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A *Schistosoma mansoni* epitope recognized by a protective monoclonal antibody is identical to the stage-specific embryonic antigen 1

(carbohydrate epitope/autoantibodies/olfactory epithelium/mouse embryos)

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ABSTRACT **In infections with the parasitic trematode *Schistosoma mansoni*, a component of the host defense is directed against the invading larval form, and carbohydrates on the surface of these larvae are targets for the immune attack during the early stages of infection. To identify such carbohydrate epitopes, which may be suitable for immunization against schistosomiasis, we have previously generated monoclonal antibodies to surface antigens; some of these confer protection to naive mice when passively administered. Here we show that one of the protective antibodies recognizes a determinant present in both the parasite and its mammalian hosts. The immunohistochemical distribution of this determinant in the head of embryonic mice was found to be identical to the stage-specific embryonic antigen 1 (SSEA-1), an epitope abundant in preimplantation embryos, several adult tissues, and malignant tumors. Oligosaccharides containing the SSEA-1 trisaccharide Galβ1-4(Fucα1-3)GlcNAc inhibit antibody binding to parasite antigen. SSEA-1 antibodies generated from mice immunized with rodent neural antigens bind to the surface of the schistosome larvae and mediate antibody-dependent cellular cytotoxicity. SSEA-1 antibodies are also elicited during human schistosomiasis infection, and this autoantibody response may be involved in the development of the natural immunity against the parasite.**

Chronic schistosomiasis affects approximately 200 million people worldwide. In humans (1) and experimental animals (2, 3), natural infection with the parasitic trematode *Schistosoma mansoni* induces a partial resistance to reinfection, and antibodies reactive against the surface of invading larvae may play an essential role in the development of this immunity (4). The majority of these antibodies recognize carbohydrate epitopes associated with surface glycoproteins of M_r 17,000, 38,000, 160,000, and >200,000 (5). Whereas some of the anti-carbohydrate antibodies confer protection against infection (6–10), others, which are designated blocking antibodies, inhibit the protective antibodies from attacking the parasite (11–13). Despite the significance of the carbohydrate epitopes for immunity and their potential use as a vaccine against schistosomiasis, their precise chemical structures have not yet been determined.

We have generated (14) several monoclonal antibodies to the four major species of surface glycoproteins on *S. mansoni* cercaria and schistosomula, the invasive larval stages in the definitive vertebrate host; they recognize at least five distinct carbohydrate epitopes (9). Some of these antibodies confer protection against infection when passively administered to naive mice (6, 9). Because limiting amounts of parasite antigen make further chemical and immunological characterization of these epitopes difficult, other sources known to contain a variety of different carbohydrate structures were screened to detect cross-reactive epitopes. Here we describe the results of such a screen, the characterization of a protective epitope through comparison with the same epitope in embryonic mice.

METHODS

Antibodies. The monoclonal antibodies E.1, E.3, and E.5 were prepared from mice immunized with *S. mansoni* eggs as described (6, 14, 15); both E.1, which is of the IgG2b isotype, and E.5, an IgM, are protective in mice *in vivo,* whereas E.3, an IgG3 isotype antibody, is nonprotective. Each of these three antibodies recognizes a different carbohydrate epitope. The well-characterized anti-stage-specific embryonic antigen 1 (anti-SSEA-1) monoclonal antibody 7A, which was generated from mice immunized with fetal rat brain, was a gift from M. Yamamoto (16). The two monoclonal antibodies 2H1a and 3B11, which are here shown to recognize the SSEA-1 epitope, were generated from mice immunized with murine retinas (U.C.D., unpublished data), as was the neurofilament antibody R3 (17); all antibodies raised against rodent antigens are of the IgM isotype. Sera from patients with chronic schistosomiasis were collected in Caatinga do Moura, Bahia, Brazil, and were used as pools of at least four patients. The normal human serum consisted of a pool of sera from three noninfected donors living in Boston. Anti-schistosome rabbit antisera were prepared by immunization with a saline-soluble homogenate of adult worms (6); normal rabbit serum was taken from the same rabbits prior to immunization. Both patient and rabbit sera were separated by protein A-Sepharose chromatography into binding and nonbinding fractions.

