Immunopathology of *Schistosoma mansoni* Infection

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INTRODUCTION

Schistosomiasis mansoni is a disease of the tropics. According to a survey of the World Health Organization, the disease is endemic in 51 countries, affecting about 100 million people (169). The causative agents are digenetic trematode worms, also called blood flukes, that live in pairs and are attached to the walls of the host mesenteric veins. The male and female worms have a complicated life cycle alternating between aquatic snails (intermediate host), in which asexual reproduction takes place, and humans (definitive host), in whom sexual reproduction occurs. Human infection is initiated during water exposure (planting, fishing, washing, and swimming), when the free-swimming fork-tailed cercariae penetrate the intact host skin. In the skin, cercariae transform into another larval form, the schistosomulum, that is adapted to live in the higher osmotic environment of the body. Host reaction in the skin is variable and may result in transient dermatitis. Male and female larvae migrate through the skin, blood, and lungs; the worms grow and mature, descend to the liver, mate by pairing, and settle in the mesenteric venous plexus. The small adult worms (male, 10 to 15, and female, 14 to 20, mm long) live in copula, and each female produces about 300 eggs daily. The life span of the worm is 5 to 10 years, and by a variety of mechanisms it is capable of evading the host immune response (95). The worms feed on host erythrocytes and body solutes, and their presence is innocuous to the host. Some of the eggs deposited by the females into the bloodstream pass through the venule walls, cross the intestinal mucosa, reach the lumen, and are evacuated with the fecal material. Eggs that arrive in the outside environment have to reach fresh water for the embryo (miracidium) to hatch and swim to find its intermediate snail host. Penetrating the snails, the miracidia undergo a complicated metamorphosis involving assexual reproduction, and in a few weeks they emerge in the new infectious form, the cercariae. In the definitive mammalian host, eggs that do not reach the intestinal lumen are swept into the portal circulation and are trapped in the intestinal wall, the liver, or the lung and systemic organs and are surrounded by the granulomatous inflammatory response of the host. The continuous egg deposition induces a chronic inflammatory host response that is responsible for the disease syndrome. Most low or mild infections are well tolerated, with minimal symptoms; heavy infections may terminate fatally.

The variety of antigens secreted by the worms or shed during the different developmental stages of the worm life cycle (cercariae, schistosomula, adult male and female, and eggs) provide strong sustained stimuli to the host humoral and T-lymphocyte-mediated immune systems. The aims of this review are to illustrate how the vigorous host response mounted to parasite antigens may lead to the development of pathological events and to describe the regulatory mechanisms that modulate the exuberant immune responses and ameliorate excessive morbidity.

DERMATITIS

Schistosome infection is initiated after cercariae penetrate through the skin and transform into schistosomula, the larval form suited for existence in the host milieu (144). Penetration and migration of cercariae are facilitated by proteolytic enzymes secreted from cephalic glands capable of digesting epidermal keratin (135). Infected individuals exposed to *Schistosoma mansoni* cercariae experience itching within 1 h of water contact. In mild cases, a skin rash appears that consists of rounded, discrete, 1- to 2-mm erythematous papules. Urticaria that persists for several days may also be present. The itchy sensation and papular eruption may take days to disappear, and the healed papules may leave behind a pigmented spot. Severe dermatitis is manifested by an evenly distributed confluent papular reaction. If papules are infected with bacteria, pustules will appear (5, 21). Exposure to cercariae of nonhuman schistosomes causes a severe, intensely pruritic dermatitis in the reinfected host (20). This phenomenon has been extensively investigated in individuals who had been repeatedly exposed to duck schistosomes in lakes of the American Midwest (93). After penetration, cercariae of bird schistosomes die in the skin of the unnatural host and their antigens initiate a sensitization process. On primary host exposure, cercarial dermatitis, called swimmers’ itch, causes a mild itch and the appearance of 1- to 2-mm macules at the site of penetration. This reaction is transient and innocuous. After repeated exposures, the individual reacts within 1 h of exposure with intense itch, the formation of papules, and, sometimes, diffuse erythema. About 10 to 15 h later, itchy papules 3 to 5 mm in size develop and become indurated and edematous. In 2 to 3 days, vesicles that may rupture are formed. Histological examination of biopsied skin shows the cercariae confined to...
the epidermis. During the first 30 h after exposure, an extensive infiltration of neutrophils and lymphocytes occurs which by day 2 is augmented by the influx of eosinophils. The infiltrating cells kill the penetrated cercariae (50). The symptomatology and histologic picture are consistent with an anamnestic immune reaction (210). The immune basis of cercarial dermatitis in S. mansoni-infected individuals is supported by the demonstration of humoral and cell-mediated immune reactivities to cercarial antigenic preparations. Patient sera react with the outer coat of the larvae (Cercariaenhülle Reaction) (259), which can be also demonstrated by the indirect immunofluorescence (IF) reaction (241, 243).

Judging by the immediate and delayed-type hypersensitivity reactions following intradermal injection of cercarial antigens, individuals develop both reagin-type, immunoglobulin E (IgE) skin-binding antibodies (242) and T-cell-mediated reactivity to cercariae (196). The latter is confirmed by specific lymphoproliferation of peripheral blood mononuclear cells (PBMN) stimulated with cercarial antigens (85, 213).

In normal mouse skin, cercarial penetration causes, first, a mild edematous reaction with infiltration of predominantly neutrophilic cells into the epidermis and dermis (91, 274). In previously sensitized animals, the reaction is enhanced; it appears faster and the total granulocyte content of the infiltrate is increased, a major portion of the cells being eosinophils (274). Mast cells degranulate, and the vascular endothelium may show focal degeneration (27). Guinea pigs sensitized with schistosomal antigens react to cercariae with a strong basophil and minor eosinophil infiltration (18). Basophils and free basophil granules are observed in close proximity to the invading larvae (219). Rat peritoneal mast cells exposed to cercariae in vitro undergo degranulation and release histamine (64). In contrast to rodents, normal baboons do not mount an inflammatory skin response to penetrated cercariae. They develop the ability to mount a predominantly eosinophilic infiltration around cercariae only after a prolonged infection (250).

Passive transfer experiments conducted in mice with both immune sera and cells have indicated that both humoral and cell-mediated mechanisms participate in the antieccercarial skin response. The early wave of cell infiltration is evoked by the passively transferred antibodies, and the subsequent activity is attributed to T lymphocytes (91). Cercarial extracts also have been reported to activate complement (186) and exert chemotaxis on eosinophils (84). Thus, the initial nonspecific reaction to cercariae is attributed to focal damage and triggering of the complement pathway, which can generate polymorphonuclear infiltration; focal mast cell degranulation and released histamine may account for itching. On secondary or repeated experimental exposures, memory lymphocytes are triggered and elicit both humoral and T-cell-mediated, accelerated, enhanced focal reactions rich in eosinophils. It is presumed that such a reaction is protective and contributes to the attrition and death of the penetrated cercariae (83, 95, 226). It should be noted, however, that a history of dermatitis is only rarely elicited in S. mansoni-infected patients who live in areas in which schistosomiasis is endemic.

**ACUTE SCHISTOSOMIASIS**

Acute schistosomiasis is a clinical syndrome often seen in nonimmune individuals (tourists, immigrants, or the indigenous population) who have been exposed in an endemic area to a primary infection by cercariae (79, 106, 234, 300). The syndrome is sometimes called Katayama fever, described in Japan in persons who acquired a massive infection with *S. japonicum* worms. Acute disease manifestations rarely occur among reinfected individuals living in an endemic area, presumably because of their pre- and postnatal exposures to schistosome antigens via transplacental transfer (61), lactation (249), and repeated infections at an early age. This view is supported by murine experiments in which fetal or neonatal exposure to schistosome antigens induced a state of hyporesponsiveness and diminished the granulomatous responses of adult animals (152, 179).

