ARTIFICIAL INDUCTION OF CONCOMITANT IMMUNITY AGAINST *SCHISTOSOMA MANSONI* IN MICE

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Introduction

Schistosomiasis is a parasitic disease which affects men and other mammals in tropical and subtropical regions of the world. Currently, there are approximately 200 million reported cases of human schistosomiasis in 71 countries or areas (WHO Chronicle, 1976). Unlike the parasitic diseases of malaria and hookworms which currently seem to be on the decline, schistosomiasis is on the upgrade (Cheng, 1973). Schistosomiasis in humans is caused by three species in the genus Schistosoma (S. mansoni, S. haematobium, and S. japonicum). Schistosomes are dioecious digentic trematode flukes that live in the blood of their hosts. The three species of schistosomes inhabit different areas in the world with some overlap. This distribution seems to be dependent upon two factors. The first is, naturally, whether or not the parasite has been spread by infected individuals into a region. With the advent of a highly mobile world society, schistosomiasis is no longer a disease of distant lands. For instance, occurrences of infection with schistosomes have been reported in considerable numbers in metropolitan areas such as New York, Chicago, and Philadelphia (Cheng, 1973). Thus, the second factor upon which distribution of schistosomiasis is dependant, the presence of the proper snail intermediate host (which is different for different species of schistosomiasis), is the limiting factor in the incidence of the disease.

The life cycle of Schistosoma begins when the adult female, living in copula with the adult male in the portal circulation veins of the host, lays her eggs. The number of eggs laid by an individual

female is between one and several hundred per day (Edington and Gilles, 1976). After the eggs are laid in the blood vessels, they secrete proteolytic enzymes which enable them to pass through the intestinal (S. mansoni and S. japonicum) or bladder (S. haematobium) wall and pass out in the feces or urine of the host.

In areas lacking proper sanitation (often most highly prevelant in poor tropical regions where the intermediate snail host thrives), the eggs easily reach fresh water which is needed for the continuation of the life cycle. With the change in pH and temperature of the water, the eggs quickly hatch to release a free swimming, ciliated, larval from known as a miracidia. The miracidium swims in the water until it locates a suitable snail intermediate host. It then penetrates through the body surfaces of the snail and sheds its outer epithelium, becoming transformed into a second larval stage known as the mother sporocyst. The mother sporocyst develops germ balls through mitotic division which differentiate into numerous daughter sporocysts. Likewise, germ balls which develop in the daughter sporocyst differentiate into large numbers of furcocercous (fork-tailed) cercaria which are infective larval stage to the primary host. Thus, through the differentiations which occur in the snail, a large number of cercaria stem from a single miracidium. This amplification of infective forms is a common parasitic manifestation for self-preservation.

Following their development, the cercariae pass out of the daughter sporocysts through the birth pore, leave the snail, and become free swimming in fresh water. Upon location of a suitable

mammalian host, the cercariae explore the surface of the skin and attach to penetrate at irregularities (Stirewalt and Hockley, 1956) using enzymatic secretions (Stirewalt and Evans, 1952) and alterate elongation and contraction (Gordon and Griffiths, 1957). Upon penetration, the cercaria loses the furcocercous tail and undergoes other physiological changes to be transformed into the post-penetration larval stage known as the schistosomulum.

The schistosomulum migrates from the skin into the blood stream and into the lungs via the heart. From the lungs, the schistosomules migrate to the sinusoids of the liver where they mature for a period of time and then migrate to their ultimate locus of infection, complete their development, and mate. In S. mansoni and S. japonicum, this locus is the mesenteric veins while S. haematobium eventually inhabits the mesical, prostatic, and uterine plexuses. During the migration from the skin to the adult infection site, the schistosome undergoes great morphological and physiological alterations which are very significant in the host-parasite interaction. Throughout the parasite's existence in the definitive host, the schistosome feeds on human red blood cells.

Although the parasite's diet would seem to be a major debilitiating factor to the host by causing anemia, the true pathological manifestations of this disease are caused by the schistosome ova. As previously stated, the eggs are laid in the portal venous system. The migrating eggs secrete proteolytic enzymes which cause much tissue damage and evoke an inflammatory immunologic response. Giant cells and epithelioid cells surround the eggs

and are later surrounded themselves by loose fibrous tissue (Wilkcocks and Mason-Bahr, 1972). These fibrous nodules are known as granulomas. Often, the eggs calcify and the granulomas coalesce, forming lesions from which no organ in the body is exempt (Edington and Gilles, 1976). The most intense as well as most destructive locus of granuloma formation is in the liver. A textbook description of hepatosplenic schistosomiasis is virtually identical with that of severe liver cirrhosis (Warren 1967). "Increasing fibrosis in the liver causes it to shrink. The portal hypertension due to cirrhosis causes the spleen to enlarge and acites developes". (Adams and Macgrath, 1964). The implications of the resulting liver and spleen malfunctions are obvious as these organs are the most vital in the body in terms of proper metabolic function.

