# The implantation of sepharose beads in mouse livers as an aid in the study of hepatic schistosomal fibrosis

E.A.E. van Marck, S. Stocker, J.A. Grimaud, L. Kestens, P.L.J. Gigase and A.M. Deelder[1]

*Department of Histopathology and Experimental Schistosomiasis, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp (Belgium), Centre de Microscopie Electronique et de Pathologie Ultrastructurale and Laboratoire de Pathologie Cellulaire du Foie, C.N.R.S., ERA 819, Institut Pasteur, 77, rue Pasteur, F-69365 Lyon (France), and Leiden University, Medical Faculty, Laboratory of Parasitology, Rapenburg 33, Leiden (The Netherlands), 25 February 1980*

*Summary.* An experimental model of schistosomal portal fibrosis is described. Sepharose beads the size of schistosome eggs, loaded or not with soluble egg antigen (SEA) from *Schistosoma mansoni*, are injected into the coecal vein of C3H/Sn mice and become embolized in the liver. Only SEA-coated beads evoke a granulomatous reaction; this is enhanced by simultaneous priming of the mice with spleen cells from *Schistosoma mansoni*-infected syngeneic animals. The fibrosis, which ensues around the beads, is stable and is much more evident after priming. Preliminary collagen tissue immunotyping reveals the presence of collagen deposits of types I and III collagen. Type IV collagen remains unchanged in the portal tracts. The model appears to be well suited for studies of the pathogenesis of portal fibrosis.

The severity of the disease schistosomiasis mansoni in humans is essentially determined by portal fibrosis. Little is known about the quality of this fibrous tissue and about the mechanisms responsible for the collagen deposition. According to the available evidence[2] the trapping of eggs in portal vein tributaries generates collagen deposition preceded by a cellular reaction with 2 origins, 1 being the response to a foreign body and the other the hypersensitivity phenomena, in which egg-derived antigens and the host's immune response are simultaneously implicated. In this experiment a model was used in which a known quantity of soluble egg antigen (SEA) was delivered in particulate form. In this way it became possible to define the effects of purely mechanical factors and of immune-related factors, and to establish precisely the quality of the collagen deposits in the induced fibrosis using tissue immunotyping of collagen fibres.

*Material and methods.* SEA was prepared from *Schistosoma mansoni* eggs, which were isolated from the livers of infected hamsters (48 days post-infection), according to the technique described by Browne and Thomas[3]. After homogenization and centrifugation (25,000 × g; 45 min) the supernatant containing the SEA was dialized overnight against distilled water at 4 °C, and lyophilized. Composition of the recovered antigen preparation was analyzed by immuno-electrophoresis against pooled infected mouse serum; at least 20 different antigenic components could be determined in this way.

Sepharose CL-4B beads (Pharmacia, Uppsala, Sweden) were wet-sieved to obtain a fraction with a diameter ranging between 63 and 75 µm. SEA was coupled to these beads as previously described[4]; beads were activated with CNBr (50 mg/ml of beads), SEA was coupled to them (10 mg SEA/ml) and they were subsequently deactivated with 4-aminobutyric acid. Before injection, the beads were washed exhaustively with phosphate buffer solution (PBS), pH 7.2. Homogeneous binding of the antigen to the beads was checked with immunofluorescence. Sepharose beads, which had been activated and deactivated but to which no antigen was coupled, were also used. For the experimental implantation studies adult male C3H/Sn mice were utilized. About 20,000 beads in 0.2 ml of PBS were injected into the coecal vein according to a technique previously described[5]. 1 group of animals (I; n = 19) was sensitized by injection i.v. of $2 \times 10^7$ spleen cells from syngeneic C3H/Sn mice which had been infected with 120 cercariae of *Schistosoma mansoni* 8 weeks previously. SEA-coated beads were administered to these sensitized mice on the same day. A 2nd group (II; n = 18) received SEA-coated beads without previous sensitization. A 3rd group (III; n = 14) comprised unsensitized C3H/Sn mice receiving beads devoid of antigen. Animals were sacrificed 48 h, 8, 16, 30, 45, 60 and 120 days after injection. Liver specimens fixed in 10% buffered formalin were processed for light microscopy and studied on haematoxylin-eosin-saffron stained sections. On some liver samples from sensitized animals injected with SEA-loaded beads collagen typing for types I, III and IV was performed by indirect immunofluorescence using unfixed cryostat sections and specific immune reagents. Preparation of the reagents and control of the reaction have been previously described[6].