Symptoms of acute schistosomiasis usually appear 4 to 10 weeks after a heavy exposure to cercariae. This would coincide with the migratory stage of the maturing schistosomula in the lungs and liver, maturation of male and female worms, or early oviposition in the mesenteric veins. The acute (toxic) syndrome is manifested by fever, malaise, hepatosplenomegaly, eosinophilia, diarrhea, and, in some cases, edema, urticaria, lymphadenopathy, and arthralgia (106, 200, 234, 277). These symptoms are transient and spontaneously disappear with the passage of the infection to the chronic stage. However, patients may develop cardiacepatic, hepatic, or intestinal complications may appear with or without granulomatous pathology, sometimes with fatal consequences. Moderate elevation in serum globulin, serum glutamic oxalacetic transaminase, and serum glutamic pyruvic transaminase levels may occur, indicating liver involvement. In rare cases, acute hepatitis with focal necrosis and eosinophil infiltration are observed (234). The efficacy of anthelminthic therapy at this phase of the infection is equivocal (200). Treatment has been reported to diminish eosinophilia and improve liver function (192) or exacerbate symptoms (154).

The relationship of fecal egg count (indicator of worm burden) to the severity of symptoms is still unclear. Whereas in certain patients a direct relationship was established (159), in others the presence of severe symptoms without a concomitant diminution in fecal egg counts supports the notion that clinical symptoms are induced by schistosomatid adult worm and egg antigens. In experimental studies, sequential histologic examination of infected mice showed severe reactive hepatitis around the first eggs deposited in the liver. Microvacuolated hepatocytes were visible adjacent to the eggs, coagulative necrosis was evident, and the area was surrounded by eosinophils (9).

The exact mechanism(s) responsible for the pathogenesis of acute schistosomiasis is still unknown. Both nonimmune and immune mechanisms may participate in the pathogenic process. Experiments in vitro demonstrated that schistosomula directly activate the alternate pathway of complement (245). Thus, conceivably, the antigens released in large amounts during the migration, maturation, or death of schistosomula may trigger serum complement, with resulting anaphylactoid reactions. At the immune level, immediate-type and immune complex-mediated hypersensitivity reactions can be invoked as possible pathogenic factors. Because IgE antibody levels are elevated at the acute phase of the infection and may correlate positively with the magnitude of the infection (159), heavily infected individuals may develop anaphylactic reactions following parasite antigen-induced degranulation of IgE-coated mast cells (215). Clinical manifestations seen in patients, i.e., skin rash, eosinophilia, pulmonary edema, or diarrhea, are consistent with this contention (36, 106, 234). The temporal relationship between the appearance of symptoms and the release into the circulation of large amounts of worm antigens derived from
was found (159). Another study that used the $^{125}$I-labeled Clq-binding assay to detect circulating complexes measured binding activity in the sera of 14 of 15 acutely infected persons, but only in sera of 2 of 11 chronically infected persons. Serum IgM levels in acutely infected persons were elevated over those measured in chronically infected individuals. Complement fixation tests with adult worm antigens showed significantly higher titers in acute-phase compared with chronic-phase sera. Analysis of the precipitated complexes detected moderate amounts of IgM and IgG antibodies, but no antigens could be found. No correlation was seen between the magnitude of Clq binding and liver and renal function tests (176). Serial determinations of labeled Clq-binding activity in acutely infected patients revealed peak binding ability with maximally elevated IgM levels and eosinophilia by 10 weeks after exposure. These levels correlated positively with the number of eggs excreted in the feces and the index of symptom severity. Following antihelminthic therapy with niridazole, Clq-binding values as well as IgM and IgG levels returned to normal in a small number of patients (158).

Overall, the available evidence indicates that acute schistosomiasis is accompanied by strong host immune responses. The pronounced hypergammaglobulinemia found in humans (16, 26, 106) and mice (251) and the occurrence of autoantibodies in the latter (134) during the acute phase of the disease are indicative of nonspecific polyclonal B-cell stimulation. Whether polyclonal B-cell stimulation occurs in human schistosomiasis and whether it contributes to the immunopathology of the disease remain to be elucidated. Because in a certain number of patients circulating complexes have been found (but thecomplexed antigens have not been identified), it is possible that the complexes are composed of immunoglobulin-rheumatoid factors (63). Conceivably, both immune and nonimmune mechanisms contribute to the pathogenic process. Whether there exists a preponderant immune factor such as immune complex-induced serum sickness that is responsible for the acute clinical manifestations seen in schistosomiasis is still to be confirmed.

**CHRONIC SCHISTOSOMIASIS**

**Renal Pathology**

In Brazil, chronic schistosomiasis is classified into the mild intestinal, hepatointestinal, and severe hepatosplenic (HS) forms of the disease (14). Patients with the mild intestinal form constitute >90% of the infected population. They usually carry a light worm burden and may be asymptomatic or have abdominal pain, transient diarrhea, and bloody stools. Less that 10% of the infected persons develop HS disease, which is manifested by hepatosplenomegaly, Symmers’ clay pipestem fibrosis, portal vein obstruction, portal hypertension, ascites fluid formation, and esophageal varices which rupture and cause repeated episodes of hematemesis (97, 141, 233, 277, 280). Among individuals with HS manifestations, 10 to 15% develop the nephrotic syndrome (14). This syndrome may sometimes be attributed solely to a concomitant bacterial infection such as salmonellosis (24), which may increase the incidence of CIC formation and directly or indirectly cause renal disease. However, the helminthic infection alone is sufficient to initiate the nephrotic syndrome.

Renal manifestations develop slowly and appear many years after the onset of HS disease (11). Histologically, renal...
tissue shows a spectrum of pathological changes, not necessarily accompanied by clinical syndrome. Early changes include mesangial expansion with amorphous periodic acid-Schiff stain-positive deposits. Mesangial cell hypertrophy and hyperplasia may follow. Basement membrane changes are not marked; subepithelial, subendothelial, and intramembranous deposits may be visible. In more advanced cases, membranoproliferative glomerulonephritis develops and focal glomerulosclerosis is evident (8, 14). Proteinuria may be linked to HS disease manifestations (177, 232). However, glomerulopathy without proteinuria occurs frequently (252) and, when associated with salmonellosis, may be caused by the bacterial rather than the helminthic infection (24).

To establish the immune etiology of schistosomal glomerulopathy, the sera and renal tissues of infected humans have been examined for the presence of CIC. Infected individuals often have elevated total levels of serum IgM and IgG (16, 26). It is probable that only a fraction of those immunoglobulins are antigen specific. In humans, CIC were assayed by immunodiffusion (187), immunoelectrophoresis of acidified dissociated complexes (230), quantitation of the amount of bound serum complement, or precipitation of complexes with polyethylene glycol (248). In one study, results of radionuclide 1Cq binding, complement fixation, and polyethylene glycol precipitation tests showed good correlation, but levels of CIC were higher in patients with mild disease than in patients with HS disease. Nevertheless, the precipitated immune complex (IC) contained schistosome antigens that reacted with specific IgM, IgG, and IgE antibodies (49). Subsequent studies showed good correlation among levels of CIC, intensity of infection, and severity of disease as manifested by the amount of circulating antigens and fecal egg excretion (61, 136, 230, 247). Fixation of complement by CIC was confirmed (248), and high levels of C3d breakdown product were found in the sera (136). However, correlation was not always found between the levels of CIC and C3d because the circulating antigens nonspecifically bound complement (246).

