

EXPERIMENTAL PULMONARY SCHISTOSOMIASIS:* LACK OF MORPHOLOGICAL EVIDENCE OF MODULATION IN SCHISTOSOMAL PULMONARY GRANULOMAS

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SUMMARY

Numerous pulmonary schistosome egg granulomas were present in mice submitted to partial portal vein ligation (Warren's model). The granulomas were characterized by cellular aggregations formed within alveolar tissue. Main cellular types were macrophages (epithelioid cells), eosinophils, plasma cells and lymphocytes. These cells were supported by scanty fibrous stroma and exhibited close membrane contact points amongst themselves, but without forming specialized adhesion apparatus. When granulomas involved arterial structures, proliferation of endothelial and smooth muscle cells occurred and fibrosis associated with angiogenesis became more evident.

Granulomas formed around mature eggs in the pulmonary alveolar tissue presented approximately the same size and morphology regardless of the time of infection, the latter being 10, 18 and 25 weeks after cercarial exposure. This persistence of morphological appearance suggests that pulmonary granulomas do not undergo immunological modulation, as is the case with the granulomas in the liver and, to a lesser extent, in the intestines. Probably, besides general immunological factors, local (stromal) factors play an important role in schistosomal granuloma modulation.

KEY WORDS: Experimental schistosomiasis; Pulmonary schistosomiasis; Periovascular granuloma; Immunological modulation.

INTRODUCTION

Severe pulmonary arteritis and chronic cor pulmonale usually results when massive embolization of schistosome eggs to the lungs takes place. This occurs as a consequence of the opening of collateral porto-systemic shunts by portal hypertension². By producing a partial portal vein ligation in infected mice, WARREN¹⁸ observed that numerous schistosome eggs were diverted from the portal to the pulmonary circulation, where they caused widespread granulomas. Although this experimental model has been successfully used in some previous studies^{1, 12}, its

main histopathological features have not been thoroughly investigated.

During an attempt to study the histopathology of the lung in experimental schistosomiasis of mice we observed that: a) the periovascular reactions present in alveolar tissue are represented by cellular aggregations rather than by classical granulomas; b) the granulomas to newly arrived eggs do not change with time and thus do not seem to undergo immunological modulation; c) fibrosis is not an important feature of pulmonary

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* This paper is an abridged version of a Thesis for the Master's degree in Pathology presented to the Federal University of Bahia by Maura Raquel Ferreira Souza Vidal.

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granulomas formed in alveolar tissue of the lung; d) two different granuloma types can be formed in the lungs.

These aspects constitute the major histological characteristics of the Warren's model of pulmonary schistosomiasis and their presentation and discussion represent the objective of the present communication.

MATERIALS AND METHODS

Outbred albino Swiss mice of both sexes, 20/25g, were maintained on a pellet balanced diet and water ad libitum. They were submitted to a surgical operation, under general ether anesthesia, for partial portal vein ligation, according to CHEEVER & WARREN⁷. Mortality was high with this surgical procedure, but a total of 39 mice were available after completion of all procedures. One week later, the animals being recovered from the operation were exposed to infection with 50 *Schistosoma mansoni* cercariae each. Infection was made by the transcutaneous route. All animals used in the present study were passing viable eggs in the feces, 50 days after cercarial exposure. A group of infected non-ligated control animals was also used. The animals were killed 10, 18 and 25 weeks after infection. Ten portal vein ligated animals were used at each experimental point. Parasitological methods applied at the time of sacrifice included the oograma according to PRATA¹⁵ and recovery of adult worms by means of perfusion of the portal system by the DUVALL and DEWITT's method¹⁰.

Histology - The lungs were carefully distended by the intratracheal injection of fixatives. Millonig saline phosphate buffered 10% formalin was used for the tissues to be prepared for routine paraffin embedding technique. Histological sections were stained with Hematoxylin & Eosin, Weigert-Van Gieson for elastic and collagen tissues, Periodic Acid Schiff (PAS), Picrosirius-Red and Gomori's reticulum methods. Histological sections from formalin-fixed and paraffin-embedded liver tissue from all animals were also examined.