*Results.* 48 h after injection, in all 3 groups, beads were found randomly lodged, though wih frequent clustering, in the peripheral presinusoidal ramifications of the portal vein. The beads were occasionally surrounded by a few neutrophils and macrophages, closely adhering to their surfaces. At 8 and 16 days, group III showed no cellular reaction around the beads, except for a monolayer of macrophages or multinucleate giant cells. On the contrary, SEA-coated beads elicited in both other groups a significant granulomatous reaction comprising macrophages, epithelioid cells, multinucleate giant cells, lymphocytes and eosinophils, but no neutrophils. At this stage the sensitized group already showed fibroblasts and slight collagen deposits in the granulomas (figure 1); this was in contrast to the unsensitized group. The degree of collagen deposition appeared to be different between the mice of groups I and II sacrificed after 30, 45, 60 and 120 days. In the unsensi-

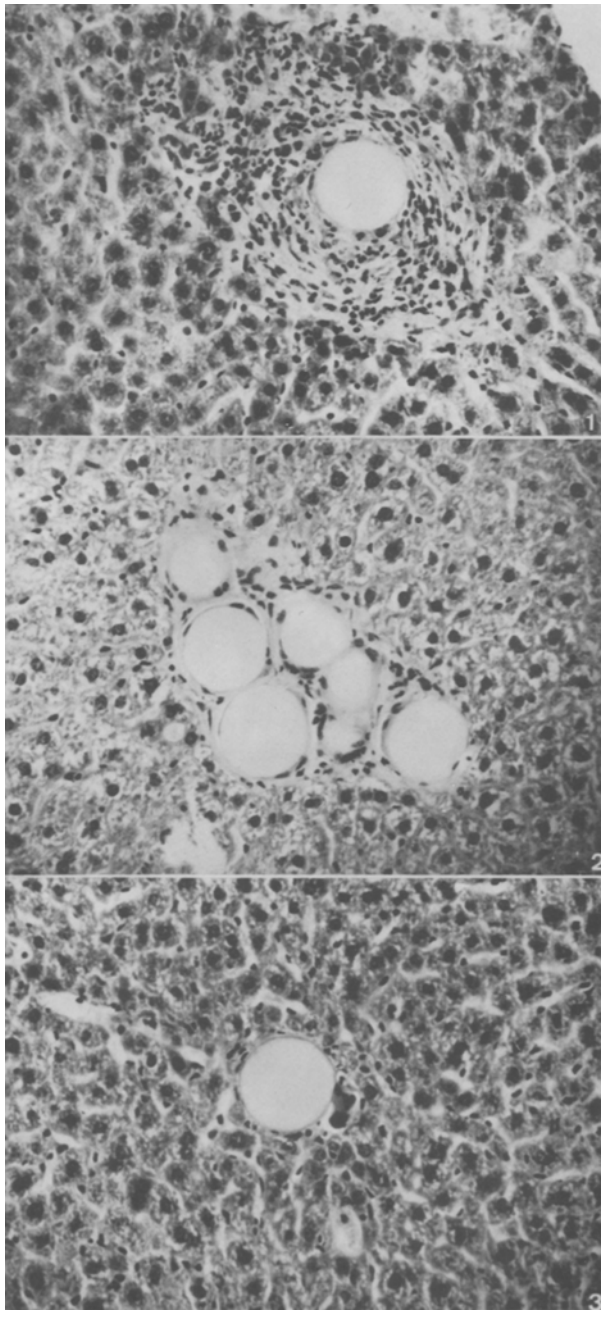
Observed histological features

| | Granulomatous reaction | | | Eosinophils | | | Fibrosis | | |
|---|---|---|---|---|---|---|---|---|---|
| | I Sepharose + SEA Sensitized | II Sepharose + SEA Unsensitized | III Sepharose | I Sepharose + SEA Sensitized | II Sepharose + SEA Unsensitized | III Sepharose | I Sepharose + SEA Sensitized | II Sepharose + SEA Unsensitized | III Sepharose |
| 48 h | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 d | ++ | + | 0 | + | + | 0 | + | 0 | 0 |
| 16 d | +++ | ++ | 0 | +++ | ++ | 0 | ++ | 0 | 0 |
| 30–120 d | + | + | 0 | + | ++ | 0 | +++ | ++ | 0 |

