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Two Distinct Pathological Syndromes in Male CBA/J Inbred Mice with Chronic *Schistosoma mansoni* Infections

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Humans chronically infected with* Schistosoma mansoni *most commonly present with the relatively asymptomatic intestinal form of the disease, whereas a small minority develop hepatosplenism characterized by severe hepatic disease with portal hypertension. Investigation of hypotheses describing the pathogenic mechanisms underlying the clinical forms of the human disease has been limited by the absence of an animal model that predictably develops such a spectrum of disease. We report that inbred male CBA/J mice that are chronically infected with S. mansoni develop two distinct syndromes, hypersplenomegaly syndrome (HSS) and moderate splenomegaly syndrome (MSS). Pathologically and immunologically, MSS and HSS remarkably parallel the intestinal and hepatosplenic clinical forms, respectively, in humans. HSS affects approximately 20% of these mice and consists of massive splenomegaly, ascites, thymic atrophy, severe anemia, and cachexia. The remaining majority of mice with MSS develop moderate splenomegaly only. Histopathological features of HSS include 1) relatively extensive hepatic fibrosis and granulomatous inflammation, 2) splenic congestion, 3) lymph node plasmacytosis, and 4) worms and eggs in the pulmonary vasculature. Immunologically, the idiotypes present on anti-soluble egg antigen antibodies from HSS mice are distinct from those from mice with acute infections or the chronic MSS infection. These idiotypic differences are similar to those observed in patients with intestinal and hepatosplenic forms of the disease and may have regulatory importance. Investigation of the cellular and molecular events that lead to the development of MSS and HSS may advance current understanding of the pathogenesis of the clinical forms of chronic schistosomiasis in humans. (Am J Pathol 1993, 142:703–714)

Chronic *Schistosoma mansoni* infection in humans is a spectral illness, and patients usually present with one of several generalized clinical forms.[1–3] The majority of chronically infected patients have an asymptomatic and ambulatory clinical status, which has been termed the intestinal (INT) form of the disease. Less than 10% of chronically infected patients exhibit the hepatosplenic (HS) clinical form characterized by hepatosplenomegaly, "pipestem" fibrosis, portal hypertension, ascites, esophageal varices, hematemesis, and often, if untreated, death. The INT and HS ends of the spectrum of human schistosomiasis are also immunologically distinct. Peripheral blood mononuclear cells (PBMC) from acutely infected and ambulatory HS patients respond more vigorously to some schistosomal antigen preparations than do PBMC of INT patients and, after curative chemotherapy, full responsiveness of INT patients against soluble egg antigens (SEAs) is regained.[1,3–5] Patients with the various clinical forms also have distinct anti-SEA antibody idiotypic repertoires, which differ in their ability to stimulate *in vitro*

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proliferation of regulatory anti-idiotypic T lymphocytes present in preparations of PBMC from patients [6–12] or spleen cells from infected mice.[9] These idiotypic differences can also be detected serologically by either polyclonal[8,9] or monoclonal anti-idiotypic antibodies.[10] These differences have led to the hypothesis that the efficacy of immunoregulatory mechanisms, as well as the intensity of infection[13] and the immunogenetics of the host,[3,11,14,15] contributes to the clinical outcome of chronic *S. mansoni* infection in humans [1,3,5,11,16,17].

The direct investigation of this hypothesis has been hindered by the apparent lack of an animal model that represents the spectral presentation seen in chronic human infection. Among inbred and outbred mouse strains experimentally infected with *S. mansoni*, a number of differences in the severity of infection-induced pathology and in the immune response to worm and egg antigens have been described.[18–27] Chronically infected mice are often referred to as being hepatosplenic[23,28–31] because of their development of hepatosplenomegaly, increased portal pressure, collateral circulation, and esophageal varices, relative to normal mice and mice in the acute stage of infection.[28,29,31,32] However, no clear spectrum of pathology or correlative immunology has been defined at any given time point in chronically infected mice.

We now report that, in groups of male CBA/J mice, each exposed to an average of 45 cercariae of *S. mansoni*, a spectrum of morbidity develops as infections become chronic. In approximately 20% of these mice, a distinct pathological syndrome develops that grossly consists of massive splenomegaly, ascites, thymic atrophy, severe anemia, and cachexia. The histopathological features of the syndrome are splenic congestion, lymph node plasmacytosis, and extensive liver fibrosis associated with a relatively large percentage of liver mass being occupied by fibrotic granulomatous lesions. These features parallel many of those exhibited by HS patients, and this general similarity is also manifested in parallel immunological differences. Immunologically, mice with this more severe syndrome (hypersplenomegaly syndrome) (HSS) express an anti-SEA antibody idiotypic profile distinct from that of acutely infected mice and the other 80% of chronically infected mice that do not develop this syndrome but maintain a definite, but more moderate, splenomegaly (moderate splenomegaly syndrome) (MSS). In this report we describe the initial parasitological, gross pathological and histopathological, and immunological characteristics that define these two syndromes in male CBA/J mice with chronic *S. mansoni* infections.

Materials and Methods

Animals and Infections

Male CBA/J mice (6 to 8 weeks of age) were obtained from The Jackson Laboratory (Bar Harbor, ME) or through a National Cancer Institute contract [IGA V101 (134A)P-77014] and were maintained in the American Association for the Accreditation of Laboratory Animal Care-approved animal care facility of the Veterans Affairs Medical Center. Mice were infected by subcutaneous injection of 45 cercariae of a Puerto Rican strain of *S. mansoni* maintained in *Biomphalaria glabrata* snails. Replicate studies involved the use of mice from various shipments, which were infected with different pools of cercariae.

Examination of Gross Pathology

Mice infected for different lengths of time and age-matched, uninfected, control mice were examined in the following manner. A blood sample was collected in a microcapillary tube, from a minimal tail snip, for hematocrit determination. Mice were weighed and anesthetized with ether. A larger blood sample for serum collection was taken from the retro-orbital plexus, and the mice were sacrificed. Through a medial abdominal incision the presence or absence of visible ascitic fluid was noted. The liver, spleen, and mesenteric lymph nodes were removed, weighed, and fixed in 10% buffered formalin. Finally, the presence or absence of retroperitoneal fat was noted for each mouse, as an indication of cachexia.

