Ann. Soc. belge Méd. trop.
1975, 55, 4, 373-377

PRESENCE OF THE CIRCULATING POLYSACCHARIDE ANTIGEN IN THE LIVER OF MICE INFECTED WITH *SCHISTOSOMA MANSONI*

by

E. VAN MARCK

Summary — Evidence is offered on the presence of the circulating polysaccharide schistosoma antigen in Kupffer cells of experimentally infected mice using an indirect immunofluorescent technique.

KEYWORDS : Bilharziasis; Schistosoma mansoni; Polysaccharides; Fluorescent Antibody Technique.

Circulating antigen in mice and hamsters heavily infected with *Schistosoma mansoni* has been described by Gold and co-workers (1969), Berggren and Weller (1967), and more recently by Bawden and Weller (1974). This antigen was further characterized by Nash and co-workers (1974) and identified as a polysaccharide with a molecular weight of about 100.000. The origin of this antigen was shown to be in the intestinal lining cells of the cecum of the adult worm by Nash (1974) and by von Lichtenberg and co-workers (1974), both using an indirect immunofluorescence technique.

Present paper deals with the presence of the antigen in the liver of infected mice.

Material and methods

Young adult outbred albino mice were exposed to an infection with 100 cercariae through tail-immersion for 30-45 minutes after anesthesia with chloral hydrate. The cercariae were obtained from snails kept in the laboratory and infected with miracidia from a strain of Feira de Santana (Bahia). Fourty-four days after infection mice were killed by cervical dislocation. Adult worm counts revealed a mean of 26 worms of both sexes.

Tissue samples of liver, spleen and kidney were fixed in Bouin overnight and routinely imbedded in paraffin. Sections at 4μm were stained with H. E., P. A. S. and P. A. S. after diastase digestion.

Immunofluorescence studies were carried out on unstained Bouin fixed sections of liver, spleen and kidney after deparaffinization and hydration in PBS (phosphate buffered saline) pH 7.2. Slides were layered for 30 minutes with a 1/8 dilution of a specific rabbit antiserum for circulating polysaccharide adult worm antigen, washed three times in PBS for 5 minutes,

layered for 30 minutes with a 1/10 dilution of commercial fluorescein conjugated goat antirabbit gammaglobulin (Microbiological Associates Inc., Bethesda, Maryland) to which was added a 1/10.000 solution of Evans blue, washed again three times for 5 minutes in PBS, and mounted in buffered glycerin.

Adult *Schistosoma mansoni* worms, after fixation in Rossman's fixative overnight and paraffin embedding were sectioned at 4 μ and the unstained sections treated in the same way for immunofluorescence.

Control sections included normal uninfected mouse liver, spleen and kidney treated with antiserum plus conjugate, sections of infected mouse liver, spleen and kidney treated with normal rabbit serum plus conjugate, antiserum alone, conjugate alone, none of the aforementioned.

Slides for immunofluorescence were viewed on a Zeiss standard microscope 18 under dark-field and using a Zeiss FITC filter, heat filter, barrier filter 53, with a halogen bulb 100 W 12 V as a light source. Photomicrographs were reproduced from slides taken with a Kodak High Speed Ektachrome colorfilm.

Results

Normal light microscopic slides revealed numerous mature eggs surrounded by granulomas in the acute stage throughout the liver. Extracellular P. A. S. positive material confined to the granulomas was seen. Kupffer cells were prominent, most of them containing a moderate amount of brown pigment. Some Kupffer cells exhibited a faint diastase resistant P. A. S. staining of their cytoplasm. Spleen sections showed hyperplasia of the white pulp with prominent germinative centers. Scattered pigment laden reticular cells were present in the red pulp. No lesions could be detected in the kidney sections.

Immunofluorescence study of adult worms revealed apple-green fluorescence exclusively confined to the walling of the worm's intestine (figure 1), as described earlier by Nash (1974) and by von Lichtenberg and co-workers (1974).

Liver sections displayed yellow autofluorescence of egg-shells and occasional greater pigment deposits in granuloma macrophages. Almost all Kupffer cells exhibited a fairly strong apple-green specific fluorescence of their cytoplasm (figure 2). No specific fluorescence could be detected in the eggs, granulomas nor hepatocytes.

An occasional reticulum cell in the red pulp of the spleen showed doubtfull fluorescence.

