HEPATOSPLENIC SCHISTOSOMIASIS MANSONI: AN IMMUNOLOGIC DISEASE*

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HEPATOSPLENIC schistosomiasis is a disease caused by a misplaced product of a parasitic worm. This elicits in the host an inappropriate immunologic reaction, resulting in inflammation, scarring, and obstruction to bloodflow.

Schistosoma mansoni and *S. japonicum*, the two of the three major human schistosomes which are associated with hepatic disease in man, infect more than 100,000,000 people in Africa, South America, and the Caribbean.[1] Schistosomes are trematodes, organisms which alternate generations between snail intermediate hosts within which they multiply and mammalian definitive hosts within which they do not undergo direct replication. Under natural conditions, therefore, human definitive hosts carry different levels of infection; in most cases worm burdens are low, and it appears that only those with heavy worm burdens will develop overt disease of the liver. Hepatosplenic schistosomiasis is characterized clinically by enlarged liver and spleen, portal hypertension, and esophageal varices; liver function is usually normal. Pathophysiologically, there is an intrahepatic presinusoidal block to portal bloodflow, in the presence of normal total hepatic bloodflow. There is marked portal fibrosis, but the lobular architecture is intact and the parenchymal cells of the liver are normal. Many aspects of the pathogenesis of this syndrome have remained in question, including the parasite factor which initiates the changes, the role of the host's response to the parasite, and the way in which these factors result in altered hepatic circulation.

The development of an animal model of hepatosplenic schistosomiasis has elucidated all the above questions.[2] Mice are exposed to varying numbers of the infectious schistosome cercariae. These organisms pene-

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trate the skin, migrate to the lungs, and then to the liver. Here they grow into adult worms and mate. They pass down into the mesenteric venules, where the pairs of worms produce large numbers of eggs daily for many years. The eggs secrete enzymes, which facilitate penetration through the tissues into the lumen of the intestine. More than 50% of the eggs remain in the body, a large proportion of them breaking free in the blood vessels to be sieved out in the liver. The worms ingest red blood cells and regurgitate insoluble break-down products of hemoglobin which are taken up by the reticulo-endothelial cells of the liver. When the worms die they pass upward into the liver as large emboli. It had been postulated by different investigators that each of these factors is responsible for the development of hepatic disease.

Mice with *S. mansoni* or *S. japonicum* infections develop hepatosplenic disease characterized by hepatomegaly, splenomegaly, portal hypertension, esophageal varices, and relatively normal liver function. In early infections there are large discrete granulomas scattered throughout the liver; in long-term infections there is marked periportal fibrosis which resembles in microscopic appearance the classical lesions described in man.[3] Ultramicroscopically, the hepatic parenchymal cells of infected animals show no significant changes in subcellular constituents.[4] Total hepatic bloodflow remains within normal limits in mice with advanced hepatosplenic schistosomiasis.[5] It has recently been demonstrated that this is due to compensatory hypertrophy of the hepatic arterial system.[6]

Using the development of the hepatosplenic syndrome as an endpoint, we initiated a series of studies to determine the parasite factor which was responsible for the development of disease. By manipulating the infection, we were able to study the effect of eggs (bisexual infections), of toxins produced by living worms (unisexual infections), and of dead worms (drugs). Hepatosplenic schistosomiasis appeared only in animals in which eggs were produced.[7] Studies with a drug which reversibly stops egg production by the worms confirmed these findings.[8] The question then arose as to whether the eggs alone or the host's inflammatory and fibrotic reaction to the eggs could be held responsible for the alterations in hepatic circulation. Microcirculation studies in living animals revealed that schistosome eggs trapped in the interlobular portal venules obstructed blood flow only partly.[6] The granuloma which formed around the egg caused extensive damage to the hepatoportal vascular system. This lesion was initially avascular, but as healing and

scarring occurred there was neovascular formation which was wholly arterial in origin. Thus the inflammatory and fibrotic reaction in the host was the main factor responsible for obstruction to portal venous flow and its sequelae—splenomegaly and the establishment of portal-systemic collateral circulation.