Preparation of Tissues for Microscopic Examination

Tissues fixed as described above were embedded in paraffin and sectioned. Liver sections were stained with hematoxylin and eosin and with Masson's trichrome stain.[33] Spleen and lymph node sections were stained with periodic acid/Schiff reagent. Microscopic observations were made and recorded for tissues from both normal and infected mice. Quantitative measurements of granuloma size and the overall area of fibrotic tissue were evaluated on hematoxylin/eosin- and Masson's trichrome-stained tissues, respectively, by using a BioQuant image analysis system (Bioquant Inc., Nashville, TN) as described previously.[34]

Worm Recovery and Enumeration

The method used for perfusion, worm recovery, and enumeration was that described by Colley and Freeman.[21,25] Mice were sacrificed by intraperito-

neal injection of 0.5 ml of a mixture containing 3 ml of sodium pentobarbitol (60 mg/ml; Diamond Laboratories) and 12 ml of perfusion fluid (0.05 mol/L sodium citrate, 0.15 mol/L sodium chloride). Perfusion fluid (15 to 20 ml) was pumped by a peristaltic pump through the left ventricle and exited via the cut hepatic portal vein. Worms were collected by filtering the perfusate through 73-μm nylon mesh. Worms were placed in a petri dish in perfusion fluid, examined by stereomicroscopy, and counted.

Quantitation of Hepatic Schistosome Eggs

Livers from infected mice were removed and weighed. A known weight of liver tissue was then digested overnight in 5% potassium hydroxide.[30] The eggs present in an aliquot of the digest were counted under microscopic visualization, and the total number of eggs per liver was calculated for each mouse.

Preparation of Human Anti-SEA Monoclonal Antibody E5

E5 is a human IgG2 monoclonal antibody with anti-SEA specificity that was originally prepared from the PBMC of a patient with the INT clinical form of schistosomiasis.[35] E5 has been shown to stimulate the *in vitro* proliferation of anti-idiotypic PBMC from patients infected with *S. mansoni*.[6,7] The E5-producing heterohybridoma, generously provided by Dr. A. Goes and Dr. B. Doughty (Texas A & M University, College Station, TX), was grown in Pristane-primed nude mice, their ascites fluids were collected, and the IgG fraction of the ascites was obtained by precipitation with 40% ammonium sulfate. The IgG precipitate was resolubilized, dialyzed, and passed over a GammaBind G-agarose column (Genex, Gaithersburg, MD) to further purify the IgG fraction containing E5. The final protein concentration of the E5-containing IgG solution was determined with a bicinchoninic acid protein determination kit (Pierce, Rockford, IL).

Preparation of Mouse Anti-SEA Antibodies from Sera of Infected Mice

Mouse anti-SEA antibodies, termed idiotypic antibodies (Id), were isolated as reported previously.[8–10,36–38] Pools of sera from mice infected for either 8 weeks or 20 to 30 weeks (further segregated based on spleen weight) were collected and stored at −20 C until absorption. SEAs were coupled to CNBr-activated sepharose 4B (Pharmacia, Piscataway, NJ), and serum pools were mixed with SEA-sepharose at 4 C overnight, with continuous rotation. SEA-sepharose beads were then washed with 0.01 mol/L Na_3PO_4, 1.0 mol/L NaCl, pH 7.2, to remove nonspecifically bound material. Bound, immunoaffinity-purified anti-SEA antibodies were eluted with 0.1 mol/L glycine-HCl, pH 2.8, and neutralized by collection directly into 0.25 volume of 0.1 mol/L sodium borate, pH 8.0. Fractions were pooled and dialyzed against phosphate-buffered saline, pH 7.4.

Preparation of Rabbit Anti-E5 and Anti-Id Polyclonal Antibodies

The method used for generating anti-E5 and anti-Id polyclonal antibodies was that described by Montesano et al.[8–10] Rabbits were immunized subcutaneously in two sites with 200 μg of E5 or Id purified from the various groups of infected mice, diluted 1:1 in complete Freund's adjuvant (Difco Laboratories, Detroit, MI). Booster injections of 100 μg of antibody diluted 1:1 in incomplete Freund's adjuvant were given twice at 15-day intervals. Fifteen days after the final injection, the rabbits were bled and the sera were obtained. Each rabbit antiserum was precipitated with 40% ammonium sulfate. The precipitate was resuspended in phosphate-buffered saline and exhaustively absorbed (10 to 16 times) on either normal human IgG-coupled or normal mouse immunoglobulin-coupled CNBr-activated sepharose 4B. Absorptions were considered complete when the rabbit antibodies were shown to react by enzyme-linked immunosorbent assay (ELISA) with E5 or the immunizing mouse Ids but not with normal human immunoglobulin or normal mouse immunoglobulin, respectively.

Anti-Id ELISA and Competitive ELISA

Anti-Id ELISA was performed by reacting various dilutions of anti-E5, anti-8-week Id, and anti-MSS Id with purified E5, 8-week Id, or MSS Id antibody, respectively, that had been bound to Immulon II microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA). Specifically bound rabbit anti-Id antibodies were detected by reaction with biotinylated goat anti-rabbit antibody (Sigma Chemical Co., St. Louis, MO), followed by avidin-peroxidase (Sigma) and 2,2′-azino-di(3-ethylbenzthiazoline sulfonate) substrate (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) addition.[39] The resulting optical density of each well was measured with a MR 600 microplate reader (Dynatech). The dilutions of rabbit serum at which half of maximum specific reactivity was reached were 1/320 for the rabbit anti-E5 and anti-8-week Id reagents and 1/160 for the rabbit anti-MSS

