Review Article

Schistosomiasis and liver fibrosis

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SUMMARY

Schistosoma mansoni *infection invariably results in liver fibrosis of the host. This fibrosis may be represented by small focal areas of chronic inflammation and excess extracellular matrix deposited in periovular granulomas, distributed in variable numbers at the periphery of the portal vein system. This is the outcome of 90% of the infected population in endemic areas. Conversely, a minority of infected individuals develop extensive disease with numerous granulomas along the entire extension of the portal spaces. This latter situation is mainly dependent on special hemodynamic changes created by a heavy worm load, with the subsequent production of numerous eggs and represents a severe form of a peculiar chronic hepatopathy. Thus, host–parasite interactions in schistosomiasis help us to understand a number of important features of liver fibrosis: its initiation and regulation, the significance of accompanying vascular changes, the dynamics of fibrosis formation and regression with antiparasitic treatment; host genetic and immunological contributions, and the pathophysiology of portal hypertension.*

Keywords *fibrogenesis, fibrosis regression, hepatic fibrosis, periovular granuloma, portal vasculopathy,* Schistosoma mansoni

INTRODUCTION

A number of different species of schistosomes cause disease in man, but this chapter is solely concerned with schistosomiasis caused by *Schistosoma mansoni*, which is the only schistosome species to be found in Brazil.

Schistosoma mansoni is a digeneic intravascular parasite that lives within the venous portal-mesenteric system of man, where a single female worm can release an estimated number of 300 eggs per day. The liver therefore, is a focal point of pathogenic insult and subsequent pathological damage in schistosomiasis. The degree of pathology is variable, and can run from the presence of a few scattered isolated periovular granulomas distributed throughout the liver tissue, to systemic portal fibrosis with granulomatous chronic inflammation and severe obstructive vascular lesions; the reason for two differing outcomes depends in the main on the parasite load. It is unusual for an individual with a high worm load not to develop severe disease, but the development of the advanced forms of schistosomiasis have never been observed in an individual with a low worm burden. Therefore, although worm burden is of primary importance in pathogenesis, other factors such as host genetic background, conditions of infection and re-infections, and parasite strains, must also play a role (1).

The periovular granuloma is formed in a permissive host when a mature egg is trapped within the living tissue. It is an essentially inflammatory and fibrosing lesion, that soon becomes encapsulated by the formation of concentric collagen rings at its periphery. When the egg emerges from the female worm, it is still immature and causes no tissue reaction. It takes about 5–6 days for an embryo – the miracidium – to differentiate and to start eliminating lytic and antigenic secretions through micro pores present in the egg-shell.

This elementary lesion starts within a blood vessel, usually a pre-capillary venule. So, the vascular endothelium is the first line of damage and responds by proliferation. Factor-VIII-positive endothelial cells are present in great numbers within schistosomal periovular granulomas (2).

Additionally, studies in *in vitro* have revealed that soluble egg antigens (SEA) induce proliferation of endothelial cells (3) and also up-regulate vascular endothelial growth factor (VEGF) and angiogenesis (4). When vascular proliferation occurs, it involves not only endothelial cells but also their associated pericytes, which are actin-containing cells with high plasticity and the capacity to become transformed into myofibroblasts, an important matrix-synthesizing cell involved in vascular remodelling (5).

Schistosome eggs that do not successfully pass through the intestinal mucosa towards the lumen are usually carried by the portal vein blood flow to the liver until they stop inside small pre-sinusoidal vessels. When observed in a histological section, the granuloma then appears to be located within the liver parenchyma not in a portal space, as the smallest artery and bile duct segments presumably present before are involved and destroyed within the lesion. During a mild/low worm burden infection, if the arrival of new eggs in the liver is kept at a low pace, a balance may develop between the immune response to new eggs and the resorption of older lesions with mild or no clinical manifestations. This is what usually happens in endemic areas where approximately 90% of those infected present with mild clinical manifestations or are asymptomatic. Conversely, when a histological section reveals the presence of only a few schistosomal periovular granulomas located within medium-sized or larger portal spaces, where segments of an artery and bile ducts can be recognized, it serves as an indication for the presence of so-called advanced hepatic schistosomiasis. This means that diffuse destruction of peripheral portal vasculature has occurred and that this is sufficient to increase intra-hepatic portal pressure, thus allowing newly increased flow and the consequent arrival of eggs to be lodged into small veins branching off from medium–large sized portal trunks. Fibrosis may then involve large portal spaces and gives rise to one of the most pathognomonic or typical lesions in human pathology, systemic schistosomal periportal fibrosis (6), the morphological substratum of the clinical condition known as hepatosplenic schistosomiasis.