In addition to the personal physical health aspect of schistosomiasis, this disease has several implications in other areas of community health. For instance, individuals who do not have access to sanitary water supplies (usually as a result of poverty) often must obtain water and bathe in waters which are infested with cercaria. If an individual contacts the disease, he is not able to work as efficiently, thus increasing poverty for himself, his family, and his community. Many times, the infestation in a body of water will become so severe that it must be shut off from public use. Closures of public water supplies cause a further demise in the welfare of the local community. Like the incidence of schistosomiasis, these closures have also been on the rise in recent years (WHO Chronicle, 1980). The real mystery of schistome

infections is their ability to survive and even thrive in the normally aseptic and immunologically hostile environment of the circulatory system. The definitive method by which they evade the host's immune response is not known. However, there are many theories which are very plausible in describing this phenomenon and the actual immune escape mechanism is probably a combination of two or more of these theories.

The first of these theories is the concept of host antigen absorption suggested by Smithers, et. al., (1968). In this theory, the adult worm evades the immune response by absorbing host antigens onto its tegument and thereby appearing non-foreign to the immune system. The primary activity of the immune system is regulated by its ability to distinguish self from non-self. Thus, if the immune system cannot identify something as foreign, it cannot mount an immune response against it.

The second theory for immune evasion is like the first in terms of making the parasite indistinguishable from self. However, in this theory, the parasites are postulated to have the ability to mimic host antigens. This theory was suggested by Damien (1964) and is referred to as molecular mimicry.

A third possible immune escape mechanism of shcistosomes also involves the adult worm tegument which is the site of attack when schistosomes succub to immunity (Hockley and Smithers, 1970). The adult worm tegument is composed of an unusual double-layered membrane which is probably continouosly shed (Ogilvie and Wilson, 1976). If this were the case, any host immunologic attack on the worm tegument

could simply be sloughed off before is was able to do any real damage. Extending the possible efficiency of the worm one step further, the worms may not shed the entire outer membrane but may only shed those areas of tegument which are directly under immune attack (Kemp, Brown, Merrit, and Miller, 1980).

A fourth and even more advanced theory of immune evasion is that of secretion of soluble factors by the adult worm which disrupt the natural function of the host's immune response. It has been shown that schistosome hosts often have a depressed ability to respond immunologically to antigens unrelated to schistosomiasis (as well as to schistosome antigens). Specifically, the regulation of IgG production is often awry in parasite-infected hosts and the production and action of suppressor T cells in enhanced (Ogilvie and Wilson, 1976).

Even more interesting than the possible mechanisms of the adult worm immune evasion is the occurence of concomitant immunity. It has been shown that although the mammalian host is incapable of successfully combating adult worms and eliminating them from its body, a secondary exposure to cercariae is met with a powerful resistance to reinfection (Smithers and Terry, 1965). As in tumor immunology, in which concomitant immunity was originally demonstrated (Gershon, Carter, and Kono, 1967), inter-host transfer of schistosomes has shown that adult worms provide the major immunologic stimulus for this acquired immunity against reinfection (Smithers and Terry, 1967) and that host worms are not affected by this immunity, even though they induce it. The reduction of the number

of schistosomula recovered from the lungs of infected mice challenged 12 weeks after primary exposure as compared with the survival of primary infection schistosomula has been shown to be between 50-60% (Sher, Mackenzie, and Smithers, 1974) and up to 90% (Perez, et. al.,1974).

This type of immune response by the host is very important to the survival of the parasite in the host. The barrier to continuous infection affected by concomitant immunity prevents overcrowding of the parasite in the host. If the population of adult schistosomes was not controlled, the extreme pathology incurred by the high infection would decrease the longevity of the host and therefore that of the parasite as well.

The most likely explanation for the occurence of concomitant immunity is that the young schistosomula have not developed the ability to evade the host's immune response and are consequently killed by it. An experiment reported by McLaren et al. (1975) has shown that schistosomula are able to evade an in vitro immune response as early as 4 days postinfection while 3 hour schistosomula are killed. An alternate theory for the mechanism of concomitant immunity, which is unpleasant from the immunoparasitological viewpoint, is the possibility that the adult worm may produce a substance which is nonimmunogenic but is toxic to the larvae (Kemp, 1977).

If the mechanism of concomitant immunity is indeed immunological (as most schistosomologists believe), it may be possible to artificially induce concomitant immunity and thereby protect

a potential from even a primary infection.

