest that antiidiotype 31-3B6 can structurally mimic *S. mansoni* antigens. It also points out that such mimicry is imperfect in that many Ab₃ anti-worm antibodies produced by this immunization are not specifically inhibited by either the purified antigen or the monoclonal antibody 31-3B6.

Characterization of antiidiotype 31-3B6. Antiidiotypic antibodies can be functionally classified into three discrete subpopulations: Ab₂α, Ab₂β, and Ab₂ε (33–35). Ab₂α and Ab₂β, originally classified by Jerne et al. (34), describe the population of antiidiotypic antibodies that recognize determinants present in the variable antibody region and those that represent internal images of antigen, respectively. The Ab₂β antiidiotypic antibodies, therefore, have been postulated by numerous investigators to be valuable as reagents for vaccine development. The Ab₂ε subpopulation is a defined (35) group of antiidiotypic antibodies that react with antigen and idiotype of an antibody directed toward the same antigen.

With regard to potential vaccine development of antiidiotypic antibodies, studies were performed to detail the presence of Ab₂β antiidiotypic antibodies in the polyclonal xenogeneic rabbit antiidiotype used in the present studies. Initial studies, detailing the presence of Ab₂β antibodies or the internal image of antigen, utilized a competitive solid-phase RIA. In the studies, the ability of rabbit polyclonal antiidiotype antibody to compete with purified antigen to bind 31-3B6 (Ab₁) antibody was determined. It was determined that only a minor proportion of rabbit antiidiotype could mimic purified antigen in binding to Ab₁. As shown in Table V, 5 μg of purified antigen could only inhibit at the 5% level the binding of rabbit antiidiotype to Ab₁ (31-3B6 antibody). These data, therefore, indicate that only a minor subpopulation of rabbit antiidiotype could function as Ab₂β molecules.
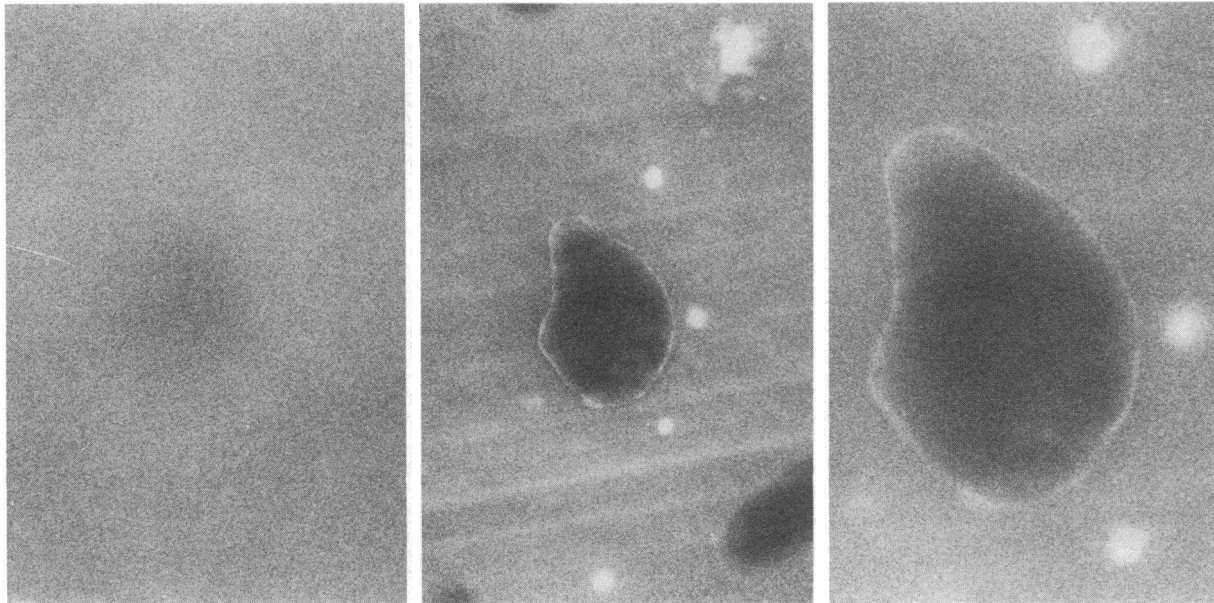
Figure 2. Immunofluorescence of 4-h, mechanically generated schistosomula. (*Left*) Schistosomula incubated with mouse serum from animals hyperimmunized with rabbit serum at 37°C for 30 min followed by staining with FITC-goat anti-mouse IgG (×200). (*Center*) Schistosomula incubated with mouse serum of animals hyperimmunized with antiidiotype 31-3B6 and noted to be protected from reinfection with *S. mansoni* cercariae. These mice were noted to have anti-SWAP activity (×200). (*Right*) ×400 of schistosomula from center panel. Note the continuous membranous staining.