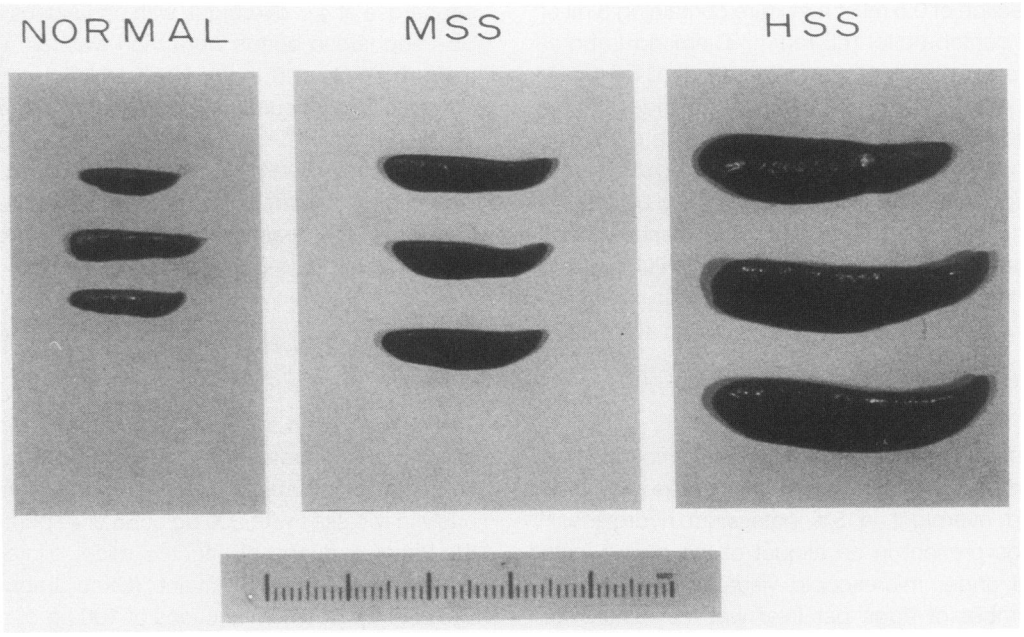

Figure 1. *Gross comparison of spleen sizes of normal uninfected, MSS, and HSS mice. The weights (in mg) of each of the spleens (from top to bottom) are as follows: normal, 60, 110, and 70; MSS, 210, 230, and 270; HSS, 780, 1160, and 1180. The scale bar shows length in mm.*

Id antibodies. Competitive ELISAs were performed according to the method of Montesano et al.[8–10] Rabbit anti-E5 or anti-Id reagents at the aforementioned dilutions were preincubated with various dilutions of whole serum or various concentrations of purified anti-SEA Ids from MSS, HSS, or 8-week-infected mice. The preincubated rabbit sera were then tested for their ability to bind the idiotypes expressed on the respective specific antibodies, ie, E5, 8-week Id, or MSS Id. The enzyme/substrate detection system used in this competitive ELISA was the same as used in the anti-Id ELISA.

Results

Gross Characterization and Incidence of HSS

During the course of investigations that required the examination of large numbers of male CBA/J mice with chronic infections (>20 mice each time), it was observed that an appreciable, and relatively predictable, number of these mice had a distinctive syndrome, termed HSS, with several grossly observable features. The most striking of these features is massive splenomegaly beyond the level of splenomegaly

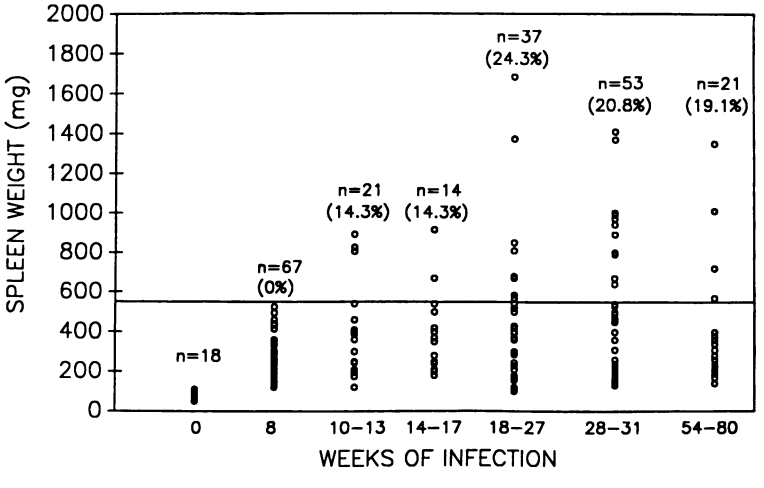

Figure 2. *HSS at various periods of infection. At each infection time point, the indicated number of mice were killed, the spleens were weighed, and the presence or absence of each feature of HSS was noted. The distribution of individual spleen weights at each infection time point is plotted. The line at 550 mg indicates that mice with a spleen weight of 550 mg uniformally exhibit all other features of HSS. The same is true of mice in which the percentage of body weight of the spleen is 1.2% (see text). The percentage of mice with a spleen weight of 550 mg at each infection time point is indicated in parentheses.*

seen in the majority of mice with chronic infections (Figure 1). Another gross feature characteristic of HSS is cachexia, which is most readily apparent by the absence of retroperitoneal fat. HSS mice were also observed to have 2 to 5 ml of ascites, whereas ascites was never seen in mice with MSS. HSS mice have a markedly reduced mass of grossly visible thymic tissue, compared with MSS mice. In a survey of >200 mice infected for 8 to 80 weeks, it was found that HSS appeared to develop after 10 weeks of infection and that there was a critical spleen mass of 550 mg and/or 1.2% of total body weight, at or above which all four gross features of HSS were always present. Approximately 14% of mice infected for 10 to 17 weeks had HSS, and in mice infected for between 18 and 80 weeks the presence of HSS increased to between 19 and 24% (Figure 2). HSS mice are also profoundly anemic. In a group of mice infected for 20 to 25 weeks, a strong inverse correlation was observed between spleen weights and hematocrit values (Figure 3; Pearson's $r = 0.91$; $P < 0.001$). This relationship between mice with severe anemia and those with HSS usually allowed for the identification of HSS mice before sacrifice by the relative pallor of their muzzles and paws.