Before attempting to artificially induce a state of concomitant immunity, the theory why concomitant immunity exists *in vivo* must be examined. Most likely, due to their common genetic material, the adult worms and schistosomula have some sort of common antigenic determinant on their teguments. An antibody specific for some such common antigeni determinant would therefore direct an immune response against both an adult worm and a schistosomulum. As the adult worm has the abilty to evade host immune responses,it would be only slightly or not at all affected by this immune response. The young schistosomule, however, would be unable to escape the host's immune action and therefore most likely be killed. Thus, when a host is sufficiently sensitized to an antigen present on the schistosomule's tegument, it repels the infection. In natural infections, the host's immune system is not quick enough to respond to such an antigen and mount an immune response directed against the schistosomule before the parasite is able to develop immune evasion capabilities.

Therefore, in order to attempt to artificially induce concomitant immunity, an antigenic determinant from the schistosomule could be isolated and injected into a non-infected "host" in order to attempt to sensitize that individual to the antigen prior to the cercarial attack so that when such an attack occured, the individual's immune response could easily repel it. Thus, it seems as if the problem could be solved with simple vaccination techniques using cercaria to sensitize individuals against cercaria. However. whole schistosomules that have been either killed or irradiated and then

injected into an individual fail to induce proper immunological sensitization against cercaria to prevent infection.

It would therefore seem that in order to evoke the host's immune system to sensitivity,a specific antigen must be used. Also, in order to concur with *in vivo* models, this specific antigen must also be associated with the adult worm. However, because of the adult worm's evasive abilities and the lack of definitive knowledge of how these abilities work, it is not clear if the host even mounts a successful antibody response against the adult worm. The purpose of this research is to discern whether there are antigens on the worm that the host recognizes as foreign and mounts an antibody response against. Understanding in this area can lead to a more full understanding of concomitant immunity and therefore a greater possibility of artificially inducing it.

MATERIALS AND METHODS

Parasite life cycle.

Adult worm pairs of a Kenyan strain of *Schistosoma mansoni* were obtained from percutaneously infected Texas Swiss mice after passage of the parasite through a Puerto Rican strain of *Biomphalaria galabrata* snails. The mice were killed with chloroform, bled by cardiac puncture, and then perfused with phosphate buffered saline (PBS) following severance of the hepatic portal vein. The worm pairs in the perfusion were collected as were any worm pairs found in the mesenteries upon examination. The worms were stored in PBS at -70° C.

Serum antibodies.

The blood collected in the cardiac puncture from the infected mice was allowed to clot overnight at 4°C. The following day, the blood was spun down in a clinical centrifuge to separate the serum from the clotted red blood cells. Infected mouse serum (IMS) was also stored at -70°C.

In order to collect the IgG's from the serum, the IMS was passed over a Protein A (*Staphylococcus aures* origin) Sepharose affinity column which had been "cleaned" with pH 3.0 citrate buffer and re-equilabrated with pH 8.0 phosphate buffer. After addition of the IMS, the column was washed with pH 8.0 phosphate buffer at a rate of 8 drops per minute until no more protein was detectable in the wash by a Beckman 35 spectrophotometer at 280 nm. The bound IgG's

were eluted with pH 3.0 citrate buffer (25 ml) followed by a re-equilabration of the column with pH 8.0 phosphate buffer containing .02% sodium azide (25 ml). The IgG's were collected in 2 ml fractions directly into test tubes containing 2ml 1 M Tris buffer. The fractions containing protein (IgG determined by 280 nm spectrophotometry) were collected and dialyzed overnight against pH 8.0 PBS containing .02% sodium azide. The following day, this solution was concentrated down with polyvinal pyrolidon and dialyzed overnight. After the second dialysis, the protein content of the IgG sample was determined by the Lowry method using bovine serum albumin as the protein standard. 10 ul phenylmethylsulfonyl fluoride (PMSF) per ml IgG solution was added. The IMS IgG sample was stored at -70°C.

Antigen-specific affinity column.

After isolation of approximately 50 mg of IMS IgG's, there was enough of this protein to use as the ligand in a CNBr-activated Sepharose 4B column of a 10 ml volume according to the Pharmacia affinity column preparation technique (Pharmacia, 1979). 2.85g of the dry CNBr-activated Sepharose 4B was weighed out and swollen for 15 minutes with 1mM HCl and washed on a sintered glass filter (200 ml per dry gram). The gel was then washed with coupling buffer (0.1M $NaHCO_3$, 0.5M NaCl, pH 8.3--5ml per gram dry gel) and immediately transferred to a solution of the ligand (IMS IgG dissolved in coupling buffer). This mixture was shaken overnight at 4°C. Following this, the remaining reactive groups which did not bind IgG

were blocked by the addition of 1M glycine (pH 8.0) and incubated overnight at 4°C. In order to remove excess uncoupled ligand that remains after coupling, the adsorbent was washed with alternate high (coupling buffer, pH 8) and low (0.1M acetate buffer, pH 4) pH solutions five times. The ligand-bound gel was then washed with coupling buffer and poured into a column which was stored at 4°C in the presence of coupling buffer containing .02% sodium azide.