Kidney sections were negative for fluorescence.

All control sections were also uniformly negative.

Discussion

Our finding suggest that one of the biological pathways of the circulating polysaccharide antigen of *Schistosoma mansoni* consists in storage in the Kupffer cells of the liver. Breakdown and further processing remain unexplained.

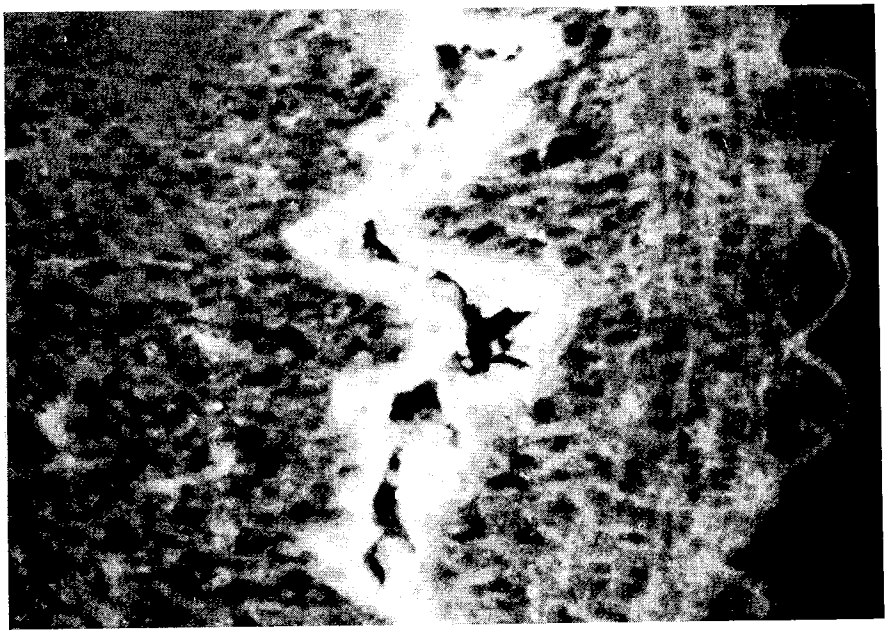

Figure 1
Adult worm section showing specific immunofluorescence of the walling of the intestine. Original magnification 400 times.

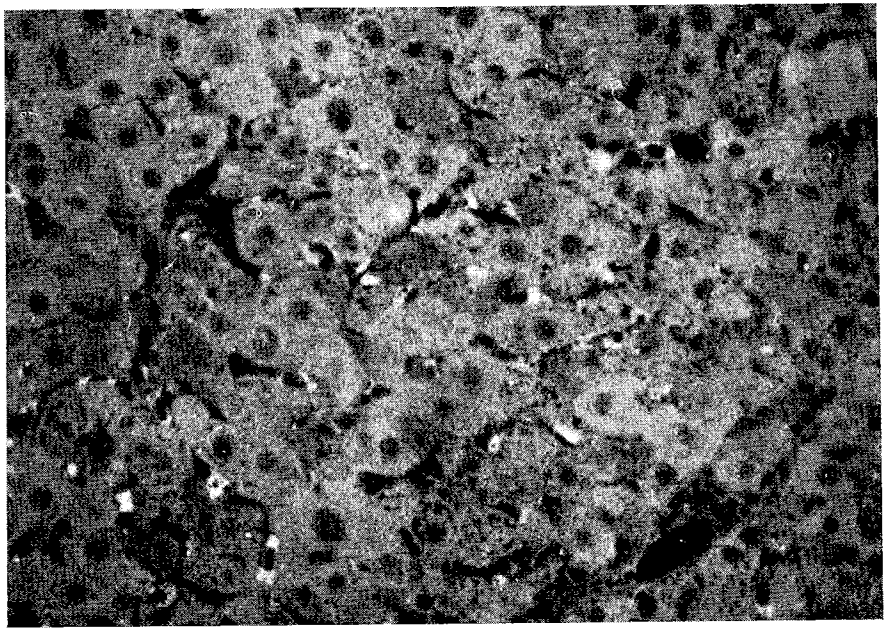

Figure 2
Liver section showing specific immunofluorescence of the cytoplasm of Kupffer cells, visible as white dots. Original magnification 250 times.