Histological Comparison of Spleens, Lymph Nodes, and Lungs from MSS and HSS Mice

Microscopic examination of sections of spleens taken from MSS and HSS mice showed that both groups had white pulp hyperplasia with secondary follicle formation and mantle zone expansion (Figure 4, A and B). A distinguishing feature of the spleens from HSS mice was a greater degree of red pulp expansion and extensive sinus distention by erythrocytes, leukocytes, and megakaryocytes (Figure 4, C and D). Examination of the mesenteric lymph nodes of both MSS and HSS mice showed that both groups had similar degrees of expansion of primary and secondary follicles in the cortex. In 100% of the lymph nodes examined from HSS mice ($n = 6$), the medulla was consistently expanded by sheets of plasma cells, which often extended into the cortical interfollicular areas. This finding of extensive medullary plasmacytosis was present in only approximately 50% of lymph nodes of MSS mice ($n = 6$). Histopathological observations of the lungs of HSS mice showed that at least 60% of these animals had shunted at least one adult worm to their lungs, and all HSS mice had many eggs in their pulmonary vasculature. In contrast, the lungs of MSS mice did not contain any worms and only rarely were eggs seen in the lungs.

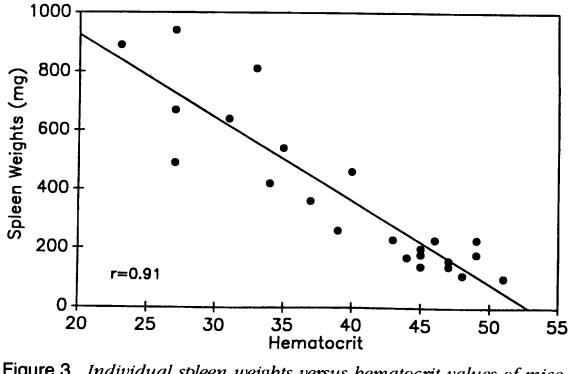

Figure 3. *Individual spleen weights versus hematocrit values of mice with infections of 20- to 25-week duration. Linear regression analysis revealed a correlation between spleen weight and anemia (Pearson's* r *= 0.91,* P *< 0.001).*

Gross and Histological Comparison of Livers of MSS and HSS Mice

The livers of mice with MSS were reddish brown in color, with multiple yellow-white, pinpoint-sized, punctate areas visible on all serosal and cut surfaces. The serosal surfaces had a slightly nodular texture. The livers of mice with HSS had a lighter red-gray color, with multiple dark green areas. The yellow-white punctate lesions seen in MSS livers were present at a higher density in HSS livers. The serosal surfaces of HSS livers were somewhat more nodular than those of MSS livers. The mean volumes of individual hepatic granulomas formed around newly deposited eggs were determined for groups of MSS and HSS mice, and no significant difference was found (Figure 5A). However, quantification of the mean percentage of granulomatous tissue and associated fibrosis in the livers of these same groups showed that the HSS mice had a significantly greater ($P < 0.001$) percentage of their liver tissue involved in granulomatous and fibrotic areas than did the MSS mice (Figure 5B). The qualitative microscopic appearances of MSS and HSS livers were remarkably distinct, secondary to this difference in the quantity of granulomatous and fibrous tissue. At low power, the granulomas in MSS livers were single dispersed fibrotic lesions (Figure 4C). In contrast, the granulomas in HSS livers were usually coalescent and only rarely were isolated lesions seen (Figure 4D). Thus, predominately intimal fibrosis of large branches of the portal vein, often without adjacent granulomas, and marked periportal fibrosis were consistently seen in HSS livers but only rarely in MSS livers (Figure 4, E to H).

Quantification of Worm Burden and Eggs per Liver in MSS and HSS Mice

The numbers of mature worms and eggs per liver were counted in individual mice with HSS and MSS.