These two main presentations of fibrosis in hepatic schistosomiasis: the periovular granuloma and periportal fibrosis will be discussed in more detail below.

THE PERIOVULAR SCHISTOSOMAL GRANULOMA

The focal reaction formed around the mature schistosome egg – the periovular granuloma is well described although the morphology may vary with time. This process is controlled by several factors, not all of which are known. Andrade and Warren (7) observed that during early infec-tion in the mouse, the periovular granulomas in the liver were large, edematous, rich in oeosinophils with a more or less predominant necrotic centre and with a ragged periphery. With time, whilst older granulomas were involuting, those formed around newly arrived eggs appeared smaller, fibrotic and with a predominance of macrophages over oeosinophils at the periphery of the granuloma. This modulation over time was considered protective for the host, as the increased portal pressure registered during early infection progressively decreased with time although the number of eggs per gram of liver tissue markedly increased. A number of groups focused their research on identifying the factors involved in the modulation of the schistosomal granuloma. Miracidial secretions contain antigenic materials of a complex carbohydrate character and a host of enzymes (8). A major antigenic fraction was characterized as SEA (9). Soluble egg antigen was shown to elicit a host CD4 response, which was initially Th1-biased, but soon switched to become highly Th2-polarized. (10). Furthermore, the egg-induced granulomas and the accompanying hepatic fibrosis were observed to depend on cytokine regulation with interleukin 10 (IL-10) playing a central role (11).

Interestingly, a number of studies revealed such modulation as an exclusively hepatic phenomenon, as pulmonary and intestinal granulomas formed around mature eggs did not change in either size or morphology with time (12). One particular feature that may explain this aspect of the liver pathology in schistosomiasis may be related to the participation of the neuro-endocrine system of the liver in inflammatory and fibrosing processes (13,14). Preliminary studies revealed that the schistosomal granuloma in the mouse liver contains cells that are reactive to neuropeptides, neural molecules and to leptin and its receptor (14). Further investigations are therefore necessary to confirm whether the hepatic neuro-endocrine environment can play a decisive role in schistosomal granuloma modulation. (15).

The functional significance of the periovular granuloma in schistosomiasis has been a topic of debate. First suggestions were that the granuloma served to destroy the egg and the miracidium inside it. Oeosinophils, with their granules of major basic protein, were implicated in miracidium killing (16,17). Chemotherapeutic studies, however, revealed that the eggs remained alive inside the granulomas for up to 7 days after the death of all the worms, (a lapse of time, which is considered within the miracidial life-span). Furthermore, this finding was observed in both early and late infections, that is, regardless the cellular composition of the granuloma, whether it contained numerous or a few Oeosinophils. Therefore, the periovular granuloma appeared functionally important to protect the host tissue from enzymatic injury, rather than a reaction to destroy the miracidum (18).

PORTAL 'PIPESTEM' FIBROSIS

The most prominent feature of liver pathology in schistosomiasis is represented by a process of portal fibrosis that extends from the smallest to the largest portal spaces. It forms a typical gross finding that can be appreciated not only at the surface of the liver at autopsy but also from images obtained by ultrasound or magnetic-resonance techniques (19,20). When observed on the surface of a human liver at autopsy, whitish plaques are observed in clear contrast with the dark brown well preserved hepatic parenchyma. This typical finding has sometimes being referred to as 'pipestem' fibrosis, since Symmers (21), a British pathologist working in Egypt, described it in 1904, and compared each plaque at the cut surface of the liver with the section of a clay-pipe-stem.