Immunohistochemistry. Mouse embyros at different stages of development were removed from the uterus and fixed in a solution of 4% phosphate-buffered paraformaldehyde with 0.13% picric acid; their heads were cut at 10–12 μm on a cryostat. The sections were incubated with monoclonal antibodies in the form of hybridoma culture supernatants or with antisera at a range of dilutions and were processed for indirect immunofluorescence with fluorescein-conjugated goat antibodies against mouse, rabbit, or human immunoglobulin.

S. mansoni schistosomula were prepared as described (6) and incubated alive at 4°C for 45 min with hybridoma culture supernatants containing the monoclonal antibodies. After being washed in Hanks' balanced salt solution containing 20 mM Hepes (pH 7.4) and 1% bovine serum albumin, they were incubated with a 1:100 dilution of rabbit anti-mouse immunoglobulin antibodies for 30 min, washed again, incubated

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Abbreviations: SSEA-1, stage-specific embryonic antigen 1; GM-CSF, granulocyte/macrophage colony-stimulating factor.

with a 1:100 dilution of fluorescein-conjugated goat anti-rabbit immunoglobulin antibodies for 30 min, and fixed with phosphate-buffered 4% paraformaldehyde/0.13% picric acid for 1 hr at 4°C.

Sugar-Binding Assay. Hybridoma culture supernatants containing the monoclonal antibodies E.5, 7A, 2H1a, 3B11n, E.1, and E.3 were incubated for 45 min in the presence of increasing concentrations (0.02–2000 ng/ml) of lacto-*N*-fucopentaose III (BioCarb, Lund, Sweden) and then applied for 30 min to polyvinyl chloride microtiter plates coated with soluble egg antigen (50 ng per well) prepared from purified *S. mansoni* eggs as described (6). The plates were washed and incubated with ^{125}I-labeled rabbit-anti mouse immunoglobulin antibodies (500,000 cpm per well) for 30 min. The wells were washed and cut out, and the bound radioactivity was measured. The percentage of binding was determined by calculating $100 \times (a - b)/a$, where a and b are the amount of radioactivity bound in wells incubated in the absence and presence of lacto-*N*-fucopentaose III. The experiment was carried out in duplicate copies, and each value represents the mean percentage. The standard deviations were less than 7.