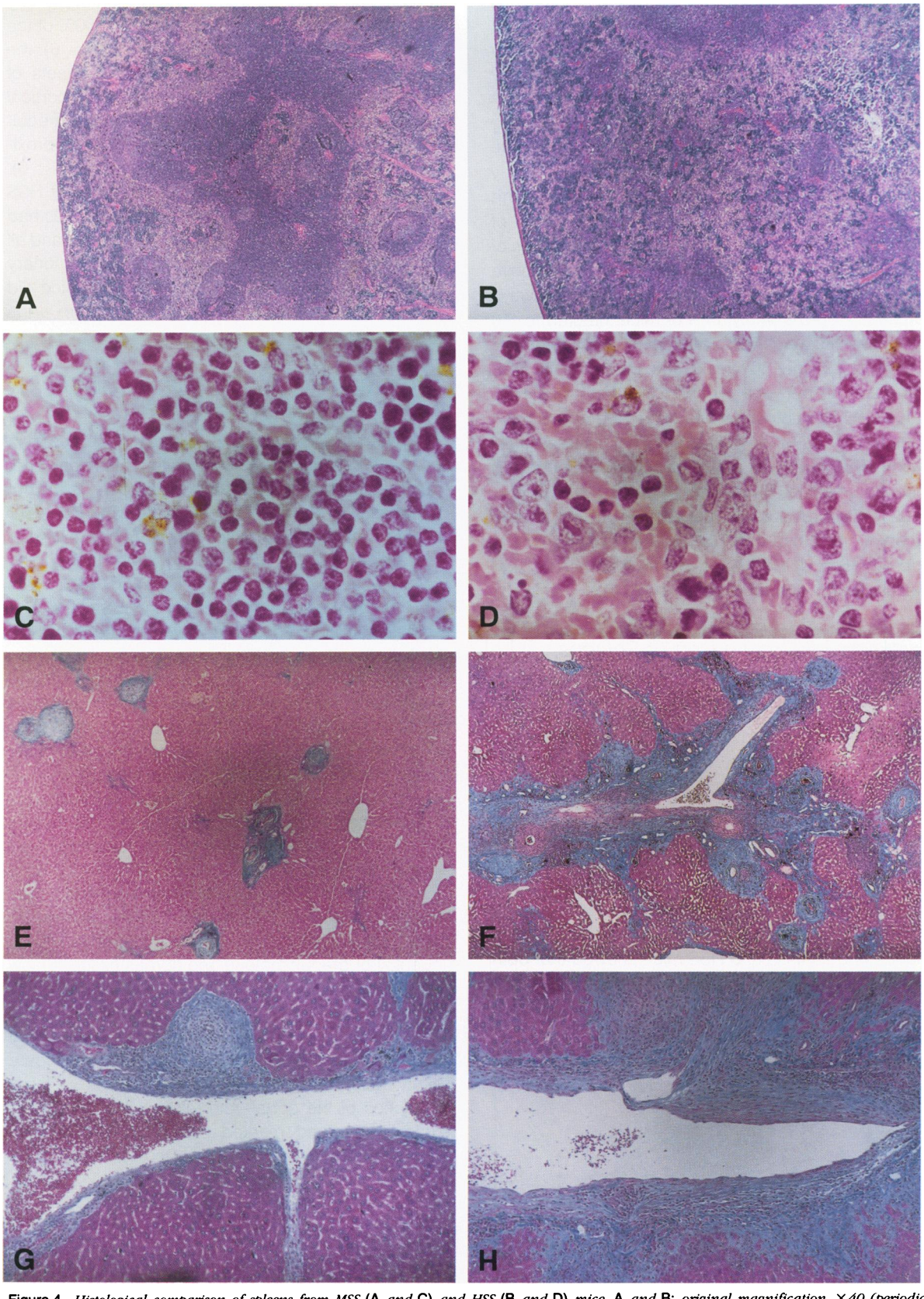

Figure 4. *Histological comparison of spleens from MSS* (**A** *and* **C**) *and HSS* (**B** *and* **D**) *mice.* **A** *and* **B**: *original magnification, ×40 (periodic acid/Schiff stain);* **C** *and* **D**: *original magnification, ×400 (hematoxylin and eosin stain).* **E** *to* **H**: *microscopic appearance of livers from mice with MSS and HSS. Granulomas in MSS* (**E**) *and HSS* (**F**) *livers; original magnification, ×40. Intrahepatic portal veins in MSS* (**G**) *and HSS* (**H**) *livers; original magnification, ×100.* **E** *to* **H** *are all stained with Masson's trichrome stain.*

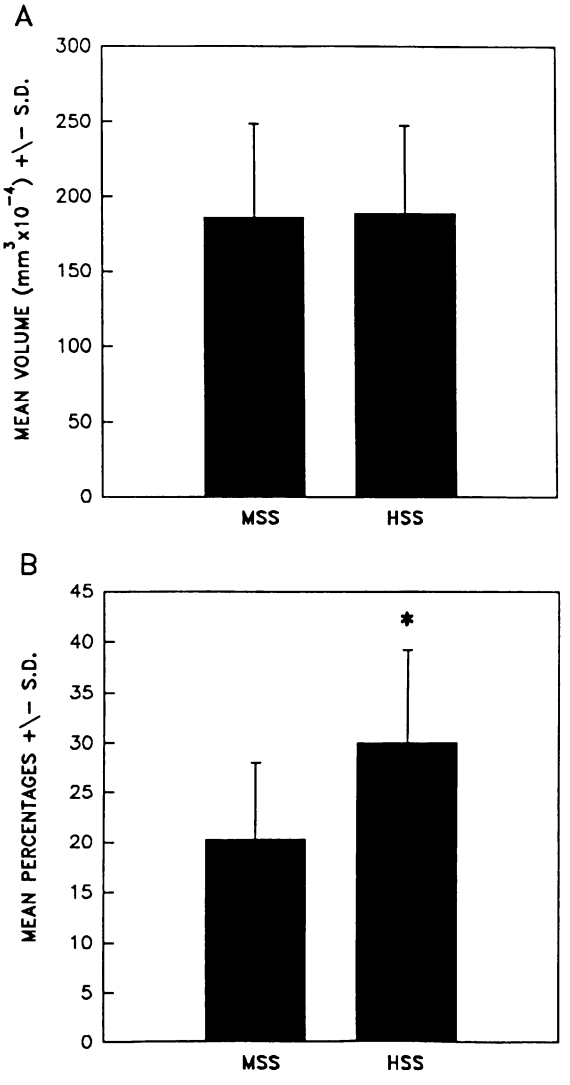

Figure 5. A: *Mean volumes of new individual granulomas in the livers of MSS and HSS mice. The mean granuloma volume for each mouse was calculated from at least 25 computer-aided video micrometric measurements of Masson's trichrome-stained liver sections. Granulomas were considered new if the section contained a viable miracidium. Group mean values ± standard deviations are plotted* (n = 19 *mice in each group).* B: *mean percentages of granulomatous tissue and granuloma-associated fibrosis in the livers of MSS and HSS mice. The mean percentage of granulomas and fibrosis in liver tissue for each mouse was calculated from at least 25 computer-aided video micrometric measurements of Masson's trichrome-stained liver sections. Calculated group mean values ± SD were 20.2 ± 7.8% for MSS mice* (n = 19) *and 30.0 ± 9.2% for HSS mice* (n = 19). *, $P < 0.001$.