As previously mentioned, this extensive portal fibrosis is associated with a schistosome infection with a heavy worm burden, as it is the presence of numerous schistosome eggs that cause a sufficient peripheral block of small portal vessels. This first results in an increase in portal pressure, thus allowing the blood flow to be partially diverted towards collaterals branching off from larger veins. As a continuous arrival of new eggs occurs, there will then be subsequent damage to veins of larger diameter, plus peri-portal granulomatous inflammation and inter-granulomatous fibrosis. The main functional consequence of this sequence of events is portal hypertension, which is soon followed by splenomegaly and porto-systemic collateral circulation, notably oesophageal varices, a dangerous lesion that may spontaneously rupture and lead to host death by acute anaemia. The hepatic parenchyma maintains its usual acinar structure, and this is reflected in patients exhibiting a normal liver function despite the signs of portal hypertension, a contrast with the situation in cirrhosis. This may be temporary; however, as in some cases this functional equilibrium may deteriorate with time in some patients, and the picture of 'compensated' hepatosplenic schistosomiasis may in turn 'decompensate' with the presence of ascites, muscular loss and hepatic failure. This unfavourable sequence of events is mainly because of episodes of gastric bleeding following the rupture of oesophageal varices, as the sudden drop in blood pressure leads to focal parenchymal ischaemic necroses to be followed by post-necrotic scarring. To better understand this problem, a brief description of the vascular changes that result in portal fibrosis is important.

Vascular changes

It is a common mistake to think that hepatic granulomas are pathogenic because they precipitate fibrosis, which then obstructs blood flow, increases portal blood pressure and ultimately promotes development of a portal-systemic venous shunt. Indeed, fibrosis represents an increase in extracellular matrix of the interstitial connective tissue that normally supports blood, lymphatic vessels, nerves and also biliary ducts in the liver. Thus, liver fibrosis *per se* usually causes no problems in an organ with such considerable functional reserve as the liver unless the structure being supported by such fibrosis is damaged. Schistosome eggs are important pathogenic factors because they directly cause vascular damage and obstruction. It has been demonstrated since long ago that individuals with classical Symmers′s pipestem fibrosis of the liver, may be asymptomatic and have no splenomegaly (22). A recent study made in humans with advanced schistosomiasis, using histology, morphometry, ultrasound and magnetic-resonance imaging, failed to correlate the degree of portal fibrosis with the severity of clinical presentation (23).

Fibrosis in schistosomiasis was also found to be under immunological regulation as it is closely related to and under the influence of the same cytokine patterns as the schistosome-induced egg immuno-pathology. The Th2 cytokines and in particular IL-13 and the IL-13 receptors (IL-13Rα2) seem to play a key role in hepatic fibrogenesis associated with schistosome infection (24).

Schistosomal fibrosis is accompanied by numerous vascular changes within the host. The best way to appreciate such changes is to examine plastic vascular casts in which the three vascular systems of the liver have been injected at the same time, with different colours (25). The portal vein then appears reduced and appears as the least prominent of the three vascular liver sectors, with considerable reduction of the fine peripheral branches. (Figure 1) Around the larger branches, that sometimes appear tortuous, there appears a network of fine vessels, sometimes producing an appearance of fine hairs around the main vessels. Large or medium-sized portal veins frequently present a sudden diminution of diameter, sometimes terminating as a point of a pencil. Short-circuit communications with a neighbouring branch were also observed. Whilst the hepatic vein system casts showed no changes and were comparable with those obtained from normal livers, the hepatic arterial system presented considerable hypertrophy and hyperplasia, especially at the site of the peri-biliary vascular plexus (Figure 2).