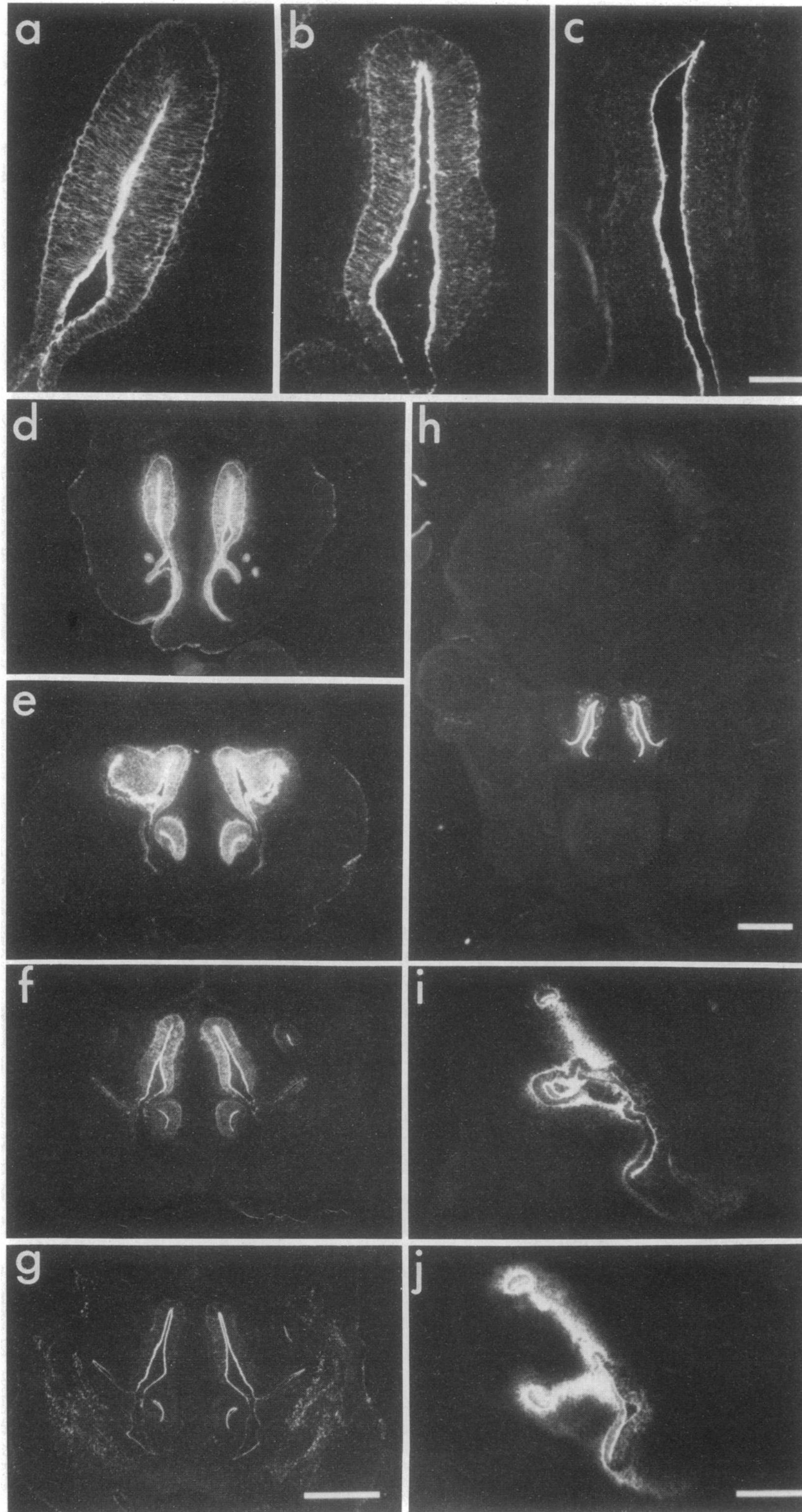

FIG. 1. Immunohistochemical localization of antigens recognized in the embryonic mouse head by the anti-schistosome monoclonal antibody E.5, schistosome antisera, and the SSEA-1 antibodies 7A (16), 2H1a, and 3B11n. An indirect immunofluorescence assay was performed to detect binding of the antibodies to tissue sections of embryonic mice at different stages of development. (*a–c*) Sections of olfactory epithelium from embryonic day 13 mice stained with monoclonal antibodies 3B11n (*a*) and E.5 (*b*) and sera obtained from Brazilian patients with chronic schistosomiasis (*c*); because chronic human sera contain other antibodies reactive with embryonic brain, the signal is relatively weaker than with the monoclonal antibodies. (Bar = 250 μm.) (*d–g*) Frontal sections through the heads of mouse embryos obtained embryonic day 11 (*d*), day 13 (*e*), day 14 (*f*), and day 15 (*g*), labeled with monoclonal antibodies 3B11n (*d*), 2H1a (*e*), E.5 (*f*), and sera from rabbits immunized with purified *S. mansoni* egg glycoproteins (*g*). Note the bright labeling of the olfactory epithelium with the vomeronasal organ at its medioventral aspect. (Bar = 500 μm.) (*h*) Frontal section through the head of an embryonic day 13 mouse stained with monoclonal antibody 7A (16); in addition to the brightly labeled olfactory epithelium, note the labeled lid folds and dorsal portions of the cerebral hemispheres. (Bar = 500 μm.) (*i* and *j*) Sections of the inner ear from an embryonic day 14 mouse stained with E.5 (*i*) and 2H1a (*j*). (Bar = 250 μm.)