Electron Microscopy - For electron microscopy, the intratracheal injection was made with 2% glutaraldehyde in 0.1M phosphate buffer, pH 7.4, followed by the post-fixation of tiny fragments in 1% osmium tetroxide, resin embedding and ultramicrotome cutting. Ultra-thin sections mounted in copper grids were contrasted with uranyl acetate and lead nitrate, and examined in a Zeiss-EM-109 electron microscope at 50 Kv.

Morphometry - For morphometric studies, sections of the liver and lung from 5 animals at each experimental point were examined by digitalized morphometry, by using an electronic drawing board (Summasketch II, Summagraphics, Connecticut, USA) connected to an IBM-PC-AT compatible computer and the Sigma-Scan Measurement System (Jandel Scientific, San Francisco, USA) and a Zeiss Optical Microscope in which a camera lucida was attached. For morphometric calculations, only periovular granulomas having a mature schistosome egg in their centers were considered. They were regarded as spheres having normal size distribution. The size of the section was registered and measurements of the granulomas were made with a 10X objective, the system being calibrated for this particular amplification. The overlay images of the granulomas on the tablet were traced in their structural contours with the cursor connected to the microprocessor to compute the respective areas in μm^2 . The following parameters were calculated: size, volume density and numeric density. Size was represented by the surface area of the granulomas in the histological sections. Volume density was calculated as the quotient of the total granuloma profile area to the total section area per animal. The number of periovular granulomas per unit volume studied (numeric density) was estimated by applying the Weibel and Gomez's formula²⁰. Statistical evaluation was made by means of one way analysis of variance (ANOVA) and the linear regression test.

RESULTS

An average of 2 worm-pairs were recovered from the portal system of each animal. Effectiveness of portal vein ligation was monitored by the presence of peri-esophageal varices and by the finding of numerous pulmonary periovular granulomas in histological sections. Numeric density for pulmonary granulomas in ligated animals reached 4,600/mm³ as compared to 80/mm³ in non-ligated controls ($p < 0.0001$).

Granulomas located within alveolar tissue were observed after 10, 18 and 25 weeks after infection. Those formed around eggs containing a fairly preserved miracidium exhibited about the same sizes regardless the duration of infection, as demonstrated morphometrically (Fig. 1a), and statistically verified by linear regression

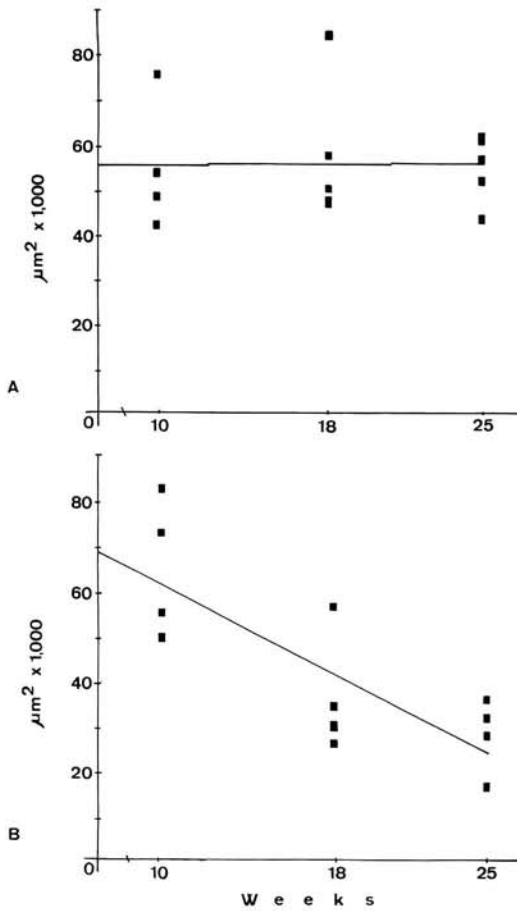


Fig. 1 - Morphometric data on schistosomal granuloma sectional areas at different times of infection. A - Pulmonary granulomas: linear regression test showed p value = 0.9421, the slope being not significantly different than zero. B - Hepatic granulomas: p value = 0.0016, the slope is significantly different than zero.