The question if this antigen is present in his native form or as an immune-complex is difficult to answer. Poor antigenicity (Nash *et al.*, 1974) would favor the first hypothesis. Earlier findings of Andrade and co-workers (1971) demonstrating the presence of host-gamma-globulin within the sinusoids lining cells in infected mice on the other hand do not rule out an immune-complex nature of these deposits.

In the same way, the identity of the antigenic material we describe here with the small particles encountered by Ramadan (1971) in granulomatous phagocytes in his electron-microscopic study, remains to be investigated.

So far, with the employed technique, no evidence was found of the presence of the antigen within the spleen nor kidney. As in schistosomiasis one is faced with renal lesions occurring in patients with very heavy infections (Andrade *et al.*, 1968, 1971) it is tentative to think of this antigen as an etiologic factor after saturation of the Kupffer cells. Investigation in progress, interfering with the processing of the antigen or its combined form in the liver, aims at clarifying this point.

Acknowledgements — *Prof. Dr. Zilton Andrade is kindly acknowledged for his stimulating discussions and all laboratory facilities. Dr. Nash is also kindly acknowledged for supplying the specific rabbit antiserum against the schistosome antigen.*

Résumé — **Présence de l'antigène circulant polysaccharide dans le foie de souris infectées de** *Schistosoma mansoni.*

La présence dans les cellules de Kupffer de souris infectées expérimentalement de l'antigène circulant polysaccharide est démontrée en utilisant une technique d'immunofluorescence indirecte.

Samenvatting — **Aanwezigheid van het circulerend polysaccharide antigeen in de lever van muizen besmet met** *Schistosoma mansoni.*

De aanwezigheid van het circulerend polysaccharide schistosoma antigeen in Kupffer cellen van experimenteel besmette muizen wordt bij middel van een indirecte immunofluorescentietechniek beschreven.

E. Van Marck : Assistant to the Department of Pathology of the Prins Leopold Instituut voor Tropische Geneeskunde, Antwerpen, Belgium.
Work performed in the Department of Pathology (Director : Prof. Zilton Andrade) of the Federal University of Bahia, Salvador, Bahia, Brazil (address for reprints).
Received for publication on April, 21, 1975.

REFERENCES

Andrade, Z. A., Andrade, S. G. and Sadigursky, M. (1971) : Renal changes in patients with hepatosplenic schistosomiasis. Amer. J. Trop. Med. & Hyg., **20**, 77-83.

Andrade, Z. A. and de Queiroz, A. C. (1968) : Lesões renais na esquistossomose hepatesplênica. Rev. Inst. Med. Trop. São Paulo, **10**, 36-40.

Andrade, Z. A., Paronetto, F. and Popper, H. (1961) : Immunocytochemical studies in schistosomiasis. Amer. J. Pathol., **39**, 589-598.

Bawden, M. P. and Weller, T. H. (1974) : *Schistosoma mansoni* circulating antigen : detection by complement fixation in sera from infected hamsters and mice. Amer. J. Trop. Med. & Hyg., **23**, 1077-1084.

Berggren, W. L. and Weller, T. H. (1967) : Immunoelectrophoretic demonstration of specific circulating antigen in animals infected with *Schistosoma mansoni.* Amer. J. Trop. Med. & Hyg., **16**, 606-612.

Gold, R., Rosen, F. S. and Weller, T. H. (1969) : A specific circulating antigen in hamsters infected with *Schistosoma mansoni.* Amer. J. Trop. Med. & Hyg., **18**, 545-552.

Lichtenberg, F. von, Bawden, M. P. and Shealey, S. A. (1974) : Origin of circulating antigen from schistosome gut : an immunofluorescent study. Amer. J. Trop. Med. & Hyg., **23**, 1088-1091.

Nash, T. E. (1974) : Localization of the circulating antigen within the gut of *Schistosoma mansoni*. Amer. J. Trop. Med. & Hyg., **23**, 1085-1087.

Nash, T. E., Prescott, B. and Neva, F. A. (1974) : The characteristics of a circulating antigen in schistosomiasis. J. Immunol., **112**, 1500-1507.

Ramadan, M. A. (1971) : Sequestration of parasitic excretory products in *Schistosoma mansoni infection*. Z. Tropenmed. Parasitol., **22**, 397-405.