Further studies identifying the binding characteristics of polyclonal antiidiotype 31-3B6 are presented in Table VI. These data show that all animals protected from *S. mansoni* infection by antiidiotypic administration portray binding activity to absorbed polyclonal rabbit antiidiotype as determined

Table IV. Competitive Inhibition of Binding of Ab₃ Antibody to Antigen (SWAP) by Murine Monoclonal Antibody 31-3B6

Animal no.	Percent inhibition
1	19±26
2	3±4
3	16±3
4	9±1
5	6±2
6	21±18
7	4±5
8	15±3
9	32±5
Mean±SE	13±6

Percent inhibition of binding of Ab_3 molecules contained in serum from antiidiotype immunized mice to SWAP by 31-3B6-monoclonal antibodies. The limiting dilution of Ab_3 combining serum which bound to SWAP was determined and used throughout the assay. Various concentrations of 31-3B6 (10–5,000 ng) were then used to inhibit the binding. Binding of IgG Ab_3 molecules to SWAP were detected by an IgG heavy chain specific goat antisera covalently linked to alkaline phosphatase. 31-3B6 is not bound in this assay since it is a murine IgM. Data is expressed as mean percent inhibition of triplicate wells, background inhibition observed in the same dilution of control mouse serum.

by ELISA. As noted earlier, however, this induced Ab_1 population is apparently serologically heterogeneous. In inhibition studies, utilizing Ab_1 antibodies as blocking reagents for the 31-3B6-antiidiotype interaction, a range of 5 to 12% inhibition of binding was noted by the various individual serum samples. Therefore, these studies confirm the previous findings that immunization with polyclonal antiidiotype 31-3B6 results in the establishment of an expanded antibody repertoire.

Finally, experiments were performed that would indicate the nature of the expression of the internal image of antigen of $Ab_2\beta$ molecules. In this regard, the expression of the 31-3B6 (id-anti-id immune network) was investigated in the rat model of *S. mansoni* infection (36, 37). In this animal model of schistosomiasis, the Fisher rat strain developed resistance to reinfection following exposure to low numbers of cercariae (comparable to the level of infection in the present study).

*Table V. Inhibition of Binding of Rabbit Anti-idiotype Antibody to 31-3B6 (Ab₁) Antibody by Purified Antigen**

Inhibitor‡	% Inhibition of binding
ng	Σ±SD
5,000	5±0.2
1,000	2±0.1
100	0±0.1

* Inhibition of binding of antiidiotypic antibody by purified antigen as measured by a solid-phase radioimmune assay. Varying quantities of inhibitor were added with a limiting quantity (1:5 dilution) of absorbed rabbit antiidiotype and incubated for 2 h in the presence of monoclonal antibody 31-3B6.
‡ Nanograms of affinity purified 31-3B6 antigen (68 kD glycoprotein).