The peripheral destruction of the portal vein system associated with the occlusion or amputation of some of the medium-sized branches accounted for the presence of portal hypertension. The hepatic arterial changes were considered compensatory for the diminution of the portal vein flux. They constitute a crucial vascular alteration in advanced schistosomal liver fibrosis, responsible for
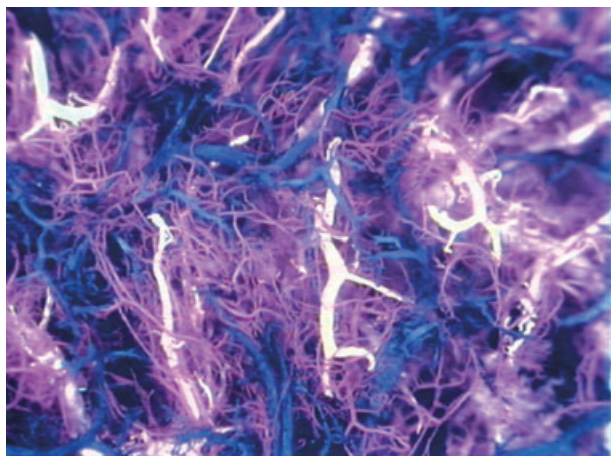
Figure 1 Vascular hepatic changes observed in a human liver in a case of advanced schistosomiasis post-injection-corrosion of the three systems with vinyl acetate. The portal vein is yellow, the hepatic artery is red, and the hepatic vein is blue. Whilst the portal vein appears severely damaged and reduced the hepatic artery is prominent and the hepatic vein system appears within normal limits.
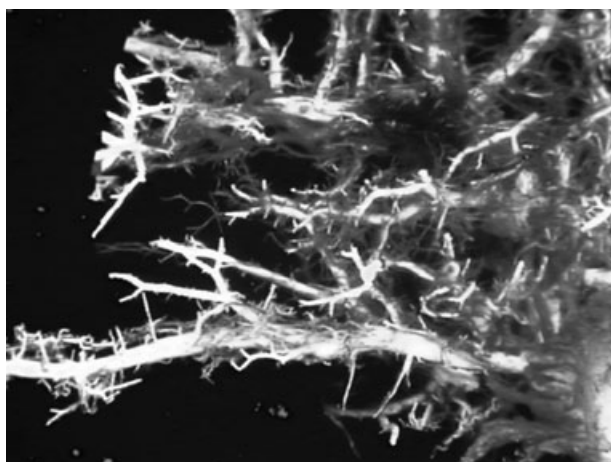

Figure 2 Vascular hepatic changes observed in a human liver in a case of advanced schistosomiasis post-injection-corrosion of the three systems with vinyl acetate. Demonstration of the irregularities in diameter and in the general disposition of the portal vein. Hyperplasia and hypertrophy of the hepatic artery system appear prominent.

increasing sinusoidal pressure and for inducing sinusoidal capillarization, changes that are important functional elements in the so-called decompensated phase of liver portal fibrosis in schistosomiasis; when signs of hepatic failure may complicate the clinical picture of hepatosplenic schistosomiasis. A further disadvantage of compensatory hepatic artery hypertrophy is that it renders the parenchyma to be dependent to a greater extent on arterial blood once the flux from portal vein blood is much reduced. The situation becomes critical when the general blood pressure suddenly drops, as can happen following massive gastric haemorrhage from the rupture of oesophageal varices. If the patient survives, the focal areas of ischaemic parenchymal necrosis undergo post-necrotic scarring (6). These focal areas containing liver cell regenerative nodules surrounded by fibrous bands and septa usually occur underneath the liver capsule, and when viewed later on may be erroneously diagnosed as cirrhosis.

Schistosomal portal fibrosis and inflammation (hepatitis)

In addition to the presence of periovular granulomas, the fibrotic portal spaces frequently show diffuse mononuclear leucocyte infiltration, sometimes with differentiation of lymphoid follicles and signs of interface activity ('piecemeal necrosis'). The aetiology of this chronic hepatitis is difficult to establish in human studies. Schistosomiasis itself can cause a certain degree of chronic hepatitis, probably of a reactive nature. The presence of this hepatitis in experimental animals; however, can be easily observed. It can assume prominent features and may disappear following the curative treatment of schistosomiasis (26). It is however, important to note that patients with hepatosplenic schistosomiasis are 7–10 times more susceptible to co-infection with hepatitis B virus than healthy blood donors from the same geographical area or even patients with mild schistosome infection. Patients presenting the hepatosplenic form of schistosomiasis associated with hepatitis B usually show persistent viraemia and exhibit chronic active hepatitis on liver biopsy, which can progress to cirrhosis (27). Similar findings have also been observed with viral hepatitis C. Conversely, other studies; however, failed to find a link between schistosomiasis and infections with hepatotropic viruses. This particular subject has been more thoroughly reviewed in an article that bears the title: 'Hepatitis B and schistosomiasis: interaction or no interaction?' (28). A major consideration was that most studies revealing increased susceptibility of patients with advanced schistosomiasis to hepatotropic viruses were based on hospitalized patients who may have been at greater risk of acquiring viral infections than subjects living in endemic areas. An experimental model susceptible to both schistosomiasis and viral hepatitis has been used in an attempt to clarify this discrepancy. The woodchuck (*Marmota monax*) is unique in this regard, as it is both susceptible to a B-virus-like hepatitis, the woodchuck hepatitis virus (WHV) and to *S. mansoni* (29). Concomitant viral and schistosome infections presented a simple additive pattern of lesions without any evidence of modification or aggravation of either one of the two infections. The viral load

