Deoxyribonucleic Acid (DNA) and Antibodies to DNA in the Serum of Hamsters and Man Infected with Schistosomes (35386)

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Despite extensive studies during the past 20 years, the precise mechanisms of immunity to schistosomiasis are still not known (1). Precipitating antibodies are produced to a large number of schistosome antigens exhibiting varying degrees of stage and species specificities (2–4). However, no direct quantitative relationship has been found between these antibodies and protective immunity. In contrast, studies on the infectious process and evolution of the disease state are better understood. From the time of penetration by an invasive cercaria through the period of maturity and oviposition by the adult worm there is an intimate contact between host and parasite involving extensive tissue destruction and exposure of the host to parasite-derived antigenic material. When the cercaria penetrates the host skin a series of proteolytic and collagenase-like enzymes are utilized causing local enzymic alteration of the extracellular, glycoprotein, connective tissue barriers of the host (5). The maturing worms imbibe and digest host substances as well as produce excretion and secretion antigens to which the host responds. The adult female worms can deposit several hundreds or thousands of eggs per female per day, depending on the species. These often lodge in the liver where extensive granuloma formation of immunologic specificity occurs (6). These granulomas result in extensive tissue damage implicating the schistosome egg as the primary parasitic element in the development of overt disease (7).

Workers in Brazil have recently reported that membranous glomerular changes and fibrillar thickening of the mesangium were observed in the kidneys of patients who died of hepatosplenic schistosomiasis (8). In addition, another laboratory found that 21.6% of human cases diagnosed clinically as chronic glomerulonephritis also had hepatosplenic schistosomiasis (9). Similar indirect evidence was not found between glomerulonephritis and cases of Chagas' disease or tuberculosis.

The present report concerns (a) an analysis of the base composition of *Schistosoma mansoni* DNA, and (b) the finding of circulating DNA and antibodies to DNA in the serum of hamsters infected with *S. mansoni* and humans infected with *S. japonicum*. Together with the above-mentioned work on kidney involvement, the results strongly suggest that autoimmune and/or localized *in vivo* antigen–antibody reactions may play a significant role in the pathogenesis of schistosomiasis.

Materials and Methods. Sera were obtained from female golden hamsters (*Mesocricetus auratus*) exposed via their cheek pouches to cercariae of *S. mansoni* varying in number between 550–2000 and bled by cardiac puncture at various intervals through 64 days postcercarial exposure. Thirty percent of the cercariae matured and were recovered as adults at autopsy. Sera from 10 humans with active infections of *S. japonicum* from the Philippines and sera from 7 patients with systemic lupus erythematosus (SLE) and having positive antinuclear immunofluorescence tests were also obtained.

The DNA used included that isolated from

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S. mansoni adult worms and rat liver by the method of Marmur (10), highly polymerized calf thymus DNA[2] and *Bacillus subtilis* DNA. DNA preparations were considered relatively pure if the 230:260:280 (mμ) ratio in the spectrophotometer was approximately 1:2:1. Fifty μg of DNA were considered equal to 1.0 OD at 260 mμ. The buoyant density of *S. mansoni* DNA was determined using the method described by Mandel *et al.* (11). From this, a determination of the guanine + cytosine (G + C) content was performed. A *Bacillus subtilis* bacteriophage, SPO-1, was used as a standard. Ultraviolet absorbance–temperature profiles were also performed to calculate G + C concentration (12). To demonstrate immunoprecipitates, Ouchterlony double diffusion in two dimensions was performed as previously described by Hillyer and Frick (3), with the modification that agarose was used at a concentration of 0.6% since this facilitated the diffusion of the nucleic acids through the gel.

Results. The calculated buoyant density in CsCl of *S. mansoni* DNA was 1.694. On this basis, using the linear equation of Schildkraut (11), the corresponding guanine + cytosine content was calculated to be 34.4 moles % G + C. On the basis of ultraviolet absorbance–temperature profile (12) the T_m was 83.6° which would indicate 34.9 moles % G + C. The finding that both of these methods predict the same G + C content suggests that no unusual bases occur in schistosome DNA. No satellite bands or unusual peaks were observed in CsCl density studies.

The sera of hamsters infected with *S. mansoni* exhibit one or two lines of precipitation when reacted against a purified preparation of nucleic acids from this parasite. These are clearly present at 49 days of infection but not in the sera collected at 41 days or earlier. One line was resistant to the action of deoxyribonuclease and ribonuclease, and stained with Schiff's reagent (preceded by periodic acid hydrolysis) indicating it was probably a polysaccharide. The second line was resistant to the action of ribonuclease but sensitive to deoxyribonuclease. This line visibly increased in intensity when the DNA mixture, prior to reacting with serum, was heat denatured by boiling 10 min, then cooled in ice water.