Table VI. Generation of Ab3 Antibody Molecules Derived
from Mice Resistant to Infection with S. mansoni Cercariae
on Immunization with Rabbit Antiidiotypic Antibody*

Sample[‡]	Optical density No. of immunizations[§]			
	1	2	3	4
1	0.14	0.17	0.13	0.17
2	0.30	0.47	0.42	0.59
3	0.29	0.38	ND	0.42
4	0.19	0.34	0.08	0.23
5	0.15	0.16	0.23	ND
6	0.24	0.32	0.33	0.37
7	0.13	0.38	0.43	0.37
8	0.14	0.0	0.17	0.0
9	0.17	0.0	0.21	0.16

* Binding of Ab1 molecules to absorbed rabbit polyclonal antiidiotypic antibody as measured by ELISA. No O.D. readings were obtained for serum samples derived from mice administered NRS and not protected from infection (see Table I).
‡ Sample, Animal number of mice administered polyclonal antiidiotypic antibody (see Table I).
§ Serum bleed 5 d after injection of antiidiotypic antibodies.

Therefore, serum from Fisher rats twice infected with *S. mansoni* cercariae and portraying a 50–60% level of protection to reinfection, were utilized as inhibitors of 31-3B6-anti-idiotype binding. As shown in Table VII, a maximum level of inhibition (86%) of rabbit antiidiotype binding to 31-3B6 was noted with neat rat serum. This inhibition of binding by protective rat serum was titerable and evident even in the 1:1,000 dilution of serum tested. These data strongly indicate that rabbit polyclonal antiidiotype 31-3B6 contains $Ab_2\beta$ based on the expression of 31-3B6 idiotypic repertoire in both rat and mouse models of *S. mansoni* infection.

Comparative protection induced by purified 31-3B6 antigen versus anti-id (31-3B6) immunization. Previous studies (22) have shown that a defined 68,000-D glycoprotein parasite antigen identified by 31-3B6 induces anti-schistosomal protection in naive mice without the use of adjuvants. In the following experiments, antiidiotypic antibody (31-3B6) was directly

Table VII. Inhibition of Idiotype-Antiidiotype Binding by Fisher
Rat Serum from Infected Mice*

Serum sample	Percent inhibition
	Mean±SD
Neat	86±0.06
1:10	75±0.07
1:100	66±0.03
1:1000	62±0.05

* Inhibition by binding of Fisher rat serum from twice infected rats of idiotype (31-3B6)-antiidiotype (31-3B6) as measured in a solid-phase RIA. Inhibition levels are based on immune complex formation in the presence of normal Fisher rat serum, which averaged 7,437±1,683 cpm performed in triplicate.

compared to this purified 68-kD antigen with regard to enhanced resistance to primary infection in vivo. As shown in Table VIII, subcutaneous immunizations with 5 μg of the 68-kD antigen (previously [22] determined to be the optimal immunization protocol) induced 26% protection in vivo. Mice administered antiidiotypic antibody (two subcutaneous injections with either 750 or 75 ng IBC) developed 26 and 17% protection, respectively ($P < 0.01$). This degree of protection was indistinguishable from that observed using purified antigen. Comparison of IBC values and purified antigen show that antiidiotype (31-3B6) can induce similar anti-schistosomal protective effects at significantly lower concentrations.

Discussion

In *S. mansoni*, experimental animals develop partial resistance to reinfection after a primary infection. Recent studies have suggested that an idiotype-antiidiotype immune network may be an important, naturally occurring immunoregulatory mechanism in this form of acquired resistance (38). Several investigators have tried to mimic this form of acquired resistance through the use of irradiated cercariae vaccines or artificial immunization (39–43). Purified worm antigens also have been used to stimulate resistance to primary infection in experimental animals (22, 42–44). These purified antigens have, in general, been identified by protective monoclonal antibodies that transfer passive resistance in vivo (22). One such monoclonal antibody was used by Grzych et al. (45) to produce a polyclonal antiidiotypic antibody that was able to induce a high level of resistance in rats in vivo. Rats, however, may not be the best model for human infection since they undergo self cure after infection and develop high levels of resistance following numerous immunologic manipulations (46). Mice, like humans, are quite susceptible to *S. mansoni* infection, sustain chronic infections, and provide a more challenging model to protect from reinfection through in vivo immunization (46). Natural infection of outbred mice, for example, results, at best, in 40% resistance to reinfection while rats develop solid immunity (46, 47).