***In Vitro* Killing Assay.** Purified peripheral blood eosinophils were obtained from human donors, and eosinophil-mediated cytotoxicity assays were conducted as described (18, 19) by incubating live, mechanically transformed schistosomula (100 per well, 200-μl final volume) with eosinophils at an effector cell/target ratio of 1000:1 in the presence or absence of 10 pM human recombinant granulocyte/macrophage colony-stimulating factor (GM-CSF; Genetics Institute). The antibody-dependent nature of the eosinophil-mediated killing of schistosomula was tested by applying either 50 μl of a 1:25 dilution of sera obtained from Brazilian patients with chronic schistosomiasis or 10 μl of a 7-fold concentrate of hybridoma culture supernatants containing the monoclonal antibodies 2H1a, 3B11n, or R3. Incubations of schistosomula and eosinophils without antibody and of schistosomula without eosinophils and without antibodies were tested as controls. The assay was scored by counting dead and live schistosomula in three wells after a 24-hr incubation. *P* values were calculated by χ^2 analysis.

RESULTS

In sections of embryonic mice, only E.5, an IgM monoclonal antibody that recognizes a protective carbohydrate epitope expressed on the $M_r > 200,000$ schistosome surface glycoprotein (9), gave a strong signal (Fig. 1). The E.5 epitope was present on an array of different tissues, and its expression changed with developmental age. The most intensely labeled tissue in the entire head was the olfactory epithelium as shown at lower magnification for an embryonic day 14 mouse in Fig. 1*f* and at higher magnification for an embryonic day 13 mouse in Fig. 1*b*; the surface of radial cells throughout the epithelium was labeled with accentuation of the luminal end-feet. E.5 labeling of the olfactory organ started at the beginning of placode invagination and persisted through development. The presence of the E.5 epitope in the olfactory epithelium seems conserved in vertebrates: we found a similar labeling pattern in mice, rats, and salamanders. In more caudal sections of days 14–16 embryonic mice, E.5 labeled strongly the inner ear (Fig. 1*i*). Not shown is the labeling of the eyelid, radial cells in the brainstem, and glia in the fetal optic nerve. In the adult retina, E.5 stained a population of normally placed and displaced amacrine cells and their processes in the inner plexiform layer. Labeling of the embryonic brain by E.5 showed pronounced variations during development in the form of waxing and waning patterns, some of which appeared to be inversely related to maturational gradients as detected by antibodies to the neural cell adhesion molecule (not shown).

Next we asked whether species other than mice generate antibodies against the E.5 epitope in response to schistosomiasis. Sera from Brazilian patients with chronic infections of *S. mansoni* and rabbits immunized with purified schistosome glycoproteins (15) labeled a similar pattern of embryonic mouse tissues as E.5, which included bright labeling of the olfactory epithelium (Fig. 1 *c* and *g*); no such labeling was observed with sera from normal human subjects or rabbits. When patient or immune rabbit sera were fractionated by protein A-Sepharose chromatography, the antibodies that gave the E.5-like pattern were present only in the flow-through fraction, suggesting that these antibodies were of the IgM isotype; this was verified by the use of μ chain-specific secondary antisera in the immunohistochemical assays.

The distinctive labeling of embryonic mouse tissues by the anti-schistosome antibody E.5 reminded us of the distribution of the SSEA-1 epitope (20), also known as X-hapten (21, 22). This determinant was first described in preimplantation embryos (20), but it is also found in embryonic brains (16), several adult tissues (23, 24), and malignant tumors (24). To determine whether the E.5-like pattern is indeed identical to the distribution of the SSEA-1 epitope, we compared E.5 to the well-characterized anti-SSEA-1 monoclonal antibody 7A (16) and to the two monoclonal antibodies 2H1a and 3B11n, which give a similar labeling pattern as 7A and which are here shown to recognize the SSEA-1 epitope as well (see below). All three antibodies were generated from mice immunized with rodent neural antigens, and like all reported SSEA-1 antibodies, they are of the IgM isotype. In sections of embryonic mice, the anti-rodent antibodies labeled a similar distribution of tissues as E.5, including the olfactory epithelium (7A in Fig. 1*h*; 2H1a in Fig. 1*e*; and 3B11n in Fig. 1 *a* and *d*) and the inner ear (2H1a in Fig. 1*j*). In addition to its presence in embryonic tissues, the SSEA-1 determinant is known to be expressed on the surface of tumor cells such as the human myelocytic leukemia line HL-60 (24) and mouse F9 teratocarcinoma cells (20). In immunocytochemical tests of HL-60 and F9 cells, the anti-schistosome antibody E.5 and anti-rodent antibodies 7A, 2H1a, and 3B11n all labeled intensely and specifically the surface of these cells.