Much effort has been invested in identifying the schistosome antigen components that participate in IC formation. Circulating polysaccharide proteoglycan antigens first detected in the sera of heavily infected experimental animals (29, 98, 142) and implicated in serum sickness-like symptoms in acutely infected persons (98–101, 203–206) are the major candidates for IC formation. These consist of GASP/CAA and circulating cathodic antigen (CCA), a positively charged, large sugar moiety-containing molecule; both of these originate from the gut epithelium of the worms (12). With the introduction of highly specific monoclonal antibody to CCA and of sensitive radioimmunosay and enzyme-linked immunosorbent assay, circulating CCA and CAA antigens have become detectable in the 1- to 200-ng range. These methods helped to establish a correlation between levels of CCA and intensity of infection. Using the 1Cq-binding test, a high percentage of patients with HS versus intestinal schistosomiasis could be identified. A decrease in egg excretion with a concomitant drop in CCA levels seen in praziquantel-treated patients further confirmed the significance of circulating antigen levels as possible indicators of heavy infection and HS disease (132). Antigen M was found in the blood, milk, and urine of infected patients and hamsters (58–60, 249) and appears to be related to CCA (201); its occurrence also correlates with the intensity of infection. Demonstration of soluble egg antigens (SEA) in kidney extracts from infected baboons (167) but not mice (121) and of parasite deoxyribonucleic acid in the glomeruli of hamsters (160) provides presumptive evidence that these antigens may enter the circulation in some but not all animal species.

Renal tissues of humans obtained by biopsy or at autopsy were examined for determining the presence of immunoglobulins, complement components, parasite antigens, and IC deposits. By direct or indirect IF, lumpy deposits of IgM, IgG, occasionally IgA and IgE. C1q, and C3 were observed in the mesangial areas and capillary walls in kidneys with ongoing proliferative glomerulonephritis (11, 96, 129, 165, 188, 211, 252). The presence of specific antibodies was confirmed by acid elution of homogenized renal tissue. The eluted IgG bound strongly to adult worm gut and reacted weakly with the outer tegument of the worm (195). Localization of GASP/CAA in the mesangium and capillary basement membrane of patients with membranoproliferative glomerulonephritis and end-stage renal failure indicates a role for this antigen in schistosome-specific nephropathy (211, 256).

Glomerular lesions affecting the mesangial areas could also be produced in heavily infected experimental animals. The renal lesions of infected hamsters consisted of mesangial and endothelial hypercellularity (161). Glomeruli of heavily infected hamsters in one study, results of immunodiffusion of IgG, occasionally IgA and IgE. C1q, and C3 were observed in the mesangial areas and capillary walls in kidneys with ongoing proliferative glomerulonephritis (11, 96, 129, 165, 188, 211, 252). The presence of specific antibodies was confirmed by acid elution of homogenized renal tissue. The eluted IgG bound strongly to adult worm gut and reacted weakly with the outer tegument of the worm (195). Localization of GASP/CAA in the mesangium and capillary basement membrane of patients with membranoproliferative glomerulonephritis and end-stage renal failure indicates a role for this antigen in schistosome-specific nephropathy (211, 256).

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damage is caused by CIC that are shunted from the liver to be filtered at the renal level. Because of the partial blockage of the portal circulation and development of collaterals, the normal hepatic clearance of antigens and CIC would become defective; therefore, these substances would arrive in greater amounts in the kidney (14). Partial ligation of the portal vein and induction of portosystemic collateral circulation facilitated deposition of CIC within the mesangium of the portal circulation and development of collaterals, the of these antigens are degraded and excreted in the urine as clearance of any CIC is dependent on the physicochemical comparison with that in normal controls, probably due to sequestration of antigens complexed with antibody appears to be sequestered by Fc mechanisms (223). GASP complexes that are cleared rapidly by the liver (199). Anti-GASP IgM and IgG antibody levels diminish in chronically infected patients, and that change may favor the formation of intermediate complexes which are potentially nephritogenic in slight antigen excess (205, 261). The extent of hepatic clearance is also influenced by the cell types and receptor mechanism(s) involved. In the infected host, the bulk of CIC or antigens is sequestered by phagocytic Kupffer cells and perhaps by granuloma macrophages of the liver (199, 244), but sequestration of antigens complexed with IgA antibodies by hepatocytes must also be considered (223). GASP complexed with antibody appears to be sequestered by Fc receptor-mediated phagocytosis, whereas PSAP-containing complexes may be removed by means of galactose receptors that interact with the glycosyl residues on the antigen (198). Supposedly, if the hepatic clearance mechanisms are faulty, then circulating antigens or CIC can reach the glomeruli and be deposited on the capillary endothelium. As the infection progresses, the gut-associated antigens initially deposited on the glomerular basement membrane may advance into the mesangium of the glomeruli (104). The size and charge of the antigens are decisive for their localization and possible excretion. Thus, negatively charged CAA may be less prone to sequestration and degradation than the neutral CCA molecule (104). As yet, no information is available on the binding or toxic properties of the parasite polycarboxylate proteoglycan antigens. It is conceivable that some parasite-derived substances may be inherently toxic to glomerular endothelium or mesangial cells. In that case, mesangial hypertrophy and proliferation may ensue from deposited ingested antigens alone, without being complexed with antibodies. Indeed, conclusive proof for deposited IC causing glomerular alterations is still missing. Moreover, a clear relationship between the amount of CIC and glomerulopathy has not been established (51). Whether CIC are deposited from the circulation or IC are formed by the binding of antibody onto antigens deposited on capillary endothelium or glomerular basement membrane is still undecided (166). Data on the identity of antigens and isotype of antibodies and on the ability of complexes to bind complement are still sketchy. Nor is it known whether complement is bound solely by antigen-antibody complexes or directly by antigens and what consequences such alternate pathway complement activation may have on kidney pathology. Inconsistent findings, i.e., the presence of antigen but no antibody or that of immunoglobulin but no antigen, weaken the otherwise cogent hypothesis that implicates IC in the induction of renal pathology. Application of highly specific monoclonal antibodies for the identification of antigens within complexes, better IC elution techniques, definition of antibody isotypes, and examination of the interaction among antigens, IC, and mesangial cells should provide more direct proof for the participation of IC in schistosoma-induced renal pathology.

**Egg-Induced Granulomatous Pathology**

The chronic granulomatous host response around disseminated parasite eggs, aggravated by fibrosis, is the major contributor to the pathology of the disease (83, 200, 226, 228, 255, 279). Granuloma formation is initiated by antigens secreted by the miracidium through microscopic pores within the rigid egg shell (257). Eggs deposited in the circulation of the mesenteric venous plexus disseminate mainly into the intestinal tract and the liver, where each one evokes a granulomatous response. With advanced pathology, collateral circulation develops, eggs disperse systemically, and renal, pulmonary (7), or central nervous system granulomas form. Central nervous system granulomas can also arise from ectopic migration of worm pairs. Hepatic granulomas are initiated after the eggs lodge in the presinusoidal capillary venules. As the granuloma grows, the vesels are first disrupted, with the eventual displacement of hepatic parenchyma. The intestinal tract is the second major site for egg deposition. The mucosa becomes congested, the granulomatous regions increase in connective matrix content, and, rarely, the intestinal wall becomes thickened and constricted (112). In some humans, colonic polyps appear. These are frequent in Egyptian patients, but are absent in infected Brazilian individuals (68).