SEA, soluble egg antigen; 0, absent; +, present; ++, intermediate; +++, maximal.

tized group the granulomatous reaction was almost devoid of fibrosis whereas the sensitized group showed evident collagen deposition (figure 2). With time, a decrease in the cellularity of the granulomas was observed while the collagen became denser. The animals in group III remained unchanged as compared with their condition after 8 and 16 days (figure 3). Except for some foci of parenchymal necrosis, which were easily distinguishable from the periparticular reaction, no significant changes were found in the liver lobule at any time. The table summarizes the features described above. The experiment confirmed that different collagen types could be identified around SEA-coated beads, reactions for types I and III being positive in collagen deposits while type IV (basement membrane) collagen remained limited to perivascular and peribiliary tracts without any participation in collagen deposits. (figures 4 and 5).

*Discussion.* Hepatic fibrosis is the most serious complication of human intestinal schistosomiasis. It has been investigated mainly by experimental infections, which, in some models, e.g. chimpanzees[7], are able to induce lesions quite similar to those observed in humans. The multiplicity of immunogenic factors and reactive mechanisms makes the elucidation of the pathogenesis hazardous, however. Pulmonary granulomas obtained by injection of eggs[8] and of antigen-loaded bentonite particles[9] in the caudal vein of mice have greatly contributed towards a better understanding of the genesis of these granulomas but less to that of hepatic fibrosis. Injection of eggs in ramifications of the portal vein is technically more difficult, but nearer to the conditions of natural infections[10]. Still, schistosome eggs are objects with a complex structure acting both as a foreign body and as a combination of numerous, more or less antigenic, constituents. The fact that the injected eggs are,



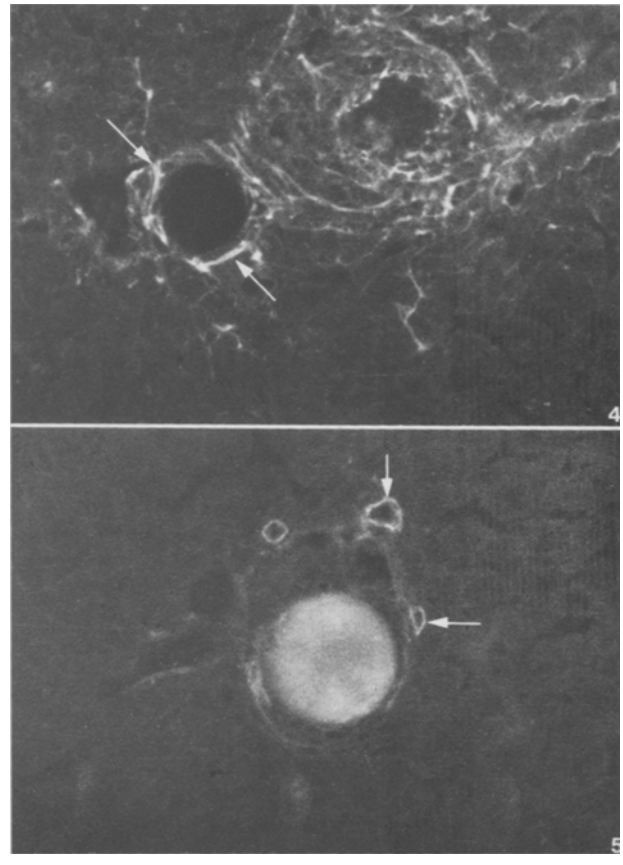


Fig. 1. Granulomatous reaction with incipient fibrosis surrounding a SEA-coated sepharose bead (16 days after injection) in the liver of a sensitized animal. Haematoxylin-eosin-saffron. ×215.

Fig. 2. Collagen deposits surrounding SEA-coated sepharose beads (45 days after injection) in the liver of a sensitized animal. Haematoxylin-eosin-saffron. ×215.

Fig. 3. Absence of any inflammatory reaction around an uncoated sepharose bead in mouse liver (30 days after injection). Haematoxylin-eosin-saffron. ×215.

Fig. 4. Collagen immunotyping (type I) around SEA-coated sepharose beads (16 days after injection). Indirect immunofluorescence. Type I collagen (→). ×295.