The antibodies, however, appear to lack specificity. At least four groups of sera from hamsters infected with *S. mansoni* produced one or two precipitates against heat-denatured calf thymus DNA and *Bacillus subtilis* DNA. Moreover, these lines coalesce and show reactions of identity when compared with *S. mansoni* DNA extracts.

The sera from patients with systemic lupus erythematosis (SLE) gave one or two lines of precipitation against native and/or heat-denatured DNA from *S. mansoni* adult worms and from *B. subtilis* DNA. The precipitates coalesced when the DNA used was from two sources.

Of 10 humans infected with *S. japonicum*, 8 were reacted against rat liver DNA. Six of these had one precipitin, and 2 were negative. The precipitin coalesced with precipitins from humans with SLE. Two additional sera from humans with schistosomiasis japonicum were tested against *S. mansoni* and calf thymus DNA. They had the strongest anti-DNA precipitins of all sera tested including SLE sera. These lines were stainable with pyronine Y and azure b. Two precipitins were observed against calf thymus DNA; four precipitins were observed against *S. mansoni* DNA. One of these precipitins gave a reaction of identity with both DNA sources.

One of the sera from patients with SLE gave a line of precipitation with the serum of hamsters infected with *S. mansoni*. This serum was a pool collected from 3 hamsters infected for 55 days with *S. mansoni*. Further analysis showed that the hamster serum, not the SLE serum, reacted as if it had circulating DNA since it precipitated 2 of 3 sera from hamsters having anti-DNA precipitins. Thus it is tentatively concluded that circulating DNA is present in the serum of some hamsters infected with *S. mansoni*. This is presently being studied further both qualitatively and quantitatively.

Discussion. The G + C content of schistosome DNA is in the region of 34.6 moles % If one accepts the upper 30s as the moles % G + C of the mammalian hosts of schistosomes (13), then it is remarkable how close the two

[2] Worthington Biochemical Corporation.

types of bases are in terms of base pairing. There is recent evidence that schistosomes incorporate nucleotides from the host, rather than synthesizing them *de novo* (14). Their nucleotide spectrum was qualitatively similar to that found in mammalian cells.

The finding that heat-denaturation of the DNA increases the precipitates observed indicates that the antibodies are probably active against the bases and not to the deoxyribophosphate backbone. Since the present study indicates that there are probably no unusual bases in *S. mansoni* DNA, on the basis of two different assay methods used to measure G + C concentrations (12), and other workers have shown that the minimal antigenic active site is a pentanucleotide, then the lack of specificity is not surprising (21).

Circulating DNA has been demonstrated in the serum of patients with systemic lupus erythematosus (15). Circulating antigens in hamsters infected with *S. mansoni* have recently been reported (16, 17). Although one antigen had an ultraviolet absorption peak of 260 mμ, they found it to be resistant to deoxyribonuclease and ribonuclease. They concluded, thus, that the antigen was a polysaccharide. Thus it is of interest to find circulating DNA in hamsters infected with schistosomiasis. It is possible that this antigen could cross the placenta of infected mothers and cause tolerance of schistosome antigens in the newborn. Tolerance in offspring of infected mothers has been experimentally demonstrated (18). It is also known that precipitins to schistosomes cross the placenta (19).

The nature of the immunogen, be it of parasite or host origin, which elicits the formation of anti-DNA antibodies is still not known. However, together with the work on kidney involvement in schistosomiasis, it suggests some parallels with systemic lupus erythematosus (20). Thus schistosomiasis may be a disease which exhibits autoimmune phenomena and/or host damaging antigen–antibody–complement complexes which contribute to glomerulonephritis and perhaps other previously obscure aspects of schistosomal disease.

Summary. (i). The guanine + cytosine content of *S. mansoni* has been shown to be 34.4 moles % on the basis of its buoyant density in CsCl and 34.9 moles % on the basis of its ultraviolet absorbance–temperature profile.

(ii). Antibodies to DNA have been demonstrated in hamsters infected with *S. mansoni* and humans infected with *S. japonicum*. These antibodies show a broad specificity and react with DNAs of parasite, bacterial, and mammalian origin.

(iii). Circulating DNA has been demonstrated in the serum of hamsters infected with *S. mansoni*.

(iv). It is suggested that schistosomiasis, in some instances at least, exhibits autoimmune or immune-complex disease.

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