In clinical schistosomiasis, ethical as well as technical considerations (availability of tissues from biopsy or autopsy material) prevent longitudinal granuloma studies in individuals. Consequently, there is a dearth of knowledge on the composition, size, and modulation of granulomas in humans. Histologic examination of rectal tissue from chronically infected individuals has shown the regular cellular composition of granulomas (lymphocytes, macrophages, eosinophils, etc.), with a wide range of granuloma sizes around freshly laid, embryonated eggs. Examination of both the patients' granuloma sizes and the SEA-specific proliferative responses of their PBMN revealed a positive correlation, confirming the murine experiments (42, 81, 238). The granuloma-forming ability of PBMN from infected persons was also investigated in vitro, using freshly produced eggs from adult worm pairs maintained in culture (114). Recently infected persons have increased capacity for granuloma formation compared with their chronically infected counterparts. The OKT4+ T4 subset was involved in the generation of the granulomas (113).

There is strong evidence that the vigorous host granulomatous response rather than the direct action of a parasite egg antigen(s) is responsible for the pathologic tissue manifestations in schistosomiasis. Microcirculation studies in murine livers showed that the granulomas that form around eggs lodged in the presinusoidal capillaries impede hepatic
blood flow (33). Blockage of the portal blood system then induces portal hypertension. A comparative study of various animal species demonstrated a clear relationship between granuloma size and portal hypertension. Lightly infected mice that show extensive disease symptoms also develop portosystemic collateral circulation (66). Quantitative recovery of worms from autopsied patients has indicated that in some cases heavy worm burden induces collateral circulation through which eggs disseminate into the lungs, where they cause granuloma formation, pulmonary arteritis, and cor pulmonale (67). An additional contributory factor to portal hypertension is Symmers’ fibrosis, which develops around branches of the portal veins and appears mostly in adults after many years of infection (68). In heavy infections, continuous granuloma formation and fibrosis together with elevated portal pressure and intense immunologic activity lead to the development of hepatosplenomegaly, initially without derangement in liver function. This is the so-called compensated state of HS disease. In a certain number of patients who progress to decompensated HS disease, ascites fluid forms in the peritoneal cavity and esophageal varices develop. Rupture of varices causes esophageal and gastrointestinal bleeding episodes, which are usually fatal. In such patients, liver function may also lead to hepatic coma. Because jaundice is frequently present in such patients and persistent hepatitis B surface antigenemia is common (25), liver pathology in decompensated cases may be compounded by viral hepatitis (235).

Studies from several geographic locations indicate that severe morbidity appears in only a small percentage of the chronically infected population. Individuals with HS disease usually but not always carry a heavy worm burden, as ascertained by fecal egg counts (67, 92, 178, 253). Whereas this observation is uncontested, the questions remain as to why such individuals permit the development of a very heavy infection and how their immune responsiveness relates to the subsequent morbidity. Besides frequency of exposure (278), the genetic predisposition of individuals has been scrutinized. Development of severe morbidity differed in persons of various racial backgrounds (32). A tentative relationship between the development of HS disease and the occurrence of blood group A has been proposed (55, 182). Similarly, a correlation has been observed between susceptibility to hepatosplenomegaly and the expression of HLA-A1 and HLA-B5 histocompatibility antigens (1). These studies need further extension before conclusive evidence of correlation between genetic background and morbidity is obtained.

Observations in infected mice indicate that granuloma formation may be under local control within the various organs. Marine liver granulomas usually attain the largest size. The cellular constituents of the hepatic granuloma are as follows: lymphocytes, plasma cells, macrophages, epithelioid and giant cells, eosinophils, neutrophils, mast cells, and fibroblasts (254, 257). The sizes and cellular compositions of the granulomas may vary according to host species (3), organ site (283), phase of evolution or involution of the lesion (193), and immunoregulatory influences (sensitization and modulation) (37). The experimentally induced pulmonary granuloma is significantly suppressed by antimacrophage (46) and antieosinophil (189) sera, indicating that these cells are the major mediators of this inflammatory response.

Examination of intestinal granuloma sizes in lightly infected mice has revealed interesting differences. Whereas during the chronic stage of the disease granulomas of the liver and colon spontaneously diminished, the smaller ileal granulomas did not change in size (283). Moreover, passive transfer of spleen cells from chronically infected donors to acutely infected recipients decreased liver and colon granuloma sizes without affecting the ileal lesions. Isolated and dissociated intestinal granulomas analyzed in tissue culture showed that liver, but not colonic or ileal, granulomas produced macrophage migration inhibition factor. Moreover, the T-lymphocyte, mast cell, and eosinophil cell numbers within hepatic granulomas were much higher than those in colonic or ileal lesions (284). These observations indicate that the granulomas of the intestinal tract are under regional (ileal or colonic) control, which may regulate the size, composition, and perhaps timing of resolution of the lesions.

In addition to causing vascular obstruction and subsequent fibrosis, schistosome granulomas may be potentially harmful to adjacent parenchymal cells. The cell populations that comprise the granulomas are in a metabolically activated state, capable of secreting a number of tissue-destructive substances. During phagocytosis, cell activation, or cell death, such substances may diffuse from the granulomas and damage tissue parenchyma cells. Indeed, healed granulomas usually leave behind scar tissue, indicating focal damage and repair (271). Granulomas isolated from murine livers and cultured in vitro under aerobic conditions have been shown to secrete lysozyme and β-glucuronidase, as well as collagenase and elastase (224, 264). Macrophages isolated from dispersed hepatic granulomas also secrete neutral proteases (265), prostaglandins, leukotrienes, superoxide anion (76), interleukin-1, and alpha/beta interferon (123). Neutral proteases and superoxide anion can degrade or oxidize cell membrane constituents, with potential tissue damage. The possible tissue-destructive role of these agents in schistosome granulomas is still to be elucidated.

Experiments in infected mice that intended to find a correlation between morbidity and genetic background have yielded equivocal results. Examination of immunopathologic parameters (portal hypertension, hepatic granuloma size, and hepatosplenomegaly) in various inbred strains of mice showed strain dependence which was not linked to the H-2 region of the major histocompatibility complex. The F1 generation obtained from crossing high and low responders as well as backcrosses showed pathologic manifestations that indicated a polygenic influence (130). Another group has examined mortality, spleen index, dermal response, and lymphoproliferation in congenic mice and concluded that the H-2 haplotype and especially the 1 (immune response gene) region influence mortality and the intensity of humoral and T-cell-mediated granulomatous responses. Mouse strains with identical H-2 genes but genetically different backgrounds showed differences in the immune response (78, 102) (Table 1).