analysis ($p = 0.9421$). Periovascular granulomas around mature eggs formed in the liver were seen to decrease in size (Fig. 1b; $p = 0.0016$) and to change morphology with passing time. Pulmonary granulomas exhibited similar histological appearance during the complete observation period. Granulomas were represented by densely packed collections of cells. Macrophages (often appearing as epithelioid cells) and eosinophils were the predominant cell-types, but plasma cells, lymphocytes, fibroblasts and a rare neutrophil polymorphonuclear leukocyte were also observed (Fig. 2). The fibrous and fibrillar stroma was scanty. A few wavy and short reticulum fibers appeared scattered among the cells and tended to be more concentrated at the periphery of the granulomas (Fig. 3) in contrast to hepatic periovascular granulomas (Fig. 4). Elastic tissue was absent.

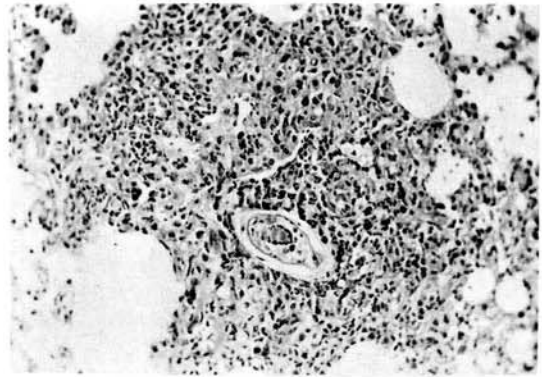


Fig. 2 - Example of a granulomatous reaction formed around a *S. mansoni* matured egg. There is a rather cellular aggregation, with many macrophages and eosinophils, supported by scanty extracellular matrix. Hematoxylin & Eosin, x200.

More rarely, fresh periovascular granulomas were formed in close proximity to or within segments of pulmonary arterioles. Then, muscular, endothelial and elastic arteriolar tissues appeared as an integral part of the lesion (Fig. 5). These granulomas exhibited proliferation of endothelial cells with new blood capillary formation (angiogenesis), and a more conspicuous proliferation of fusiform cells, usually accompanied by extracellular matrix deposition, with predominance of collagen fibers.

Hepatic granulomas presented a prominent stroma and, when undergoing involution, were accompanied by evident fibrosis that left relatively large pigmented scars.

Ultrastructurally, the compact collections of in-

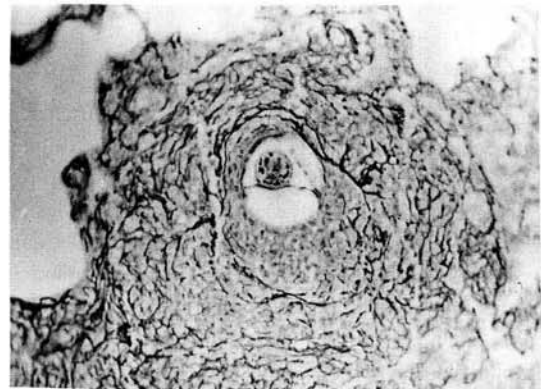


Fig. 3 - Periovascular granuloma in the lung of a mice showing delicate reticulin framework. Compare with the hepatic granuloma in the Fig. 4. Gomori's reticulum method. x200.

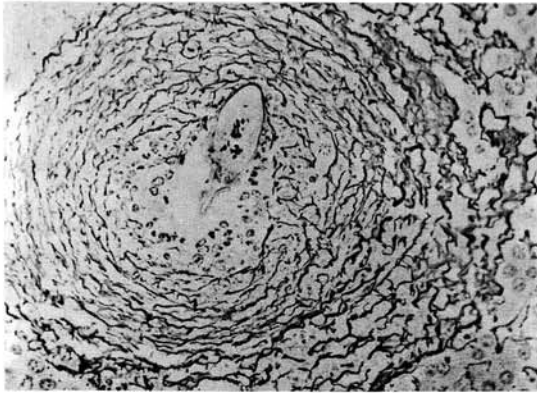


Fig. 4 - Abundant reticulin fibers present in a hepatic granuloma formed around a mature *S. mansoni* egg. Gomori's reticulum method, x200.