Worm antigens.

196 worm pairs were homogenized in 4ml of SNAG buffer (.0175M PBS, 2mM EDTA, 0.1% sodium azide, 10 ul/ml PMSF) and the resulting worm slurry was sonicated for 10 minutes and centrifuged for 30 minutes in a Sorvall RC-58 refrigerated superspeed centrifuge at 30,000g. The supernatant was collected with care not to collect the lipid layer floating on top of the supernatant. The pellet was re-suspended in 4ml of SNAG buffer and the solution was again centrifuged, supernatant collected, and pooled with the first supernatant. The worm homogenate solution was placed in dialysis tubing, concentrated with polyvinal pyrolidon, and dialyzed overnight against PBS containing .02% sodium azide. The protein content of the worm homogenate was determined by the Lowry method. Worm homogenate was stored at -70°C with 10 ul/ml PMSF.

Specific antigen isolation and characterization.

After equilibration of the CNBr-activated Sepharose 4B antigen specific affinity column with coupling buffer, 250ul of 2.85ug/ml

worm homogenate were loaded onto the column and were allowed to interact with it for 30 minutes. The column was then washed with coupling buffer at a 10ml/hr flow rate until all nonspecific protein was washed away. The presence or absence of protein coming off the column was determined by the use of an ISCO UV monitor at 280nm attached to a chart reader. The protein was then eluted with 0.1M glycine/0.2M HCl buffer at pH 2.5. The eluted protein solution's pH was immediately raised to pH 7.5 with NaOH. The specific antigen solutions were dialyzed, concentrated, and dialyzed as in the previously described manner and were stored at -70°C with 10ul/ml PMSF. The column was regenerated between runs by washing with 10 column volumes of 0.1M Tris/HCl with .5M NaCl pH 8.5 followed by washing with 10 column volumes of 0.1M sodium acetate with .5M NaCl pH 4.5 and reequilibrated and stored at 4°C with coupling buffer containing .02% sodium azide.

Analysis of specific antigens.

The molecular weights of the isolated specific antigens were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis by comparing the bands stained by Comassie Blue from the specific antigen sample with those from standard molecular weight markers.

RESULTS

Nine successful runs (eleven in all) of the CNBr-activated Sepharose 4B column were performed. Protein content of the solutions of specific antigens from the individual column runs was too small to be detected by the Lowry method. Therefore the protein solutions were all pooled, concentrated with polyvinal pyrolidon, and dialyzed overnight against PBS pH 8.0 with .02% sodium azide. On the following morning, it was noted that a precipitate had formed in the dialysis tubing. The solution was centrifuged and the precipitate was separated from the supernatant. The pellet was resuspended in 3.5 0.1mM Tris buffer and the protein content of both the supernatant and pellet solutions determined. 13.5 ml of supernatant had 68.10 ug/ml and 3.5 ml of resuspended pellet solution contained 145.21 ug/ml for a total protein content of approximately 1.4 mg. Both supernatant and pellet solutions were analyzed by SDS-PAGE and yielded the following bands:

Pellet Bands (MW)	Supernatant Bands (MW)
66,000	67,000
57,000	39,250
42,000	
40,000	

Aworm homogenate control was also run alongside and contained all the bands in the supernatent and pellet solutions as well as many other bands.

DISCUSSION

From the results obtained, showing the isolation and characterization of worm antigens with a specificity for IMS IgG's, it can be stated that the immune system of the mouse has the ability to recognize antigenic determinants on the worm as non-self and therefore the purpose of the research was fulfilled. However, the ability of the mouse immune system to recognize some worm antigens is a small thing when the potentials for the results obtained are realized. For instance, with the specific antigens isolated in this experiment, antibodies specific for these antigens could be made in larger amounts maintaining their specificity as opposed to the very heterogeneous IgG preparation of IgG's obtained in the protein A column separation of serum components. These IgG's could then be used to probe the proteins formed by the mRNA's of a library of DNA from the adult worm. Through this, the DNA that is responsible for the manufacture of antigens that the immune system can recognize can be isolated and cloned to produce a large amount of the specific antigens which can then be tested for the ability to sensitize the immune system against cercarial attacks (protection studies) and thereby artificially induce a state of concomitant immunity.

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