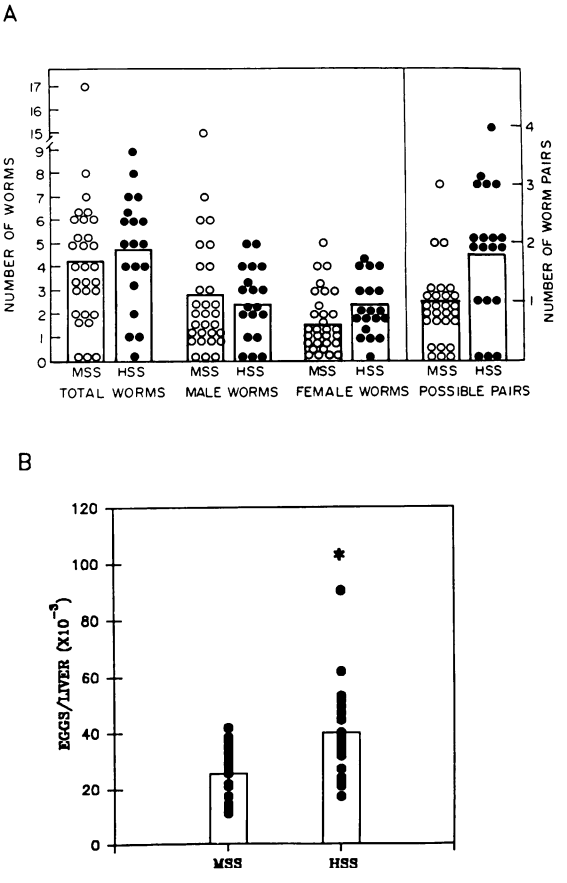

Figure 6. A: *Comparison of worm burden in MSS and HSS mice. Points correspond to the number of worms in individual mice in each group (MSS,* n = *30; HSS,* n = *19). Group means are indicated by bars superimposed over the points. Group mean values ± SD are as follows: total worms: MSS = 4.2 ± 3.2, HSS = 4.7 ± 2.5; male worms: MSS = 2.7 ± 3.0, HSS = 2.4 ± 1.6; female worms: MSS = 1.5 ± 1.3, HSS = 2.3 ± 1.2; possible pairs: MSS = 1.0 ± 0.7, HSS = 1.8 ± 1.1. Only the difference in the number of possible pairs is statistically significant ($P < 0.05$).* B: *total eggs per liver in HSS or MSS mice. Points correspond to the number of eggs per liver in individual mice in each group (MSS,* n = *20; HSS,* n = *25). The mean liver egg load ± SD for each group, indicated by bars superimposed over the individual values, was 25,511 ± 9,789 eggs/liver for MSS mice and 40,143 ± 16,240 eggs/liver for HSS mice. These mean values are significantly different ($P < 0.05$).*

No significant difference between MSS (n = 20) and HSS (n = 25) groups was found in the number of male or female worms or the total number of worms obtained by perfusion (Figure 6A). The numbers of male and female worms present in individual mice were also used to calculate the number of possible, but not necessarily observed, male/female pairs infecting each mouse. In this case it was found that the group of HSS mice had a slightly but significantly greater mean number of possible pairs than did the group of MSS mice (1.8 ± 1.1 versus 1.0 ± 0.7, $P <$ 0.05; Figure 6A). The group of HSS mice was also found to have a signficantly higher mean number of eggs per liver than did the group of MSS mice ($P <$ 0.05, Figure 6B), although, as with worm burdens, considerable overlap existed between individual members of the two groups.

Competitive ELISA Analysis of Sera and Purified Id Antibodies from HSS, MSS, and Acute-Phase Mice

Idiotypic competitive ELISAs, as used by Montesano et al,[8–10] were initially performed to test the ability of dilutions of pooled sera from uninfected mice, mice in the acute phase of infection (8 weeks), or mice with chronic MSS or HSS infections to competitively

inhibit the binding of Id-specific polyclonal rabbit anti-E5 antibodies to E5, a cross-reactive Id-bearing, human monoclonal anti-SEA antibody. At dilutions of 1/10 and 1/20 sera from mice either infected for 8 weeks or with chronic MSS infections efficiently inhibited the binding of anti-E5 to E5 (Figure 7A). In contrast, inhibition of the E5/anti-E5 ELISA was not seen with any dilution of sera from mice with HSS or from uninfected mice. To more accurately control for variable amounts of antibody in the serum pools, specific anti-SEA antibodies were immunoaffinity purified from pools of sera from mice infected for 8 weeks, pools of sera from mice with chronic MSS infections, or two separate pools of sera from mice with chronic HSS infections. Protein concentrations of each antibody preparation were assayed for inhibition of the E5/anti-E5 ELISA system. As with whole serum dilutions, immunoaffinity-purified anti-SEA antibodies from mice with either 8-week infections or chronic MSS infections both contained Ids capable of inhibiting the binding of anti-E5 antibodies to E5. Also, neither preparation of anti-SEA antibodies from two distinct pools of chronically infected HSS mice had detectable inhibitory ability (Figure 7B), ie, they did not contain the cross-reactive, E5-expressed Ids shared by chronically MSS mice and acutely 8-week-infected mice.

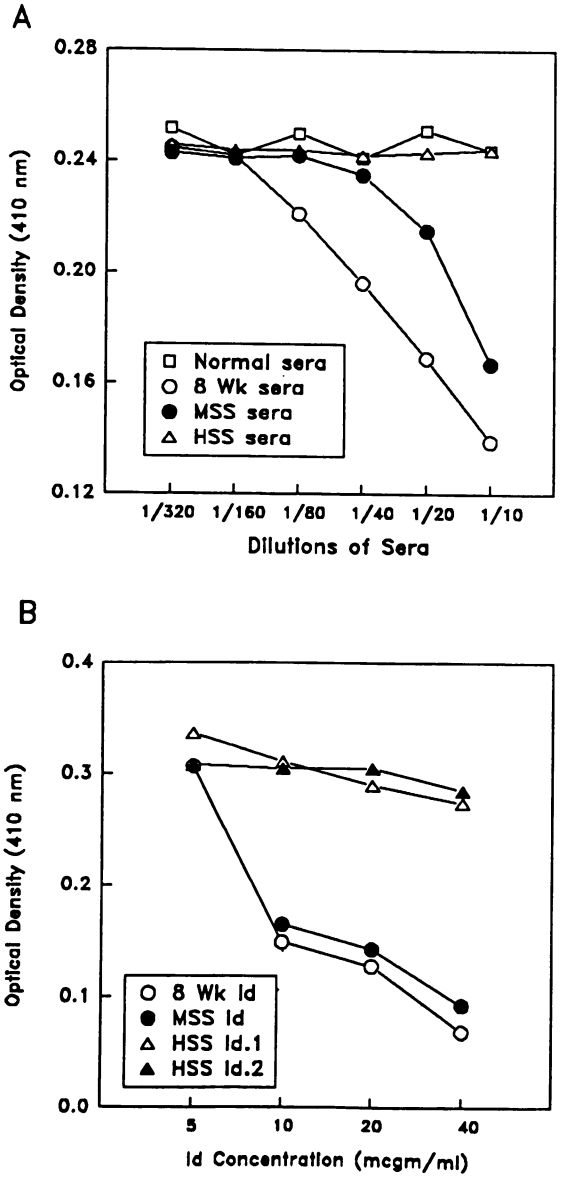

Figure 7. A: *E5/anti-E5 competitive ELISA using serum pools as competitors. The human anti-SEA monoclonal antibody E5 was used to coat the wells of microtiter plates. The ability of rabbit anti-E5 serum at a dilution of 1/320 to bind to the plate-bound E5 was evaluated by ELISA, after preincubation with serial dilutions of pooled sera from either normal uninfected mice, mice infected for 8 weeks (acute phase), chronically infected MSS mice, or chronically infected HSS mice.* B: *E5/anti-E5 competitive ELISA using anti-SEA antibodies (Id) purified from the serum pools used in* A *(Id) as competitors. The ability of rabbit anti-E5 serum at a dilution of 1/320 to bind to plate-bound E5 was evaluated by ELISA, after preincubation with the indicated concentrations of anti-SEA antibodies purified from either a single pool of sera from mice infected for 8 weeks or chronically infected MSS mice or two separate pools of sera from chronically infected HSS mice. All data are reported as the mean optical density at 410 nm of triplicate wells.*