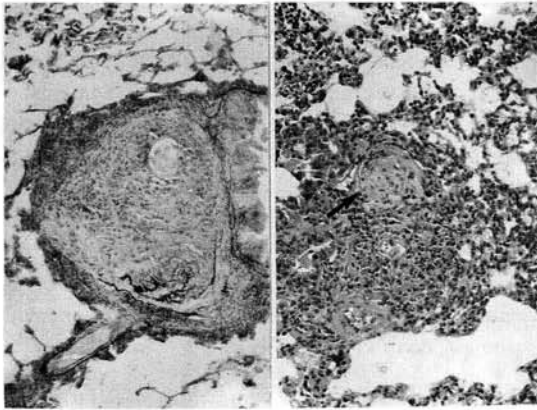


Fig. 5 - Pulmonary schistosomal granuloma involving arterioles. At left there are fragmentation of elastic tissue, smooth muscle and endothelial cell proliferation, besides the presence of macrophages and eosinophils. Orcein stain, x120. At right, an arteriole with obstruction and re-canalization, and smooth muscle and endothelial cell proliferation is seen eccentrically located within the granuloma (arrow). The egg is not present at the section level. Hematoxylin & Eosin, x120.

flammatory cells practically occurred in the absence of extracellular matrix (Fig. 6). Macrophages exhibited large cytoplasm with many mitochondria, a few lysosomes and a relatively abundant rough endoplasmic reticulum. A few tight membrane points of adhesions were occasionally seen between the external membranes of macrophages or between macrophages and other cells, such as plasma cells, but without forming specialized junction apparatus (Fig. 7).

The several cell-types were distributed at random, although eosinophils tended to form clusters. Besides

macrophages and polymorphonuclear eosinophils, which were the predominant cell types, plasma cells and small and large lymphocytes were also frequently seen. Degranulation of eosinophils was frequent. Fibroblasts were rare and appeared at the granuloma periphery. They exhibited slightly dilated rough endoplasmic reticulum and Golgi apparatus. Variable amount of collagen fibrils were seen in extracellular tissue in association with fibroblasts..

Old granulomas, as seen around disintegrating eggs or egg shells, presented signs of involution such as decreased size, a more condensed stroma and a predominance of fusiform cells. They became progressively smaller and both cells and stroma tended to disappear simultaneously, since significant fibrous scars were not observed in the lung, even after 18 or 25 weeks of infection.

Comparative morphometric data regarding the presence of granulomas in lung and liver are depicted in Fig. 8 which shows that volume density of pulmonary granulomas was significantly greater than that of the liver. As for the numerical density, which represents the number of granulomas per volume unit considered, it tended to remain stable in the lung, but to progressively increase in the liver, although the differences among the group means were not statistically significant (Fig. 9).

DISCUSSION

The experimental model of pulmonary schistosomiasis described by WARREN¹⁸ presents peculiar histological features. One of them is that the

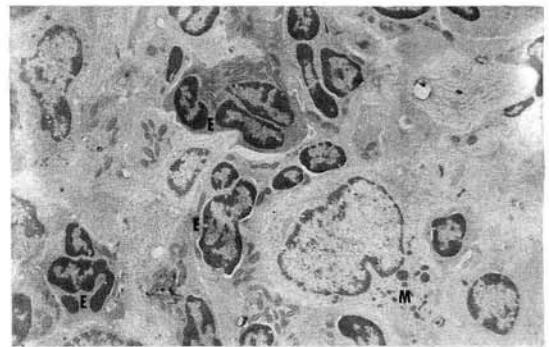


Fig. 6 - This picture shows the predominant cell types in pulmonary schistosomal granulomas: macrophages (M) and eosinophils (E). Also, the scanty stroma can be appreciated. Electron micrograph. x3,000.