Fig. 5. Collagen immunotyping (type IV) around SEA-coated bead (8 days after injection). Indirect immunofluorescence. Type IV collagen (→). ×295.

of necessity, variable in their maturation, further complicates the problem.

The purpose of this investigation was to bring into the liver particles of the approximate size of schistosome eggs, coupled to antigenic schistosome fractions. These particles had to be tolerated, stable for long periods and suitable for easy histological processing. Sepharose-CL-4B beads were eventually chosen after trials with several kinds of particles, e.g. PVC, polyacrylamide (Biogel P-150; Aminoethyl Biogel P-150) and Sepharose-4B, as they fulfilled the requirements most adequately. For the introduction of the particles into mice 2 routes were tried; the coecal vein and the splenic vein. The latter way has the disadvantage that splenectomy is unavoidable, with a risk of influencing the granulomatous reaction and disturbing the experiment. For this reason the injection in the coecal vein was used throughout. The loss of animals as a result of the procedure remained within reasonable limits, being less than 10%. Mortality within 48 h is proportional to the quantity of beads injected above a threshold of approximately 20,000 beads. It appears from the results that SEA is able to induce in mice livers granulomatous reactions with collagen deposition similar in aspect and evolution to periovular schistosomal granulomas. The initially loose collagen matrix later turns into a dense connective matrix which then remains histologically unchanged for the rest of the experiment. The simultaneous presence of type I and type III collagen in collagen deposits supports these data.[6]. Administration of a suspension of spleen cells from syngeneic infected animals enhances the reaction, which also becomes more precocious. The absence of neutrophils in the reaction around the beads, and the absence of any Splendore-Hoeppli phenomena, suggest that mainly cellular hypersensitivity reactions of type IV are involved and enhanced. The effect of immunization on collagen deposition appears even more striking, though it is not yet clear whether this difference in fibrogenesis between primed and unprimed animals is a qualitative one or merely quantitative, as a consequence of the more important granuloma formation in sensitized animals.

The absence of any collagen deposition around particles without SEA confirms that periovular fibrosis is not merely a foreign body reaction[11].

No lesions which could be traced to immune reactions have been found in the liver parenchyma. Early focal necrosis is observed but is certainly due to mechanical circulatory disturbances through embolization of the beads. The post-necrotic scarring which eventually ensues is unrelated to and clearly distinct from the collagen deposition around the beads. No significant presinusoidal thrombosis related to the beads was observed.

It appears that the model discussed is well suited to following the evolution of reactions around egg constituents in the course of time, from a well-defined moment onwards, including the sequence of the deposition of collagen types. Furthermore, it becomes possible to evaluate the participation in the granuloma formation and in collagen deposition of purified antigenic fractions, in combination or not with different modalities of immunization.

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## The change in length and width of the Sertoli cell nuclei in cytologic smears of testes with depopulation of the seminiferous epithelium

Lj. Banek and J. Posinovec

*Department of Histology and Embryology, Medical Faculty of Zagreb, Gjorgjićeva 17, YU-41000 Zagreb (Yugoslavia), 27 November 1979*

*Summary.* The appearance of the Sertoli cells in cytological smears of testes with depopulation of the seminiferous epithelium is described. The mean values of the lengths and widths of the Sertoli cell nuclei in smears differed significantly between the depopulation and the control group ($p < 0.01$).

Today it is generally considered that Sertoli cells regulate spermatogenesis. The data on the normal morphology of the Sertoli cells in testicular smears as well as on the changes of their morphology under different conditions of damage to the testes would allow insight into the state of spermatogenesis. The purpose of the present work was to describe the appearance of the Sertoli cells in cytological smears of testes with depopulation of the seminiferous epithelium (Sertoli cell only syndrome).

*Material and methods.* The material investigated comprised the cytological smears from biopsy material from 20 human testes in which the histological findings in the seminiferous epithelium showed depopulation. The control samples were obtained from 10 testes of men whose seminiferous epithelium in histological sections showed a normal aspect and also slight exfoliation. Cytological smears were made by the imprint method and stained according to the May-Grünwald-Giemsa method. The measurement of the lengths and widths of the Sertoli cell nuclei in the smears was carried out using light microscope (C. Zeiss Jena) exclusively under immersion (magnification: × 1080). In the smears of every testis the lengths and widths of 50 Sertoli cell nuclei were measured. In the investigated group (depopulation of the seminiferous epi-