was analysed and was not associated with the presence of schistosomiasis in the woodchucks. Therefore, schistosomiasis and viral hepatitis in woodchucks run parallel courses, with no apparent special features derived from the association of the two conditions. Thus, it seems that evolutionary possibilities for hepatitis virus infection in patients with schistosomiasis are the same as for the general population.

Fibrosis regression in schistosomiasis

Extracellular matrix formation and degradation are balanced processes dependent on the same cell types. The accumulation of excess matrix (fibrosis) occurs when matrix synthesis exceeds the normal rate of degradation. Chronic inflammation, a common consequence of parasitic infections, is a potent promoter of matrix formation. When the cause is removed and the inflammation subsides, degradation of excess matrix predominates and tends to result in the re-establishment of a normal or near-normal parenchyma/stroma ratio. Early on in infection, the younger the fibrosis, the faster the degradation; this process also occurs in long-standing fibrosis, albeit at a slower pace and with a peculiar morphology. At first the explanation for the prompt post-chemotherapy fibrosis regression in early schistosomiasis, as opposed to that observed later on in infection was based on the predominance of type III collagen in earlier granulomas, a collagen isotype supposed to be more easily degradable. In the liver of mice with an 8-week-old/50 cercaria infection, biochemical data revealed that type III collagen increased 22 times, whereas type I increased 11 times (30). No similar data are available for prolonged infection. Time-sequential observations with immunofluorescence microscopy revealed that both collagen isotypes disappeared gradually and simultaneously, although much more rapidly in early than in late schistosomiasis infections of mice (31,32). With time, collagen may acquire inter and intermolecular cross-links, and that may be the reason why it becomes more resilient with passing time.

It is important to note, however, that patients with hepatosplenic schistosomiasis who are given curative treatment may demonstrate regression of disease months or even years later. Data from human and experimental studies have revealed that in such situations, hepatic fibrosis regresses, accompanied by reduction in spleen size, a drop in the portal pressure, and a reduction of oesophageal varices (33–35). It can be assumed therefore, that not only hepatic fibrosis has been degraded and reduced but also considerable degree of intra-hepatic vascular remodelling had also occurred.

Recent studies show angiogenesis, formation of new blood vessels from pre-existing ones, plays a complex and extraordinary role in schistosomiasis. This may seem paradoxical, as schistosomes are intravascular parasites that cause lesions by destroying blood vessels, especially in the liver in the case of *S. mansoni* (6). Further studies revealed that angiogenesis plays a dynamic and fundamental role in the schistosomal periovular granuloma *in vivo* (36). Vascular proliferation has been shown to take place in the early periovular granuloma, but is soon gradually displaced towards the periphery, forming a vascular collar around the periovular granuloma, whereas the actual centre of the granuloma may appear almost avascular.

When there is fusion of several granulomas, as occurs in periportal fibrosis during heavy infection, both in man and in the mouse, the proliferated small blood vessels appear prominent in inter-granulomatous tissue, assuming an angiomatoid appearance. Despite the high level of vascular proliferation, many vessels are indeed destroyed and portal vein branches of different diameters can become partially or totally occluded. The vascular plastic casts obtained from the liver at such late stages of infection have revealed variable degrees of intra-hepatic portal vein obstruction, coupled with preservation of the hepatic veins and both hyperplasia and hypertrophy of the hepatic artery. Mice with a 4 month-50-cercaria infection presented with a poor portal vasculature, a 'dry-tree' appearance, which could be seen in vascular casts obtained from portal veins injected with vinyl acetate. Four months after treatment, application of the same vascular technique revealed a complete different picture with numerous small branches of vessels sprouting from and around the larger portal segments (Figures 3 and 4).

These findings give the impression at first inspection that post-chemotherapy increases in the vasculature resulted from pressure dilatation into an almost empty capillary bed after repair and dilatation of the larger portal vessels. A close microscopic observation; however, revealed that there were several focal areas of vascular proliferation associated with the presence of a positive staining for VEGF, plus the appearance of many actin-marked proliferating cells. A process of angiogenesis was therefore present during the recovery phase of hepatic schistosomiasis (Figure 5).