Though the role of granulomas in damaging tissue has been extensively studied, researchers have also explored to some extent the tissue-protective potential of this inflammatory response. Thus, infected immunosuppressed or congenitally athymic nude mice that lack immunocompetent T cells mount only feeble granulomatous responses to eggs, mostly composed of immature macrophages (6, 54, 128, 133, 227). One study showed that infected nude mice kept in aseptic environmental conditions developed less morbidity than their heterozygote littermates (227). This observation supported the notion that a granulomatous response of diminished intensity is favorable for the host. In contrasting studies, the granulomas of T-cell-depleted animals showed liquefactive necrosis in the liver and gut with severe destruction of tissue parenchyma, release of hepatic enzymes, and death (53, 108). Hepatic necrosis adjacent to the smaller
TABLE 1. Genetic influence on granulomatous inflammations

<table>
<thead>
<tr>
<th>Granuloma inducer</th>
<th>Murine strain used</th>
<th>Intensity of granuloma response</th>
<th>Genetic influence</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Schistosome eggs</td>
<td>C57BL/6J/BALB/cJ</td>
<td>Low</td>
<td>F1 generation and backcrosses with parents: polygenic influence</td>
<td>130</td>
</tr>
<tr>
<td>Schistosome eggs</td>
<td>C57BL/6/Nmri</td>
<td>High</td>
<td>F1 and F2 generations and backcrosses with parents: H-2 and other gene influence</td>
<td>70</td>
</tr>
<tr>
<td>Schistosome eggs</td>
<td>C3H.B10/C3H/Sn</td>
<td>Low</td>
<td>Congenic strains with different H-2 haplotypes: H-2 influence</td>
<td>102</td>
</tr>
<tr>
<td>BCG cell wall</td>
<td>C57BL/6/C3H/He</td>
<td>High</td>
<td>F1 generation and backcrosses with parents: H-2 and other gene influence</td>
<td>299</td>
</tr>
<tr>
<td>Killed BCG in emulsion</td>
<td>C57BL/6/CBA</td>
<td>High</td>
<td>F1 generations and backcrosses with parents: IgH and other gene influence</td>
<td>258</td>
</tr>
</tbody>
</table>

Granulomas was also observed in infected nude mice (54) and in animals treated with anti-L3T4 antiserum, which suppressed T-helper cell activity and granuloma formation (191). In the hepatic granulomas of nude mice, diffuse antigenic material was observed by IF. This lent support to the notion that one task of the well-developed granuloma is to sequester the potentially harmful protease-active (17) or other toxic (150) substances secreted by the live miracidium (272). In the absence of mature macrophages, and perhaps eosinophils, the secreted antigens, enzymes, or both diffuse into the liver parenchyma and damage hepatocytes. Another protective role envisioned for granulomas is egg destruction involving egg shell dissolution, invasion, and destruction of the embryo. The protective value for the host is the rapid elimination of the eggs, reduction in antigen contents and inflammatory episodes, and reduced tissue damage. Indeed, it was observed that mice and rhesus monkeys that received a sensitizing injection of eggs showed accelerated destruction of eggs within the liver after a challenge infection (71, 281). The major cell type that destroys the miracidium appears to be the eosinophil (35, 189, 209).

Viable eggs isolated from the liver granulomas of infected mice injected intravenously induce a primary pulmonary granuloma in normal mice (271). Analysis of this pulmonary model established the T-lymphocyte-mediated etiology of the egg granuloma. Granuloma formation is specific and occurs in an accelerated manner in mice presensitized with live eggs (45, 282). It is specific for the egg stage antigens because sensitization cannot be achieved by irritated cercariae or adult worms of a single sex. Granuloma formation is transferable to normal recipients by lymphocytes, but not by antibodies (282). It is suppressed by neonatal thymectomy (109) and antilymphocyte (110) or anti-T-helper lymphocyte (191) antiserum, as well as in thymus-deprived (133) or congenitally athymic nude mice (54, 227). Moreover, granulomas isolated from the livers of acutely infected mice release lymphokines during in vitro culture which act specifically on macrophages and eosinophils (48, 170). The requirement for T lymphocytes in granuloma formation and the correlation of the granulomatous response with other in vivo and in vitro parameters of T-cell-mediated immunity confirm that schistosome egg granulomas are T-lymphocyte-mediated immune responses.

This specific granulomatous response is induced by SEA secreted by the live miracidium (45, 153). These antigens extracted with buffered saline from homogenized eggs of the Puerto Rican strain of the worm contain numerous proteins, polysaccharides, and glycoproteins. In the absence of adjuvant, this crude preparation sensitized and induced granulomatous and dermal hypersensitivity in mice (45) and elicited dermal reactions in vivo and lymphoproliferation and lymphokine production in vitro (42, 43, 80, 81, 87, 191). SEA absorbed onto or covalently coupled to insoluble particles such as bentonite or latex also elicited pulmonary granulomatous responses in mice (47, 275). Because the importance of SEA in the immunopathology of the granulomatous disease is recognized, much effort has been invested in the isolation and identification of immunogenic and granuloma-inducing fractions. Over the years, several laboratories have used a variety of preparative methods to fractionate SEA. These include block and polyacrylamide gel electrophoresis, molecular sieving, cation exchange, and affinity and immunofinity chromatographies. These methods have yielded several antigenically active molecules that are heterogeneous in size, charge, and biologic activity (Table 2). Some anodic glycoproteins were capable of sensitizing mice for granulomatous hypersensitivity and elicited dermal responses in sensitized guinea pigs (44). At least three antigens were shown to have dermal activity in presensitized animals (52). By sodium dodecyl sulfate-polyacrylamide gel electrophoresis, at least 20 distinct protein bands were obtained from SEA, and of these 20 bands, 6 glycoproteins elicited lymphocyte blastogenic responses (62, 155). By a combination of lectin affinity and anion-exchange chromatography methods, three antigens were isolated from crude SEA. They reacted with antibodies from chronically infected mice and were named major serologic antigens MSA1, MSA2, and MSA3 (221). The highly purified MSA1, a glycoprotein with an estimated molecular size of 138 kDa, was specific for the egg stage and could be used as an immunodiagnostic reagent with sera of heavily infected humans (143, 222). It appeared to induce granulomatous hypersensitivity in normal mice (220). Another major egg glycoprotein isolated from the Egyptian strain of S. mansoni eggs had a molecular size of 70 kDa. It showed a wider antigenic reactivity, cross-reacting with antibodies directed to larval and adult stages of the parasite. Purified major egg glycoprotein elicited delayed dermal responses in acutely infected mice and, without adjuvant, induced granulomatous hypersensitivity in normal animals (151, 185). Immunoaffinity-purified intact or deglycosylated glycoproteins bound to Sepharose beads and injected into naive mice evoked hepatic granulomas, indicating that both peptide and glycosyl determinants are involved in the granulomatous response (287). Other antigenic sub-
TABLE 2. Antigenic preparations obtained from S. mansoni eggs

<table>
<thead>
<tr>
<th>Egg antigen prep</th>
<th>Biochemical properties</th>
<th>Molecular size (kDa)</th>
<th>Immunologic activity</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEA</td>
<td>Proteins, glycoproteins, polysaccharides</td>
<td>&lt;10–&gt;200</td>
<td>+ + +</td>
<td>44, 45, 62, 155</td>
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<tr>
<td>Major serological antigens</td>
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<tr>
<td>MSA1</td>
<td>Glycoprotein</td>
<td>138</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MSA2</td>
<td>Lipoglycoprotein</td>
<td>450</td>
<td>?</td>
<td>?</td>
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<tr>
<td>MSA3</td>
<td>Protein</td>
<td>80</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Major egg glycoproteins</td>
<td>Glycoprotein</td>
<td>70</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Polysaccharide egg antigen</td>
<td>Neutral polysaccharide</td>
<td>&gt;200</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>w4 Antigen</td>
<td>Protein</td>
<td>20–50</td>
<td>?</td>
<td>?</td>
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<tr>
<td>Allergen</td>
<td>Glycoprotein</td>
<td>210</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Immunoaffinity-purified antigens</td>
<td>Glycoprotein</td>
<td>&gt;40</td>
<td>+</td>
<td>+</td>
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</table>

stances obtained from fractionated crude SEA included a neutral >200-kDa polysaccharide egg antigen (34). A 20- to 50-kDa protein named w4 that has hepatotoxic effects in T-cell-deprived mice (120), and a 210-kDa allergen that reacted with IgE antibodies of infected humans (216). These antigens possess serologic activity, but their roles, if any, in granuloma induction, are unknown.