Through binding-inhibition studies with oligosaccharides (25) and the isolation and sequencing of SSEA-1 glycolipids (22), the structure of the SSEA-1 antigenic determinant has been identified as the trisaccharide Galβ1-4(Fucα1-3)GlcNAc. To test whether the anti-schistosome antibody E.5 recognizes this trisaccharide, we used lacto-*N*-fucopentaose III, an oligosaccharide containing the SSEA-1 trisaccharide at its nonreducing end, in an inhibition assay (Fig. 2). Increasing concentrations of this oligosaccharide inhibited the binding of E.5 and the anti-rodent antibodies 7A, 2H1a, and 3B11n to schistosome antigens in a dose-dependent manner; at the highest concentration of inhibitor, 2 μg/ml, levels of antibody binding were less than 30%. Lacto-*N*-neotetraose, the defucosylated analog of lacto-*N*-fucopentaose III, at concentrations up to 2 μg/ml did not inhibit antibody binding (not shown). Inhibition of 2H1a and 3B11n binding by lacto-*N*-fucopentaose III verified our previous assumption that they are SSEA-1 antibodies. Lacto-*N*-fucopentaose III did not inhibit the binding of E.1 and E.3, two anti-schistosome monoclonal antibodies that recognize a protective and a nonprotective carbohydrate epitope distinct from the E.5 epitope (6, 14), confirming that binding of E.5 and the anti-rodent antibodies to the SSEA-1 oligosaccharide was specific.

To determine whether the SSEA-1 determinant is expressed on the surface of *S. mansoni*, we tested the ability of SSEA-1 antibodies to bind to live schistosomula. Antibodies

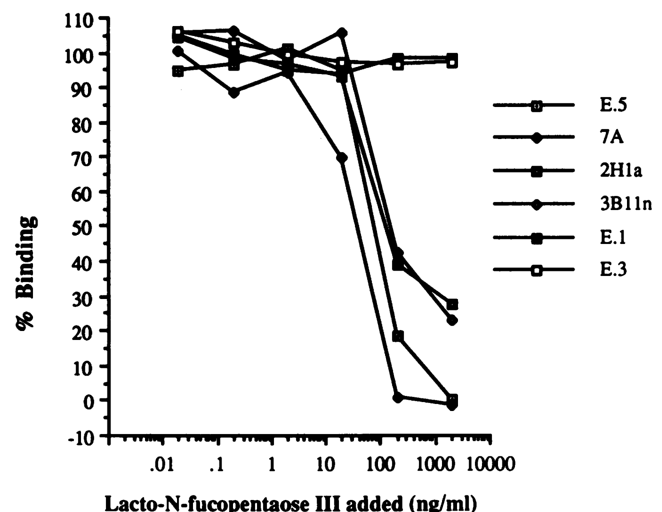

FIG. 2. Inhibition of the binding of anti-schistosome antibody E.5 and SSEA-1 antibodies to *S. mansoni* egg antigens by lacto-*N*-fucopentaose III.

E.5 (Fig. 3 *a* and *b*), 2H1a (Fig. 3 *c* and *d*), 3B11n (Fig. 3 *e* and *f*), and 7A (Fig. 3 *g* and *h*) strongly labeled the larval surface, whereas R3, an IgM monoclonal antibody against neurofilaments (17), did not (Fig. 3 *i* and *j*). Since SSEA-1 is known to be present in some tissues of adult humans (e.g., granulocytes, the nervous system, digestive tract, urinary, and reproductive systems) (23, 24), these results indicate that invading larvae express a host determinant on their surface.