This raises an interesting question as to whether angiogenesis may function along a two-way tract, being important during fibrosis production as well as during fibrosis regression and why this would happen.

There is no doubt that in fibrosis formation the importance of the pericyte/myofibroblast axis is evident (37,38). In the case of fibrosis regression, there are new data indicating that the axis pericyte-endothelium may be involved
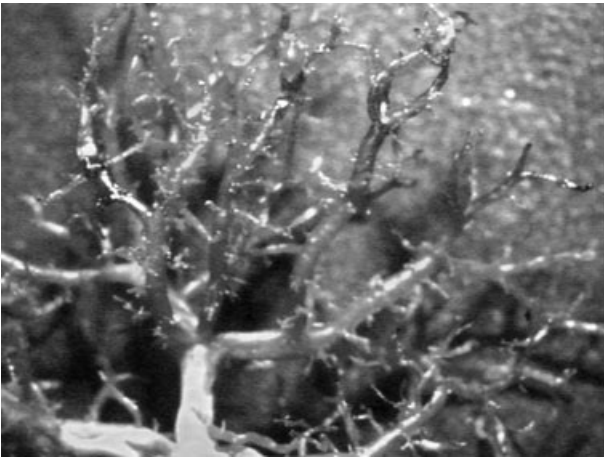
Figure 3 A vinyl plastic cast obtained from the portal vein of a mouse, which was submitted to 50 cercaria, 4 months post-*Schistosoma mansoni* infection. The larger branches are reduced in amount and size and show numerous short and small ramifications emerging in right angles from it walls creating the 'dry-tree' aspect.
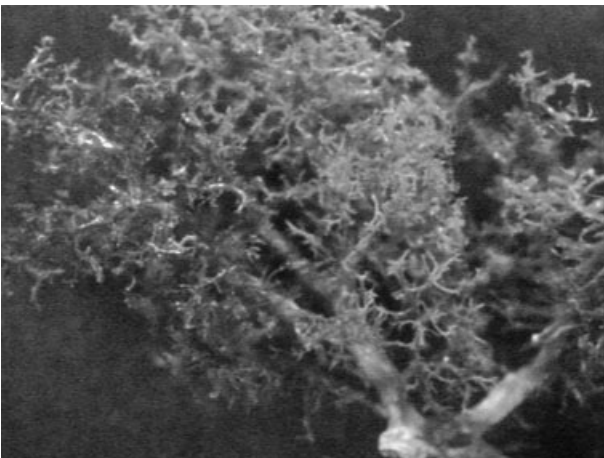

Figure 4 Portal system of a mouse infected as of Figure 3, but treated with praziquantel–oxamniquine at 4 months post-infection, and examined 4 months post-treatment. The portal vessels are now abundant in contrast with the appearance observed in Figure 3.

in remodelling, at least in the case of the damaged vasculature. As recently stated by Lee *et al.* (37) 'for a long time, the existence and role of pericytes were neglected, but during recent years these cells have gained increasing attention, not only as contractile cells but also as obligatory regulators of vascular development, stabilization, maturation, and remodelling'. These findings observed after chemotherapy of schistosomiasis in mice, described by Andrade *et al.* (36) and currently under further investigation, are strongly indicative of the role played by a
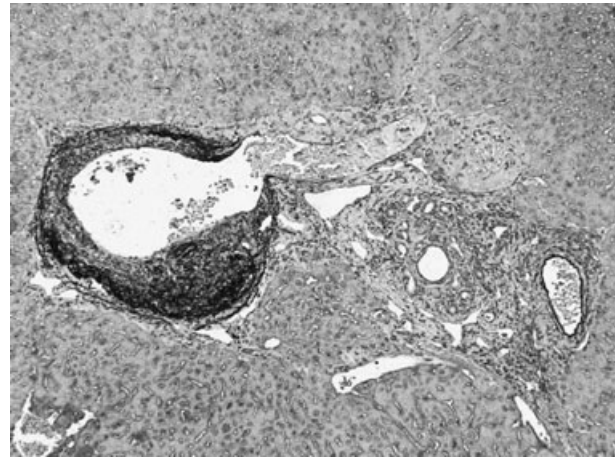