The SEA-induced circumsal granulomatous reactions are complex cellular responses that provide protection against the secretions of the disseminated eggs. The physical presence of granulomas and the substances secreted by granuloma cells cause focal tissue damage that terminates by fibrosis. Despite the chronic nature of the granulomatous condition, most infected individuals either are asymptomatic or suffer only mild symptoms of the disease. In a limited number of infected individuals, a complex syndrome develops in the form of HS disease. Many facets of this syndrome are still not understood. The outcome of HS disease depends on a series of immune and nonimmune events that include antigen processing and presentation; T-cell sensitization; lymphokine production; inflammatory cell mobilization, locomotion, and activation; monokine enzyme secretions; and collagen isotype synthesis and degradation. These functions are regulated by a variety of genes that are both outside and within the loci that regulate the immune response. Also, parasite product-host cell (parenchyma, fibroblast, and vascular endothelium) interactions may contribute to the complexity of the phenomena. Thus, it is plausible that, in addition to genes that regulate the intensity of the immune response, other genes should also influence the evolution and outcome of HS disease. Studies that analyze the genetic bases of the Mycobacterium bovis BCG cell wall-induced granulomas in mice also determined that the granulomatous inflammation is under polygenic influences (258, 299) (Table 1).

Fibrosis

Fibrosis enhances the disease pathology and contributes to the mortality of schistosomiasis mansoni. It follows the granulomatous inflammatory response (38, 116, 146, 229, 291) and occurs mostly at the site of the resolving granulomatous reactions. However, fibrous bands also appear around portal veins distant from granulomas. Portal fibrosis is partly a sequel to the chronic thrombophlebitis of portal veins. The portal vessels often show subendothelial edema, infiltration of the smooth-muscle layer with eosinophils, and local damage (148). In addition to portal fibrosis, collagen bundles are found in the space of Disse located underneath the liver sinusoids between the endothelium and the hepatocytes (147). Heavily infected patients who develop HS disease present with characteristic Symmers’ clay pipistem fibrosis, which appears as wide fibrous bands running along the portal veins (200). Liver function tests in HS patients remain normal because liver parenchyma between the fibrous bands is well preserved. Occlusion of portal venules leads to portal hypertension and to the formation of esophageal varices. Repeated bleeding episodes may cause ischemic necrosis of the liver (13). Analysis of the fibrotic process in humans has yielded some limited but interesting data. Measurement of collagen content and rate of collagen synthesis in fibrotic livers showed a 5- to 40-fold increase over that of normal livers (116, 117). In liver sections, immuno- cytochemical methods detected type I, type III, and B collagens in portal spaces, fibrous septa, and granulomas (30). Florid granulomas contained mainly type III collagen, and involving ones contained type I collagen (4).

Fibrosis has been extensively studied in the murine model of schistosomiasis mansoni. In mice, hepatic fibrosis is mostly associated with circumoval granulomas (15, 119). It is still controversial whether classical Symmers’ fibrosis develops during mild prolonged infection in this species (15). In infected experimental animals, liver egg load and total liver collagen content (measured by the amount of hydroxyproline in hydrolyzed tissue) stand in direct relationship (69). By contrast, a direct relationship between granuloma size and the degree of fibrosis could not be established. Mice splenectomized at the height of the granulomatous response responded with enlarged granulomas, but increase in granuloma size left total liver collagen content unchanged (164). Conversely, administration of Captopril, an inhibitor of angiotensin convertase, to infected mice suppressed hepatic granuloma sizes but actually increased collagen content (286). In various strains of mice, granuloma size and hepatic fibrosis were found to be unrelated. Both manifestations appear to be under independent control, involving genes both outside and within the H-2 region of the murine major histocompatibility complex (70).
TABLE 3. Host- and parasite-derived fibroblast stimulatory factors

<table>
<thead>
<tr>
<th>Source of factor</th>
<th>Biochemical property</th>
<th>Molecular size (kDa)</th>
<th>Biological function</th>
<th>Reference(s)</th>
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</thead>
<tbody>
<tr>
<td>Culture supernatant of liver granulomas</td>
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granuloma formation as well as reduced deposition of collagen fibers were demonstrated. Thus, a role for T lymphocytes in fibroblast recruitment and the induction of collagen production was indirectly confirmed (6, 191, 227). A recent report also suggests the possibility that autoimmune T cells participate in the fibrotic process. Peritoneal T cells of S. mansoni-infected mice proliferated in response to denatured collagen and formed large granulomas when the mice were injected with collagen-coated beads. Thus, during rapid collagen synthesis and breakdown in acutely infected mice, collagen-reactive T cells are generated that participate in anticolagen immune responses and thus contribute to chronic inflammation and fibrosis (293). However, initiation of fibroblast activity is not solely dependent on the cellular responses of the granuloma-bearing sensitized host. Some observations also implicate SEA as parasite factors that trigger fibroblast responses. Resting guinea pig or human dermal fibroblasts cocultured with graded doses of SEA underwent dose-dependent cell proliferation. Gel filtration of SEA yielded an active fraction with a 12-kDa molecule that stimulated fibroblast proliferation (297). Moreover, human dermal fibroblasts cultured in the presence of live schistosome eggs or SEA incorporated labeled proline into collagen. However, when larger numbers of eggs were used in the culture, cytotoxic effects were seen, i.e., cytoplasmic granulation, cell detachment, and death (40).

In conclusion, though the etiology of human pipestem fibrosis is still not understood, the host immune response appears to be involved in the overall process of fibrosis. Fibrogenesis is triggered mostly, but not solely, by the various cells that participate in the granulomatous inflammatory response. Deposition of nonfunctional fibrous tissue is a major contributory factor to hepatic pathology, HS disease, and host mortality. Fibrous tissue consists of a complex array of connective matrix material and a variety of collagen isotypes. The ratio of deposited collagens (types III/I) influences the stability and reversibility of the fibrotic condition (240). The final outcome of fibrosis is also dependent on the presence and activity of degradative proteases and blocking protease inhibitors. Reversibility of the fibrotic condition in drug-treated mice has been demonstrated (10, 194), but in humans this is a major, still unanswered question. This is especially important in endemic areas where mass antihelminthic therapy is being instituted. To evaluate the extent of fibrosis reliably, clinicians need a better tool than liver wedge biopsy. This invasive method is now being replaced by two new diagnostic tools. One is abdominal ultrasonography, which can visualize the fibrous perportal bands in the livers of patients with Symmers' fibrosis (65, 163), and the other is the measurement in the serum of procollagen III peptides as indicators of newly synthesized type III collagen (126, 237). Recent advances in the understanding of fibroblast activation pathways have provided some insight into the fibrotic process in the schistosome-infected host. Further advances should help investigators to devise means for the prevention or amelioration of fibrogenesis in infected individuals.