Antibody-dependent cellular cytotoxicity appears to be a component of the immune defense against invading schistosomula (4). Since SSEA-1 antibodies bind strongly to the surface of schistosomula, we tested whether they could direct effector cells to kill the larvae *in vitro*. When incubated with the antibodies 2H1a and 3B11n, human peripheral blood-derived eosinophils killed schistosomula at levels (10% and 15%, Fig. 4) that were statistically significant above control levels—i.e., eosinophils incubated with neurofilament antibody R3 (3%) or no antibody (2%). Sera from Brazilian patients with chronic schistosomiasis mediated a 20% level of killing. Sera from uninfected humans mediate 2% killing, like incubations without antibodies (18, 19). GM-CSF induces postmitotic phenotypic changes in human peripheral blood-derived eosinophils, which may be advantageous to the host

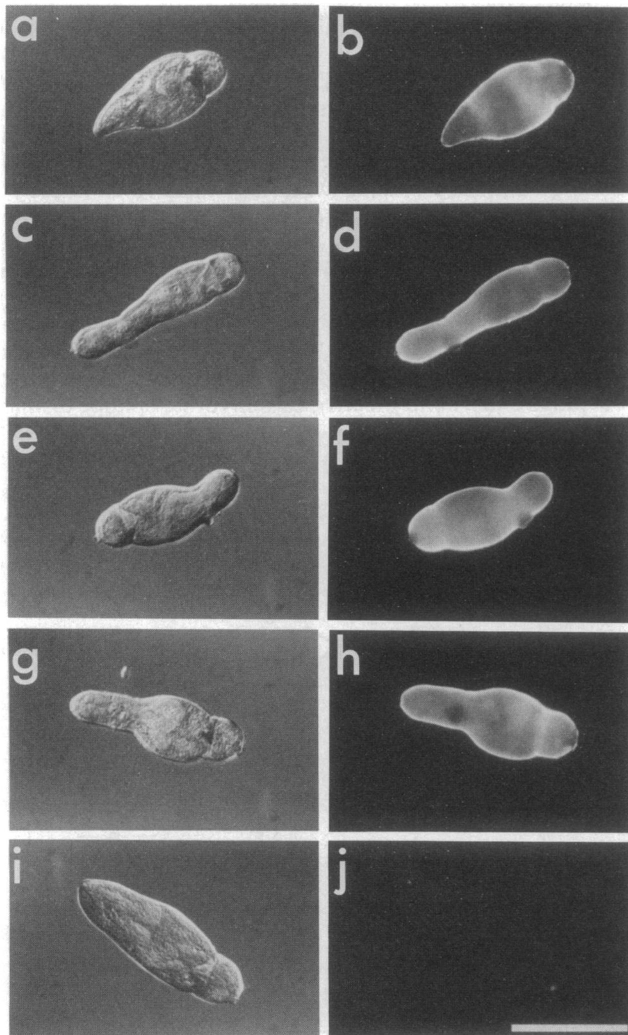

FIG. 3. Binding of the monoclonal SSEA-1 antibodies E.5 (*a* and *b*), 2H1a (*c* and *d*), 3B11n (*e* and *f*), 7A (*g* and *h*), and R3 (*i* and *j*) to the surface of live schistosomula, visualized by indirect immunofluorescence. The left column (*a*, *c*, *e*, *g*, and *i*) shows the parasites under Nomarski optics, and the right column (*b*, *d*, *f*, *h*, and *j*) shows the parasites under fluorescent illumination. (Bar = 100 μm.)

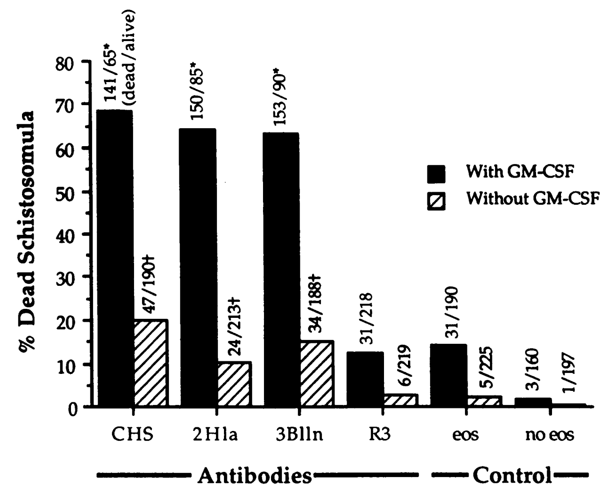

FIG. 4. *In vitro* eosinophil-mediated killing of schistosomula by SSEA-1 monoclonal antibodies 2H1a and 3B11n. Controls were incubations of schistosomula and eosinophils without antibody (eos) and schistosomula without eosinophils and without antibodies (no eos). Asterisks represent a statistically significant difference between the test proportions and control proportions in the presence of GM-CSF ($P < 0.05$). The daggers represent the same but in the absence of GM-CSF. The cytotoxicity assay has been conducted in five separate experiments, and the results shown here are from one representative experiment. CHS, sera from Brazilian patients with chronic schistosomiasis (chronic human sera).