To establish further the breadth of differences apparently present between the Ids expressed by anti-SEA antibodies from MSS and HSS mice, separate purified anti-SEA antibody preparations from these sources were tested for their inhibitory ability in polyclonal 8-week Id/anti-8-week Id and MSS Id/anti-MSS Id competitve ELISAs. The results of the 8-week Id/anti-8-week Id competitive ELISA (Figure 8A) showed that MSS Id and 8-week Id inhibited binding equally, with each preparation apparently sharing Ids in the same proportions. In this polyclonal system, the two HSS Id preparations also inhibited binding (in contrast to the results seen in the monoclonal E5/anti-E5 system) but required approximately 16-fold higher concentrations of HSS Id than of MSS Id or 8-week Id to achieve 50% inhibition. A similar degree of differential inhibitory activity between the two HSS Id preparations and the 8-week Id and MSS Id preparations was seen in the MSS Id/anti-MSS Id competitive ELISA (Figure 8B).

Discussion

This study demonstrates that male CBA/J mice chronically infected with small worm burdens of *S. mansoni* develop two distinct syndromes, MSS and HSS, which differ pathologically and immunologically and appear to be generally analogous to the INT and HS clinical forms, respectively, of chronic schistosomiasis in humans. This is the first report of the predictable occurrence of such syndromes in chronically infected mice; however, pathological variance in infected mice is not without precedent. Cheever[55] found that a minority of chronically

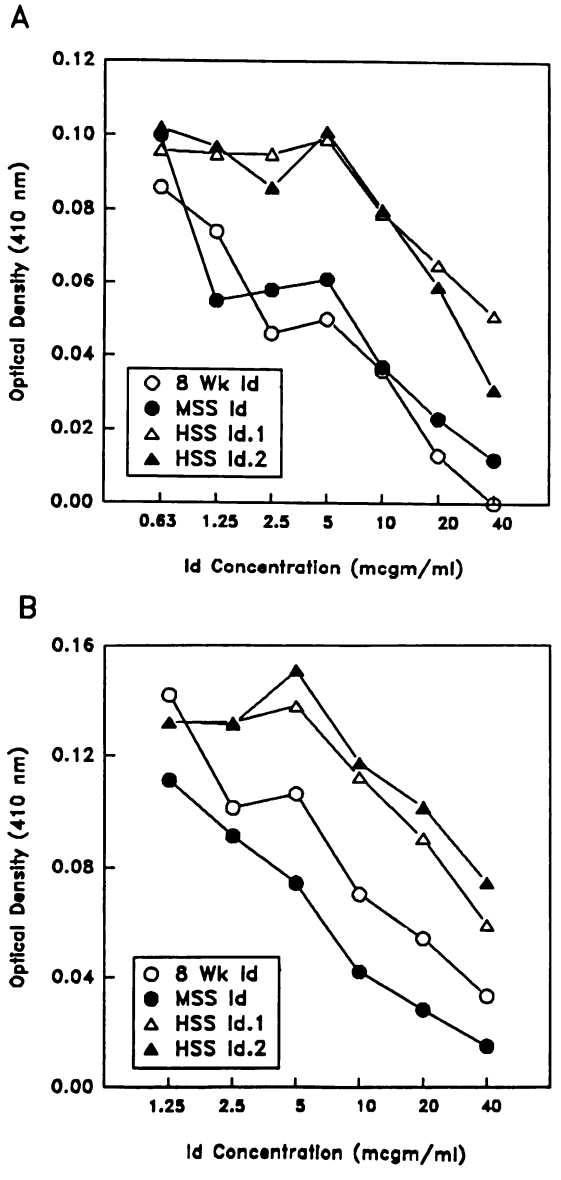

Figure 8. A: *Eight-week Id/anti-8-week Id competitive ELISA using purified anti-SEA antibody (Id) preparations as competitors. Id antibody purified from serum pooled from 8-week-infected mice was used to coat the wells of microtiter plates. The ability of rabbit anti-8-week Id serum at a dilution of 1/320 to bind to the plate-bound 8-week Id was evaluated by ELISA, after preincubation with the indicated concentrations of Id antibodies purified from either a single pool of sera from mice infected for 8 weeks or chronically infected MSS mice or two separate pools of sera from chronically infected HSS mice.* B: *MSS Id/anti-MSS Id competitive ELISA using purified anti-SEA antibody (Id) preparations as competitors. Id antibody purified from serum pooled from chronically infected MSS mice was used to coat the wells of microtiter plates. The ability of rabbit anti-MSS Id serum at a dilution of 1/160 to bind to the plate-bound MSS Id was evaluated by ELISA, after preincubation with the indicated concentrations of Id antibodies purified either from a single pool of sera from mice infected for 8 weeks or chronically infected MSS mice or two separate pools of sera from chronically infected HSS mice. Data are reported as the mean optical density at 410 nm of triplicate wells.*

infected C3H mice develop a relatively higher degree of hepatic fibrosis but apparently do not develop any of the other pathological features of HSS seen in CBA/J mice. The degree of splenomegaly, anemia, and portal hypertension that develops as a result of chronic *S. mansoni* infection also varies considerably between genetic strains of mice but has not heretofore been described to vary significantly within a given strain of mice with similar infection intensities.[18–20,22–24,28,29,31,40–42,55] Other laboratories have extensively used the CBA/J strain in studies of *S. mansoni* infection[18,19,24,43] and none have reported a HSS-like syndrome in their chronically infected populations. This could be related to differences in the routine infection intensity established in these laboratories and/or to the need to sacrifice relatively large numbers of mice to easily observe the spectrum of gross pathology we have noted in chronic infection in CBA/J mice. It could also be related to our use of male CBA/J mice, in that we have observed major differences in the morbidity and mortality seen in male versus female mice,[44] and essentially all other laboratories exclusively use females.