Monoclonal antibody 31-3B6 is an IgM that induces passive protection (25–27%) from a challenge infection with *S. mansoni* cercariae (22). 31-3B6 binds to a 68-kD glycoprotein contained in soluble adult worm homogenetics (SWAP) and also binds to the surface of 4-h old schistosomula as determined by indirect immunofluorescence (22). This 68-kD antigen has been found by electronmicroscopic studies to be located on the head glands of the schistosomula (unpublished observation). This monoclonal antibody (31-3B6) has also been used to purify the epitope containing 68 kD antigen molecule by immunoabsorbant column chromatography. This purified material induces resistance (24–38%) in mice after subcutaneous administration without adjuvant. It is not known whether the carbohydrate or protein epitopes on this molecule stimulate the protective response in vivo. To date, attempts to isolate protein epitopes of this molecule from a cDNA expression library have not been successful using either 31-3B6 or a polyclonal antiserum raised in rabbits immunized with the purified 68 kD molecule (personal communication).

Antiidiotype vaccines offer some advantages over antigen based or recombinant subunit vaccines. Antiidiotype vaccines can be effective even when the critical epitopes required for induction of resistance are carbohydrate or lipid (16, 48, 49).

Table VIII. Comparative Protection from S. mansoni Infection Induced by Purified Antigen vs. Antiidiotype (31-3B6)*

Animal No.	Dose	NRS injected	Anti-id injected		Antigen injected
			750 µg IBC	75 µg IBC	5 µg IBC
	500 µg protein	*50 µg protein*	*500 µg protein*	*50 µg protein*	
1	133	124	128	133	91
2	121	161	73	82	95
3	146	142	76	133	129
4	142	140	100	106	125
5	136	129	120	108	70
6	130	143		69	105
7		146		117	121
8		156		159	
9		133		138	
10		136		134	
11				122	
12				124	
Mean±SE	134±8	148±11	99±24	118±24	105±21
% Protection	—	—	26	17	26

* Groups of mice were immunized without adjuvant subcutaneously with either the purified 68 kD glycoprotein (identified by 31-3B6) or with antiidiotype 31-3B6 (derived from 31-3B6 immunized rabbits). Immunization was repeated 2 wk later and then all animals challenged with 500 S. mansoni cercariae. Data represents migrating larvae extracted from the lungs of individual mice. Percent protection was determined by comparing antiidiotype or antigen-treated mice with NRS controls.

Antiidiotype vaccines can be mass produced at low cost, which is a major advantage over the use of antigen-based vaccines that must be extracted from an S. mansoni life cycle. In schistosomiasis, significant evidence exists that immune responses to worm antigens are strongly regulated by immunogenetic factors (50–52). In human schistosomiasis japonica, for example, low immune responses to worm antigens (SWAP) and development of severe clinical disease appears related to certain HLA-D-phenotypes (53). Extrapolation of these observations in S. mansoni would suggest the possibility that antigen-based vaccines, even those produced through recombinant technology may not induce a protective response in the subset or individuals who most need enhanced resistance to reinfection. To this point, antiidiotype vaccines are advantageous since antiidiotype manipulation is capable of overcoming such immunogenetic barriers (8, 54, 55). Finally, antiidiotype vaccines are quite potent and provide a reasonable alternative to other vaccine strategies. In the Sendai virus model, for example, antiidiotype manipulation is even more effective than a natural infection in inducing protective responses in vivo (56).