Regulation of the Immune Response

Over the years, correlations have been sought between morbidity and the intensity of immune responsiveness of S. mansoni-infected individuals. Comparisons have been made between patients at the acute or chronic stage of the infection, and the mild intestinal versus HS forms of the disease have been examined. Schistosome antigen-induced lymphoproliferation of patient PBMN has been the most widely used immune parameter. In general, acutely infected patients have been shown to respond vigorously to adult worm and egg antigens. In contrast, the antigen-specific response is greatly diminished in chronically infected persons (83, 84, 213). In some cases, the SEA-specific responsiveness has been found to be higher than that directed to soluble adult worm antigens (SWAP). SEA could also elicit production of leukocyte-inhibitory and mitogenic factor lymphokines (140). In chronically infected patients, numerous studies have addressed the question of immune responsiveness versus intensity of infection. In examining PBMN proliferative responses to cercarial antigens, SWAP, or SEA, either no relationship (85, 267) or an inverse one has been seen in heavily infected patients (125, 131). The level of heavy infection appears to be a crucial factor, because PBMN of patients excreting 600 eggs per g of feces reacted well to SWAP after adherent cell removal, whereas those who excreted double that number of eggs failed to respond (208). The diminished reactivity to PBMN is attributed to a variety of modulatory influences detectable in chronically infected persons. Adherent cells present in their PBMN suppress the proliferation of blood lymphocytes. Adherent cell removal usually enhanced lymphocyte reactivity to SWAP, SEA, or cercarial antigens (137, 212, 262, 266). Indomethacin relieves the suppression in cell cultures, indicating that the inhibitory effect is prostaglandin mediated in persons with heavy, but not very heavy, infection (23, 208). A comparative study examining PBMN and splenocyte reactivity to SEA in splenectomized HS patients contradicted previous results and concluded that adherent cells performed an accessory rather than an inhibitory role in antigen-specific lymphoproliferation (236). Adherent cell-mediated suppression was not seen in ambulatory HS patients (266, 267). An effect of suppressor T (Ts) cells was also postulated: the Ts cells acted in both an antigen-specific and a nonspecific manner. Lymphocytes of chronically infected persons incubated with SEA generated cell populations that suppressed mitogen- or SEA-induced lymphoproliferation (89, 239). This in vitro activation of SEA-specific Ts cells was achieved both by SEA and by a protein moiety isolated from crude egg antigens; the induced suppressor activity was greater in patients with high- than in those with low-intensity infection (239). Coculture of splenic cells of splenectomized patients with autologous or allogeneic PBMN demonstrated a predominant antigen-related and a minor mitogen-related Ts cell activity (124). Exogenously added or endogenously liberated histamine exerted strong suppression over the antigen-induced and the mitogen-induced lymphocyte responsiveness of infected individuals (162). Addition of cimetidine (an H2 histamine receptor antagonist) significantly increased the PBMN responsiveness to SEA and SWAP; histamine was therefore suggested to participate in T-lymphocyte regulation (221). Histamine receptor-bearing Ts lymphocytes were also described to function in suppression of murine liver granulomas (285). Heavily infected individuals, some of whom display the HS syndrome, usually have a low T4+/T8+ (helper/suppressor T cell) ratio in the circulation due to a decrease in the T4+ cell population. Presumably, the changed ratio favors enhanced systemic immunoregulation by Ts cells (88, 137, 138, 267). Conversely, when such a ratio favors the T4+ helper effector population, a strong inflammatory and inadequate Ts regulatory influence is postulated, a condition conducive to the development of HS disease (267).

The presence of humoral circulating regulatory factors has
been also demonstrated in humans. The suppressed PBMN responsiveness to SWAP or SEA could be alleviated after autologous plasma was replaced by normal human serum (87, 143, 214, 238, 263). Some humoral factors that appeared in chronically infected persons acted in an antigen-specific suppressive mode; others acted nonspecifically, suppressing mitogen-induced lymphoproliferation (94, 171). It is therefore probable that several different circulating substances are responsible for these effects. Thus, all of the following have been implicated in suppression: shed (174) and excreted parasite antigens (57, 103), circulating immune complexes (171, 238), and immunoaffinity-purified anti-SEA idiotypic antibodies (180, 218). The latter observation is intriguing in that it indicates the existence in chronically infected patients of functional anti-idiotype-bearing T cells and of idiotype-anti-idiotype interactions.

It is still a challenging task to establish a correlation between the degree of immune responsiveness and the morbidity seen in HS patients. In such persons, IgG and IgE antibody levels to schistosome antigens remain high (51, 136). The same is true for certain circulating antigens and CIC (see preceding section). In a high percentage of HS patients, vigorous delayed skin responses to adult worm or SEA preparations are observed (56, 158). Some individuals also retain a high degree of PBMN responsiveness to SEA or SWAP (125, 157, 238). Yet the patients may have T3 lymphocytes (124), suppressor adherent cells (137), or low T4"/T8" cell ratios (137, 267) or can be completely unresponsive to schistosome antigens (125, 137).

An analysis comparing ambulatory and hospitalized HS patients revealed interesting data on immune lymphoproliferative responsiveness (86). Subdivision of patient material into moderate and high PBMN responders to SWAP, SEA, and cercarial antigens revealed that the proportion of high SEA responders was low (23%) among the mostly asymptomatic intestinal patients, moderate in the hepatointestinal group (40%), and very high (67%) in the ambulatory HS group. Responsiveness rapidly declined (20%) in the hospitalized HS group. Based on these data, an attractive hypothesis was advanced that linked immune responsiveness to SEA with the progressive development of clinical disease. During the acute phase of the infection, all patients show strong T-cell-mediated anti-SEA responses. As the infection proceeds to its chronic phase, most patients modulate their responses by a variety of regulatory mechanisms. This down-modulated anti-SEA T-cell response may prevent sustained vigorous granuloma formation and the development of overt pathologic manifestations. In a minority of patients, inadequately controlled anti-SEA responses may allow the continued strong granulomatous reaction and development of organomegaly that progresses to severe hepatosplenic morbidity. In such severe cases, patient lymphocytes may show anergy, a generalized unresponsiveness to immune and mitogenic stimuli (86). This hypothetical sequence of disease development parallels the mechanisms of granuloma immunomodulation described to occur in infected mice. Its confirmation by additional clinical data should greatly advance our understanding of the regulation of the egg-directed granulomatous response in schistosome-infected humans.

A review of the clinicoimmunological studies reveals disparate, sometimes contradictory results. Several factors may be responsible for the observed variability of data. First, the lack of standardization of the antigenic preparations and cultural conditions used provides ample room for divergent contradictory results. Second, the patients are heterogeneous and represent a spectrum of pathologic manifestations. Hepatosplenic disease is often diagnosed according to hepatosplenomegaly, which is only a partially reliable index because, in regions where concurrent bacterial, protozoan, and viral (hepatitis) infections are common, liver enlargement is widespread (25). Third, the parameter PBMN blastogenesis, chiefly used for assessment of immune responsiveness, may not be wholly representative of other T-lymphocyte functions (lymphokine production) within lymphoid organs.

In murine experiments, examination of a different immune parameter, lymphokine production and regulation, has provided an insight into the granulomatus process (37, 81). A similar approach should also be profitable in clinical analysis, because there is a dearth of information on lymphokine production in schistosome-infected humans. Production of migration inhibition factor (157), leukocyte inhibitory factor (181, 289), eosinophil stimulation promoter (173), and mitogenic factor (139) has been described in schistosome-infected patients. A careful analysis of lymphokine production and differential regulation in the intestinal, hepatointestinal, and hepatosplenic groups should provide more incisive information on T-cell responsiveness and its relationship to granuloma formation and morbidity. Finally, the relevance of data on the immune responsiveness of circulating T cells to assess the immune functions of the highly compartmentalized granuloma lymphocytes is questionable. In patients with sarcoidosis (a granulomatous disease of unknown etiology), comparison of blood and lung lymphocyte functions clearly revealed that only data on lung lymphocyte responsiveness were useful in evaluation of the pulmonary granulomatous process.