Since the reaction of the host was playing such an important role in the pathogenesis of hepatosplenic schistosomiasis, we instituted a series of experiments to determine the etiology of the granulomatous inflammation around the schistosome egg. Our interest in this area was whetted by a preliminary study in which we demonstrated that immunosuppressive drugs would markedly inhibit the formation of granulomata.[9] At this point we selected three successive goals to determine the occurrence of: 1) sensitization—a difference in response in animals previously exposed to eggs, 2) specificity—an altered response specific to the schistosome eggs, and 3) passive transfer—sensitization transferred to recipient animals with serum or with cells derived from lymph nodes and spleen. It was then demonstrated that mice injected intraperitoneally with schistosome eggs two weeks prior to intravenous challenge showed markedly accelerated and augmented formation of granulomata, that this reaction was specific in cross-sensitization studies with *Ascaris* eggs, and that sensitization could be transferred between histocompatible mice by means of cells taken from lymph nodes or spleen but not by means of serum.[10] These results strongly suggested that the schistosome egg granuloma was essentially a cell-mediated type of immunologic response or, in other words, a manifestation of delayed hypersensitivity.

Studies with a series of immunosuppressive measures provided confirmation for the above findings in that measures which primarily inhibit cell-mediated reactions, e.g., neonatal thymectomy,[11] antilymphocyte serum,[12] and advanced Hodgkin's-like tumors,[13] almost eliminated the formation of granulomata, while those which largely inhibit antibody formation, e.g., chronic x-irradiation[14] and Friend-virus leukemia,[13] had no effect. Studies done in the guinea pig revealed close correlations among granuloma formation, delayed cutaneous responses, and *in vitro* correlates of delayed hypersensitivity, such as transformation of lymphocytes and inhibition of macrophage migration—the latter being studied with soluble antigens extracted from the schistosome eggs.[15] During the period in which these reactions were being investigated no γ_1 or γ_2 antibody to the antigens involved could be detected by either

passive cutaneous anaphylaxis or passive hemagglutination, respectively.

Granulomatous hypersensitivity to the schistosome eggs is both induced and elicited by the secretion of soluble antigens through the ultramicroscopic pores in the eggshell. Eggs within Millipore diffusion chambers, placed intraperitoneally, sensitized mice to eggs subsequently injected into the pulmonary microvasculature.[16] Sensitization was also achieved by the injection of soluble egg antigens (SEA) obtained from eggs maintained in tissue culture for several days, eggs stimulated to hatch in fresh water, or eggs macerated in a tissue grinder followed by ultracentrifugation. SEA has been used in minute amounts without adjuvant to induce sensitization to granuloma formation and delayed footpad swelling in mice. It elicits delayed skin reactions, lymphocyte transformation, and production of migration inhibition factor by cells derived from the spleens of infected animals.[15] With respect to the mechanism of granuloma formation, it has been demonstrated that SEA elicits production of lymphokines by intact schistosome egg granulomas maintained *in vitro*.[17]

SEA is undergoing purification by electrophoresis, column chromatography, and isoelectric focusing. It appears to have a molecular weight above 50,000 and is destroyed by trypsin. Complete purification and characterization of this antigen will take us to the molecular level in our understanding of the pathogenesis of hepatosplenic schistosomiasis.

As has been stated in an earlier paragraph, the immunologic response of the host to the schistosome eggs has been shown to be a key event in the pathogenesis of hepatosplenic schistosomiasis mansoni. While similar reactions occur in other infectious diseases, there is a facet of schistosomiasis that uniquely qualifies it for consideration as an immunologic disease. In the case of tuberculosis, for instance, the cell-mediated responses which result in tissue damage and disease also restrain the multiplication and dissemination of the bacillus. As the schistosome egg neither multiplies nor disseminates—it lives in the tissues for only three weeks and then is slowly resorbed—the severe reaction of the host to its presence is largely harmful. Therefore, since schistosomiasis is produced largely by the host's granulomatous response to a nonmultiplying, relatively harmless, biodegradable organism, and this response is primarily a cell-mediated immunologic reaction, it is proposed that schistosomiasis is essentially an immunologic disease.

To conclude this brief essay on a hopeful note, it should be pointed

out that we have demonstrated the natural modulation of the granulomatous response in both schistosomiasis mansoni and japonica, and in the case of the latter the alleviation of disease.[18, 19] Using SEA we have been able to induce immunological tolerance to the formation of granulomata by intact schistosome eggs[20] and are now attempting to induce immunological blockade (enhancement). Thus, by interfering with specific immunopathological reactions we may be able to protect animals against development of the disease without any alteration in the state of the infection.

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