defense against helminthic infections (18, 19). When GM-CSF was added to the assay, the SSEA-1 antibodies 2H1a and 3B11n and the chronic human sera mediated levels of killing (64, 63, and 69%) that were greatly enhanced as compared with incubations without GM-CSF; these levels of killing were statistically significant above those for R3 (14%) or no antibody (12%).

DISCUSSION

Previously the SSEA-1 determinant was studied as a developmental and tumor-associated antigen in mammals. Our results indicate that the determinant is already present in cold-blooded vertebrates, such as salamanders, and in trematodes; it is present in several developmental stages of *S. mansoni*, which include schistosomula, adults, and eggs (9). The expression of the host determinant SSEA-1 on the surface of invading larvae raises questions concerning its role in the host–parasite relationship. A parallel phenomenon has been reported for the schistosome larvae that infect the molluscan intermediate host: miracidia express a surface carbohydrate epitope that is shared with freshwater snails (8). Molecular mimicry of host carbohydrate epitopes has been suggested to be an adaptive mechanism that the schistosome uses to evade host defense (26). Although this may afford an advantage to an initial infection, subsequent infections of schistosome larvae would be susceptible to attack by SSEA-1 antibodies produced in response to the initial infection. The generation of SSEA-1 autoantibodies is not an unusual phenomenon, as high titers are known to be elicited by immunization of mice with SSEA-1 glycolipids (27). Since these antibodies direct effector cells to kill schistosomula *in vitro* and, as shown for E.5 (9), confer protection *in vivo*, the generation of SSEA-1 antibodies during chronic schistosomiasis may be a component of concomitant immunity (2), a term that describes the partial resistance to reinfection induced by a chronic primary infection.

Whereas the generation of SSEA-1 antibodies may be beneficial to the host defense, a potential deleterious side effect may be the expression of autoimmunity. This ought to

be mainly directed against circulating granulocytes, which carry the SSEA-1 determinant in humans (24). Other tissue locations of the SSEA-1 epitope, such as the embryonic and adult brain, the epithelium of the digestive tract, and distal tubules of the kidney (23, 24), are not easily accessible to SSEA-1 antibodies, which occur only in the IgM idiotype, and expression of autoimmunity here would not be expected. It has been demonstrated that intravenous infusion of murine anti-SSEA-1 antibodies into patients with acute myelogenous leukemia reduces the numbers of neutrophils but is otherwise minimally toxic (28). It is possible that the mild leukopenia seen in patients with severe chronic schistosomiasis and hypersplenism (29, 30) is partly caused by the binding of SSEA-1 antibodies to neutrophils and immune sequestration by the spleen.

As mentioned above, schistosome carbohydrate epitopes can induce both protective and blocking antibody responses. Blocking antibodies have been shown to inhibit *in vivo* (11) and *in vitro* (12) the action of protective antibodies, and they are believed to prevent the expression of immunity to reinfection particularly in young children (13). Since immunization with schistosome carbohydrates will always elicit a mixed immune response, the development of a carbohydrate-based vaccine against schistosomiasis requires the identification of carbohydrate epitopes that generate only protective responses. Immunization with the SSEA-1 determinants generates exclusively IgM antibodies, which are shown here to confer protection *in vivo* and *in vitro*. Although some reports have linked the IgM isotype with blocking responses (4), others have demonstrated the ability of IgM antibodies against schistosomular surface antigens to confer significant levels of protection *in vivo* (9, 31–33). As no serious autoimmune complications have been seen with the administration of SSEA-1 antibodies (28), oligosaccharides containing the SSEA-1 trisaccharide could be tested as a potential basis for a defined vaccine that induces protective responses and does not elicit blocking antibodies.

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