In the murine model, heavily infected mice develop the HS syndrome (105, 276). In contrast, lightly infected mice tolerate the infection and during the chronic stage of the disease react with significantly diminished hepatic granulomatous responses around freshly deposited eggs (15). Because periodic injections of eggs into normal mice diminished the initially strong pulmonary granulomatous response, an immune "desensitization" process was proposed to have occurred (111). This spontaneous down-modulation of the granulomatous response was extensively analyzed by longitudinal studies at 4 to 20 weeks of the infection. At the peak of the granulomatous response (8 to 10 weeks), delayed dermal reaction and production of the lymphokines, macrophage migration inhibitory factor, and eosinophil stimulation promoter were strong. Lymphokine production declined concurrently with the waning of the granulomatous inflammation (12 weeks and onward). At that time, the specific anti-SEA antibody response was still high (42, 81). Because the diminished granulomatous response could be passively transferred from modulated donors to recipients exhibiting strong granulomatous reactions with lymphocytes but not with antiserum, the cell-mediated immune basis of the modulation has been established (82, 90). Concurrent with the waning granulomatous response, a shift in the ratio of T/B lymphocytes (favoring the latter) was observed in the blood, lymph nodes, spleen, and granulomas of the chronically infected mice (72), and the number of anti-SEA antibody-producing plasma cells in the smaller granulomas increased (39).

By means of extensive passive transfer, selective cell depletion, and cell admixture experiments, a complex network of interacting T-lymphocyte subsets was delineated. A T-helper/effector lymphocyte subset (T helper) having the Lyt1 L3T4+ markers was found to be involved in the induction and maintenance of the granulomatous response, whereas
another T subset bearing the Lyt2.3 marker was identified as the T\(_S\) that regulates the function of the former (37, 73). A third cell population bearing the Lyt1\(^+\) marker and capable of transferring granuloma suppression from modulated donors to vigorous recipients was classified as a precursor or inducer of T\(_S\) lymphocytes (37, 77). Regulation of the granulomatous response has also been examined and confirmed by the in vitro model of granuloma formation that utilizes eggs or SEA-coated beads and primed splenic lymphocytes. After a few days in culture, cellular aggregates ranging in intensity from a few cells to complete circumsomal granulomas surround the antigenic nidi. This model confirmed that Lyt1\(^+\) and Lyt2\(^+\) cells, respectively, play an inductive or suppressive role in granuloma formation (114, 115). Because admixture of T\(_{DH}\) and T\(_S\) splenic lymphocytes abrogates migration inhibitory factor lymphokine production in vitro and granuloma formation in vivo, the immune basis of the regulatory mechanism has been confirmed (74, 75).

Apparently, the effector-suppressor T lymphocytes communicate by means of soluble mediators that, in part, are products of the H-2 region of the murine major histocompatibility complex. One suppressor factor obtained from antigen-pulsed spleen cells of chronically infected mice was identified as bearing I-EC subregion determinants of the H-2 complex. It suppressed the in vitro migration inhibitory factor production by Lyt1\(^+\) cells in an antigen-specific and H-2-restricted manner (75). Another suppressor factor, added to splenic cultures of vigorous granuloma producers, recruited from a low number of precursor cells a T-suppressor population that suppressed the granuloma-inducing ability of T\(_{DH}\) lymphocytes. This suppressor factor bore the I-J\(^+\) marker and recruited I-J\(^+\) T cells (190). This finding corroborated previous observations that showed reversal of the down-modulated granuloma response by injections of anti-I-J antisera in chronically infected mice (145). The I-J\(^+\) suppressor factor preparations that reportedly suppressed in vivo granuloma formation only in I-J homologous recipients appear to be composed of two chains, one bearing the I-J phenotype and the other having idiotypic (2) or anti-idiotypic activity (225). The latter observation would be consistent with the finding that L3T4\(^+\) lymph node T lymphocytes of acutely or chronically infected mice display anti-idiotypic specificity (231). The significance of this finding in the modulation process remains to be elucidated.

In summary, granulomatous inflammation in infected mice is in a state of dynamic equilibrium maintained by inflammatory T\(_{DH}\) and regulatory T\(_S\) lymphocyte subsets. Following oviposition, egg antigens are presented by macrophages in the context of class II-A and I-E antigens, and a specific T\(_{DH}\) response is induced (122). The strong T\(_{DH}\) activity engenders inflammatory lymphokine (migration inhibitory factor and eosinophil stimulation promoter) production. Lymphokines recruit, mobilize, and activate a variety of inflammatory cells that converge around the eggs, create the granuloma, and eventually destroy the ova. Peak granuloma formation (8 to 10 weeks) is followed by the onset of modulation in which T\(_S\) cell activity prevails, resulting in diminished but not abrogated granuloma formation (14 weeks and onwards). Concurrently, local and systemic lymphokine production is decreased. The change in the quantity and quality of lymphokines accounts for the lower activation state of granuloma macrophages as reflected by receptor expression on the cell surface (288), tumoricidal activity (183), and O\(_2\)\(^-\) production (76). The modulated state is actively sustained by newly recruited suppressor T cells that regulate T\(_{DH}\) but not T\(_S\) cell function as demonstrated by the high levels of circulating antibodies (42, 81). Experimentally, the strong T\(_{DH}\) or modulated T\(_S\) influence can be reversed by a variety of interventions, proving that the respective cell populations are not eliminated, but rather are under active regulation (37). The diminished, but not abrogated, granulomatous response is advantageous to the host. Whereas the protective sequestrational role of the granuloma is retained, its smaller size and less activated cell populations lessen the possibility of tissue damage.

CONCLUSIONS

Schistosomiasis mansoni is a chronic helminthic disease of the tropics. In the majority of individuals, the infection is well tolerated but some (mostly heavily infected) persons develop HS disease often accompanied by severe morbidity and even death. Most of the host morbidity is the result of the parasite antigen-specific host immune responses that are expressed as inflammatory reactions. These include dermatis, IC formation, and granulomatous pathology. During the acute phase of the infection, parasite antigens evoke strong humoral and T-cell-mediated immune responses. As the disease advances to chronicity, the level of host responsiveness is diminished. This is conspicuous in experimental murine schistosomiasis in which the T-effector cell-mediated granulomatous response is down-regulated by T-suppressor lymphocytes with salutary effects on morbidity and mortality. In chronically infected humans, peripheral T-lymphocyte responsiveness to parasite antigens is diminished due to the appearance of a variety of immunoregulatory mechanisms. Whether such mechanisms also regulate the intensity of granulomatous responses and whether a down-regulated granulomatous response ensures a mostly symptom-free disease state are still conjectural. The chronic circumoval granulomatous inflammations and the fibrotic sequel are the main causative factors in the pathology of the disease. Whereas the granulomatous responses wax and wane, fibrosis is cumulative and mostly irreversible. In its extreme form (Symmers' fibrosis), it contributes greatly to the systemic manifestations of the disease. Advances made in the understanding of the biologic functions of purified schistosome antigens, the processes of granuloma formation, modulation, fibrous tissue production, degradation, and resorption will help to ameliorate or prevent morbidity in schistosome-infected humans.

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268


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