The massive splenomegaly in HSS appears to result from a combination of a relatively greater degree of lymphoproliferation and congestion than in MSS mice. HSS mice have a significantly greater percentage of the liver given over to granulomatous and fibrotic tissue than do MSS mice. Also, there is a remarkably distinct pattern to this fibrosis, consisting of both large bands of granulomatous and fibrotic tissue running throughout the parenchyma and intimal fibrosis of large branches of portal vein. This fibrotic pattern, particularly the portal vein fibrosis, is reminiscent of the Symmers' "clay pipestem" fibrosis seen in the livers of humans with HS schistosomiasis.[13,45–47] A similar pattern of fibrosis has been noted by Andrade[26] in outbred mice with chronic *S. mansoni* infections. As in HS patients, such fibrosis could be expected to cause increased portal hypertension. Although no direct measurement of portal pressures was undertaken in this study, strong indirect evidence of increased portal pressure in HSS mice consisted of the presence of splenic congestion,[18,19,24,42] ascites, and portal-systemic collaterals via which worms and eggs were shunted to the pulmonary vasculature. These conditions were not seen in MSS mice.

The relationship between the intensity of the granulomatous response and the development of pathogenic fibrosis in infected humans and mice[18,48] has long been enigmatic. Colley et al[1] have hypothesized that the more intense *in vitro* anti-SEA responsiveness of PBMC from HS patients is likely to be reflected *in vivo* by more exuberant egg-induced granulomatous lesions (perhaps with differential cytokine profiles), which may in turn lead to the characteristic HS hepatic fibrosis. In this study, a signifi-

cant difference between the volumes of new granulomas in HSS and MSS mice was not seen. Extrapolation of this result to pathogenesis in humans is not possible. With regard to pathogenesis in mice, this result suggests that the intensity of the granulomatous response, as measured by lesion volume, is not related to, or causal in, the development of HSS in mice. However, a possibility raised by these results and supported by other studies[18,19,24,49,55] is that lesion volume is not an accurate measure of the degree of fibrogenic and pathogenic potential of a granulomatous response. An example might be that differential immunoregulatory environments may allow the cells within granulomas of HSS mice to produce a battery of cytokines that promote a greater degree of hepatic fibrosis without forming a lesion significantly larger than that seen in MSS mice.

Anti-SEA antibodies from INT and HS patients have distinct idiotypic repertoires and anti-idiotypic T lymphocyte-stimulatory abilities. These differences in anti-SEA antibodies have been interpreted as strong correlative evidence that anti-idiotypic T lymphocytes, which are activated by certain cross-reactive idiotypes on anti-SEA antibodies, have a regulatory role in the immunopathogenesis of chronic infection in humans.[6,8–10,12,50–52] The possibility that distinct immunoregulatory environments may contribute to the development of MSS and HSS is supported by the results of the competitive ELISA studies, which show that HSS and MSS mice, like HS and INT patients, have distinct shared anti-SEA idiotypic repertoires. This establishes an immunological, as well as pathological, similarity between chronically infected CBA/J mice and humans and potentially allows for direct investigation of the role of humoral/cellular network interactions in regulating disease pathogenesis. It is of note that, without segregation of serum pools based on the MSS versus HSS differential, pooled sera from total chronically infected male CBA/J populations fail to inhibit binding in the E5/anti-E5 competitive ELISA in a manner analogous to that seen with HSS sera or anti-SEA antibodies,[9] which have an apparent overall loss of 8-week Ids. It is now clear that this is not so when only sera from MSS mice are pooled.

The number of egg-producing adult worm pairs is known to vary somewhat between individual mice infected with an equal average number of cercariae. The relationship of infection intensity to the development of MSS and HSS was investigated. Data interpretation is complicated in part by the presence of worms and eggs in the lungs of HSS mice, and conclusions could not be firmly established by enumerating eggs per liver and worms in the portal vasculature in each group. The lack of significant differences between the groups in the numbers of total, male, or female worms is offset by the significantly higher number of "possible worm pairs" in the group of HSS mice. Although statistically significant, this difference may be of little biological significance, considering that the difference is less than one pair, the groups show extensive overlap, and the calculated number of possible pairs does not necessarily reflect the true number of worms paired *in vivo*. The group of HSS mice was found to have a significantly larger mean number of eggs per liver than the MSS group. A relatively large number of eggs per liver could be due to a larger number of egg-producing worm pairs, to more fecund worm pairs (for example, perhaps due to higher tumor necrosis factor-α levels[53] in HSS mice), and/or to a slower rate of egg destruction and clearance from the liver. The worm pair data do not conclusively rule out any of these possibilities. It should also be noted that the individual eggs per liver values in each group overlap appreciably. When the number of eggs is less than approximately 50,000, it is not a reliable predictor of the development of HSS.

Cheever[13] found that at autopsy chronically infected patients with HS have statistically more intense infections, compared with patients with the INT clinical form; however, a significant number of patients with HS have infection intensities comparable to those of their INT counterparts. Hence, infection intensity is not, on a one to one basis, predictive of HS in humans. The existence of this "gray area" in the association of clinical status with infection intensity led to studies that implied that immunoregulatory[1–3,54] and/or immunogenetic[3,11,14,15] mechanisms are also important determinants of the clinical outcome of chronic *S. mansoni* infection in humans. Considered in light of the distinct pathological and immunological states of HSS and MSS mice, the less than decisive differences in the worm counts and eggs per liver data suggest that, as in humans, infection intensity may be but one of many factors affecting the development of each syndrome. The existence of these distinct syndromes in an inbred animal model may now allow for identification and investigation of further pathogenic factors related to the development of each syndrome. This will hopefully lead to testable hypotheses describing pathogenic mechanisms in human schistosomiasis.

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