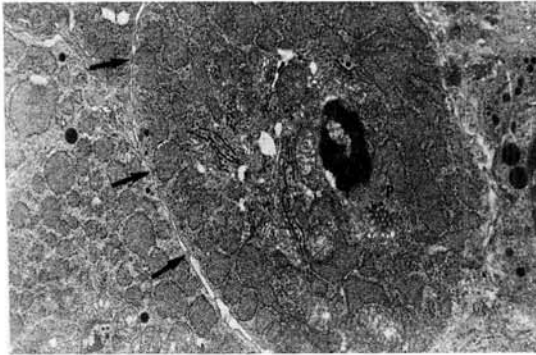


Fig. 7 - Two plasmacytes containing abundant endoplasmic reticulum secretory vesicles exhibit points of adhesion between their external membranes (arrows). Electron micrograph, x7,000.

granuloma formed within the alveolar tissue of the lung is represented by an aggregate mixture of cells with scanty extracellular matrix. CHIEVER⁶ called attention to the paucity of reticulum fibers in a lung granuloma as compared to one in the liver. Fibrosis formed in pulmonary alveolar granulomas is minimal and appears at the periphery of the granuloma. During involution such fibrosis completely disappears, together with the inflammatory cells. These features explain some peculiar aspects of pulmonary schistosomiasis, as will be discussed below.

There are two types of schistosomal granulomas in the lungs. One formed within alveolar tissue, and the other involving small branches of pulmonary arteries or arterioles. They differ morphologically, functionally and in their response to curative chemotherapy¹. Lodgement of eggs in arteriolar branches is a consequence of massive egg embolization to the pulmonary circulation. It seems to be preceded by pulmonary hypertension caused by dissemination "alveolar" granulomas², and to involve arterial smooth muscle and endothelial cells. They are more fibrogenic than alveolar granulomas, may cause significant vascular obstruction and capillary neof ormation (angiomatoid lesion), and are not completely repaired following curative chemotherapy¹. Vascular arterial lesions represent a hallmark of severe pulmonary schistosomiasis^{2, 5, 16}.

Although fibrosis is such a prominent consequence of hepatic schistosomiasis, it is not so for pulmonary schistosomiasis^{2, 5, 16}. After chemotherapy, the "alveolar" granulomas, but not the "arterial" ones, undergo complete resolution, more rapidly than those granulomas in the liver¹. A similar situation was observed in experimental intestinal schistosomiasis¹⁷.

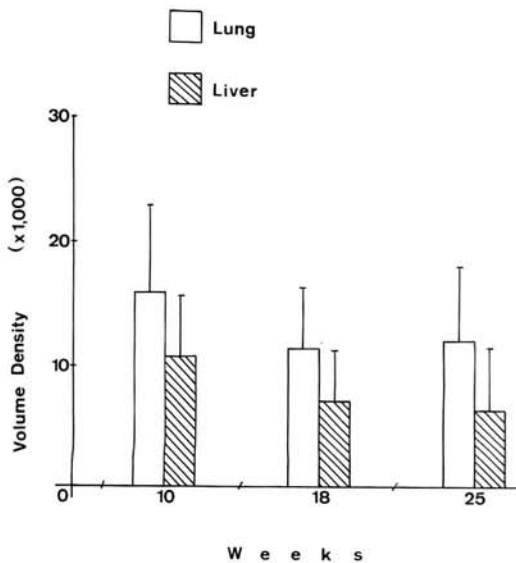


Fig. 8 - Volume density variation of pulmonary and hepatic schistosomal granulomas at different time points. The one-way analysis of variance showed that the differences among group means were significant.

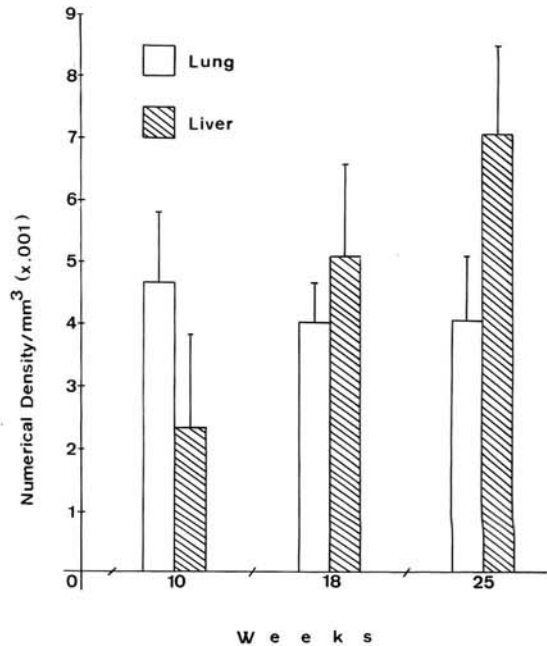


Fig. 9 - Numerical density of pulmonary and hepatic schistosomal granulomas at different time intervals. The one-way analysis of variance showed that the differences among means were not significant.