Figure 5 Portal spaces showing intense vascular proliferation at a zone of fibrosis and a medium-sized portal vessel exhibiting sub-endothelial elastic thickening with focal areas of remodelling from a mouse 4 months post-treatment for schistosomiasis. Orcein for elastic tissue, 200×.

capillary-associated, actin-containing cell in remodelling of the extracellular matrix and the associated vascular lesions. Medium-sized portal veins, which were frequently seen to be occluded by chronic inflammatory tissue in liver biopsies from mice 4 month-post-*S. mansoni* infection were observed in autopsy material from the same mice 4 months post-oxamniquine–praziquantel treatment. Other observations indicative of vascular remodelling were also observed. These included endothelial proliferation with the formation of new lumina inside the primitive vessel, focal disappearance of elastic tissue in areas of sub-endothelial elastosis and degradation of peri-vascular fibrosis (Figure 5).

These studies were further extended to include material obtained 6 months after treatment. At this time point, immunohistochemical methods for demonstrating VEGF and smooth-muscle actin were also included. The association of vascular proliferation with VEGF and the presence of actin-positive cells was clearly evident in the lesions (Figure 6). Vessels exhibiting sub-endothelial elastic hyperplasia (elastosis) frequently presented focal areas of elastic dissolution. Sometimes these lytic areas appeared as oedematous connective tissue with proliferated capillaries. Lesions involving larger branches of the intra-hepatic portal vein, which appeared occluded by a mixture of granulation tissue and focal areas of necrosis were attributed to the death of adult worms ('dead-worm lesions'). Such lesions were eventually detected in liver sections taken 6 months after treatment. They revealed no tendency towards fibrous repair, but rather one of remodelling towards the formation of numerous new vascular channels. The material obtained from treated schistosomiasis, in the
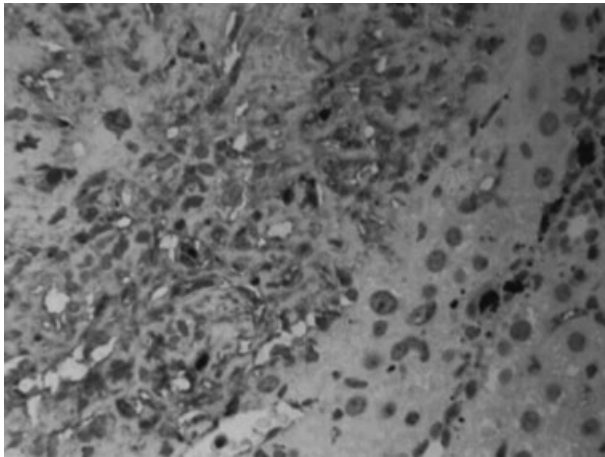
Figure 6 Intense angiogenesis at a fibrosed portal space observed in a mouse 4 months post-oxamniquine–praziquantel treatment of 4-month-old schistosomiasis infection. An edematous zone of fibrosis regression filled with small blood vessels is shown, which are made apparent by immunohistochemical smooth-muscle actin staining of the pericytes. Darker granules represent schistosomal pigment, 400×.

mouse reveals that the hepatic lesions do indeed undergo considerable remodelling with time. Obstructive vascular lesions are partially or completely repaired; the excess extracellular matrix undergoes regression at the same time that numerous small blood vessels are sprouting in several directions. When larger portal veins are obstructed by necro-inflammatory tissue, probably originating from the death of adult worms *in situ*, there appeared later on several foci of vascular proliferation (angiogenesis) instead of the classical repair changes observed with fibrous replacement in ordinary vascular thrombosis. It is probably the same cells, i.e. endothelial cells and pericytes that are at play in both instances; however, the local factors generated in both situations may be different.

CONCLUSION

There is considerable scope for further studies, which are necessary to clarify the molecular mechanisms involved in this complex process. At present, however, it is clear that the endothelium–pericyte axis is involved, which accentuates the importance of angiogenesis in both fibrosis formation, when the pericyte–myofibroblast axis is of prime importance (38,39), and remodelling of the lesions following treatment with oxamniquine–praziquantel, when the endothelium–pericyte interplay becomes evident.

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