The requirements of the induction of a protective antiidiotype-induced immune response to murine reovirus infection have been reported (57). High titer of neutralizing antibody was noted using a chronic immunization protocol that utilized a regimen of complete and incomplete Freund's adjuvant or alum. An additional requirement was the cross-linking or covalent coupling of monoclonal antiidiotypic antibody to a carrier molecule (57). These stringent requirements were not necessary, however, to produce anti-viral T cell immunity in mice (26). In addition, as recently reviewed by Hiernaux (58), numerous experimental systems of infectious diseases have utilized antiidiotypic antibody without the use of adjuvant to induce protective immunity. As in the presently reported

study, these volumes of data infer that various forms of immunity to infectious agents can be generated by using antiidiotype antibodies. However, the requirements for the type of protective immunity generated and level of protection depend on the individual experimental system.

In the present study, xenogeneic antiidiotypic antibody was used to induce varying levels of protection to a challenge with S. mansoni cercariae in outbred mice. Levels of protection ranged from 16 to 41% depending on the immunization protocol. Antiidiotype, however, remained effective even when given as a single intravenous, subcutaneous, or intraperitoneal injection without adjuvant. Although this degree of resistance (16–43%) is modest compared to vaccines for other infectious diseases, it is important to note that helminths do not multiply in the definitive host and, therefore, anti-worm vaccines can have a significant impact on disease even if only partially effective (59).

This level of resistance compares favorably to that observed in mice after the passive transfer of serum from S. mansoni infected animals (46), following adjuvant therapy (60), following immunization with irradiated cercariae vaccines (39–41), or immunization with purified antigens generally administered with complete Freund's or other adjuvant (61, 62).

This study details the direct comparison of purified antigen (68 kD glycoprotein) immunization with antiidiotype induced resistance. Antiidiotype immunization was equally effective in inducing resistance in vivo and required significantly less material (5 µg of purified antigen compared to 750 ng idiotype binding capacity).

The mechanism by which xenogeneic antiidiotype immunization induced resistance in vivo was explored by characterizing the immune response of immunized mice before their

challenge with *S. mansoni* cercariae. Antiidiotype immunized mice developed significant antibody titers, Ab₃ molecules, which reacted against worm antigens before their exposure to the parasite. These antibodies (Ab₃) also bound to living schistosomula, the presumed target of immune attack. In addition, Ab₃ binding to antigen (SWAP) could be inhibited by either monoclonal 31-3B6 (Ab₁) or affinity purified antigen in a dose-dependent manner. These observations strongly suggest that the antiidiotype 31-3B6 immunization produced an Ab₃ antibody response that portrayed Ab₁ (31-3B6-like) activity. This antigen's mimicry quality of certain antiidiotypes has been suggested as the basis for other protective antiidiotype vaccines (8). Mimicry was not, however, perfect in that antiidiotype vaccination resulted in Ab₃ molecules that bound worm antigens but were not inhibited by either 31-3B6 or the 68-kD antigen. However, the present study, which does not detail the fine specificity of the Ab₃ population, does not rule out the possibility of antibody affinity differences and cross-reactivity among the various antibody substrates.

Nevertheless, the present observations raise the possibility that antiidiotype immunization may stimulate the production of a broader immune response than purified antigen itself. The potential role of these Ab₃ immunoglobulins, which may not strictly involve the 68-kD-31-3B6 immune network in the induction of resistance to *Schistosoma mansoni*, is presently under investigation.

Conclusion. An antiidiotype-based vaccine is a viable alternative to purified antigen-based vaccines or recombinant subunit vaccines particularly if the protective epitopes are carbohydrate in nature. Antiidiotype vaccines are effective in experimental schistosomiasis when given without adjuvants and even after a single immunization. Antiidiotype administration induced an Ab₃ response that identifies the epitope-containing molecule which initiates the immune network and suggests that at least part of the mechanism of induced resistance by antiidiotype is through its molecular mimicry of antigen.

Acknowledgments

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