The difference in resolution time between granulomas in liver and lung can explain the progressive increase in numerical density with time for the hepatic granulomas, while that for the lung tended to remain stable. As for the determination of volume density, the volume of the compartment occupied by the granulomas, it was greater for the lung and this probably reflects the effectiveness of the portal vein ligation procedure in diverting schistosome eggs from portal to the pulmonary vascular system.

By looking at histological preparations of the lungs of animals with experimental pulmonary schistosomiasis, we were unable to tell whether the case belonged to early (10 weeks) or late (25 weeks) infection, since the granulomas were always similar. Perhaps the number of involuting granulomas was greater after 25 weeks, but those formed around mature eggs looked alike 10, 18 or 25 weeks after infection. This is in contrast to what happens in the liver, where the granulomas are known to diminish in size and to change their cellular composition with time³. Such spontaneous desensitization phenomenon has been extensively studied in hepatic granulomas and the evidence indicates that it is linked to a change in host immunological reactivity^{4, 8, 9, 13, 22} that may involve suppressor T cells⁷ or differential CD4 lymphocyte subset stimulation¹⁴, and may serve to protect the host³.

Granulomas in different parts of the intestines also differ in their sizes and cellular composition but undergo only mild changes related to immunological modulation during the course of infection²¹. Furthermore, granulomas in the ileum are even smaller than those in other segments of the intestines, do not depend on a suppression mechanism involving cimetidine or cyclophosphamide sensitive lymphocytes and do not undergo modulation²².

As shown here, the same is true for pulmonary alveolar tissue granulomas, although modulation has been claimed to occur in pulmonary granulomas formed after injections of isolated eggs into the tail vein of mice¹⁹.

Different aspects assumed by schistosomal granulomas in different organs is part of a general phenomenon in pathology. It is well known that soluble or fixed molecular sites present on vascular endothelium and extracellular matrix can cause

proliferation, chemotaxis, inhibition of migration, changes in phenotypic expression and mobilization of reactive cells, thus inducing selective homing of cells¹¹.

Schistosomal periovular granuloma is one of the most studied and better known type of granuloma. Its immunological aspects have been extensively investigated. Perhaps the Warren's model could be further explored concerning investigations on the role played by stromal or other local factors in modulating inflammatory responses.

RESUMO

Esquistossomose pulmonar experimental: falta de evidência morfológica de modulação nos granulomas esquistosomóticos pulmonares.

Em camundongos submetidos à ligadura parcial da veia porta e infecção pelo *Schistosoma mansoni*, os granulomas periovulares apareceram em grande número nos pulmões, comprovando a validade do modelo de Warren. Histologicamente os granulomas eram representados por agregados celulares compactos no seio de escasso estroma. Os macrófagos (células epitelióides) e eosinófilos eram os elementos celulares predominantes, vindo em seguida os linfócitos e plasmócitos. Ultraestruturalmente, as células do granuloma exibiam íntimo contacto de suas membranas, com vários pontos de adesão, mas sem formar estruturas juncionais mais específicas. Os granulomas formados em torno a ovos maduros tinham tamanho, forma e composição celular similares após 10, 18 ou 25 semanas de infecção. Este aspecto contrastava com o que aparecia nos granulomas hepáticos os quais exibiam a clássica modulação. Os granulomas que envolviam as paredes arteriolares exibiam proliferação muscular, endotelial, com formação de novas vias vasculares e fibrose.

As diferenças morfológicas observadas nos granulomas formados em diferentes locais e órgãos apontam para a importância de se considerar fatores locais (estromais) no determinismo dos padrões das respostas inflamatórias.

ACKNOWLEDGEMENT

We thank Dr. Eilson Goes Oliveira for his invaluable assistance with the statistical analysis.

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Recebido para publicação em 14/04/1993.

Aceito para publicação em 29/06/1993.