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Schistosomiasis and hepatic fibrosis regression

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ABSTRACT

Manson schistosomiasis is an important cause of hepatic fibrosis, a consequence of the highly fibrogenic nature of the mature schistosome eggs, the main pathogenetic factor of that disease. Thus, students interested on schistosomiasis are to be also interested on the subject of fibrosis formation and degradation since the very beginning of their studies. A brief review of the studies directly related to such interest is presented here to stress the progress obtained, and to point out to the need of further research.

1. Introduction

The subject of hepatic fibrosis regression has recently received considerable attention. Advanced hepatic fibrosis, especially when associated with cirrhosis, had been for a long time considered an irreversible condition. This concept has suffered a drastic change nowadays, when new and effective drug therapy against fibrosis-inducing agents is resulting in clinical improvement of chronic liver diseases, accompanied by considerable regression of hepatic fibrosis. These clinical reports have stimulated research on the causes and mechanisms of fibrosis evolution and involution, as can be noted in several recent reviews (Benyon and Iredale, 2000; Arthur, 2002; Bataller and Brenner, 2005; Bedossa and Paradis, 2003; Brown, 2000; Desmet and Roskams, 2004; Friedman, 2003; Massarrat et al., 2004).

Since a long time ago, the students of schistosomiasis have been interested on the clinical and experimental aspects of fibrosis, since it is at the roots of the pathology of this parasitic disease. Although such investigations were rarely noticed by the students of fibrosis in general, a series of fundamental contributions have indeed been accumulated along the last decades. They will be briefly reviewed herein.

2. Periovular schistosomal granuloma

As soon as more effective curative drugs for the treatment of schistosomiasis became available, some studies were made to observe their effects upon the hepatic lesions occurring in the experimental murine model. It was observed that fibrotic lesions caused by the schistosome eggs within the liver almost completely disappeared following curative chemotherapy (Gonnert, 1955). The fact that fibrous tissue could be remodeled or degraded was not a novelty, since there were numerous examples, both in physiology and pathology, such as could be observed during morphogenesis and growth, post-partum uterine involution, tooth eruption, bone resorption, tumor invasion and metastasis, penetration and migration of parasite larvae, etc. However, the concept that long stand widespread fibrosis was irreversible was hardly shaken until recently, even in regard to schistosomiasis.

However, further reports soon indicated that the post-treatment disappearance of schistosomal periovular granulomas was not so simple as it appeared. Complete and rapid resolution of fibrosis was only to be expected from the early mouse infection (8–10 weeks from cercarial exposition), but not during prolonged infection (16–20 weeks) (Cameron and Ganguly, 1964; Warren and Klein, 1969; Boros, 1983). A possible explanation came from biochemical data which showed that in mice with an 8-week old, 50-cercaria infection, type III collagen, a presumably earlier and more degradable collagen isotype, increased 22 times, while the more stable type I isotype increased only 11 times (Biempica et al., 1983). These data were soon confirmed by immunofluorescence studies (Andrade and Grimaud, 1986). However, this later study revealed in addition that both collagen isotypes disappeared gradually and simultaneously from the post-treatment hepatic periovular granulomas. A better explanation for the relative resistance of fibrosis to degradation in late schistosomal infection came from the studies by Ricard-Blum et al. (1992). Their data suggested that collagen cross-linking, which increases progressively with time, rather than collagen genetic isotype, was responsible for turning fibrosis more resilient during late schistosome infection. Pyridinoline, a cross-linking aminoacid present only in mature collagen, was found to increase markedly and progressively in the livers of schistosome-infected mice. They suggested that the neo-synthesized collagen molecules deposited in the extracellular matrix of the periovular

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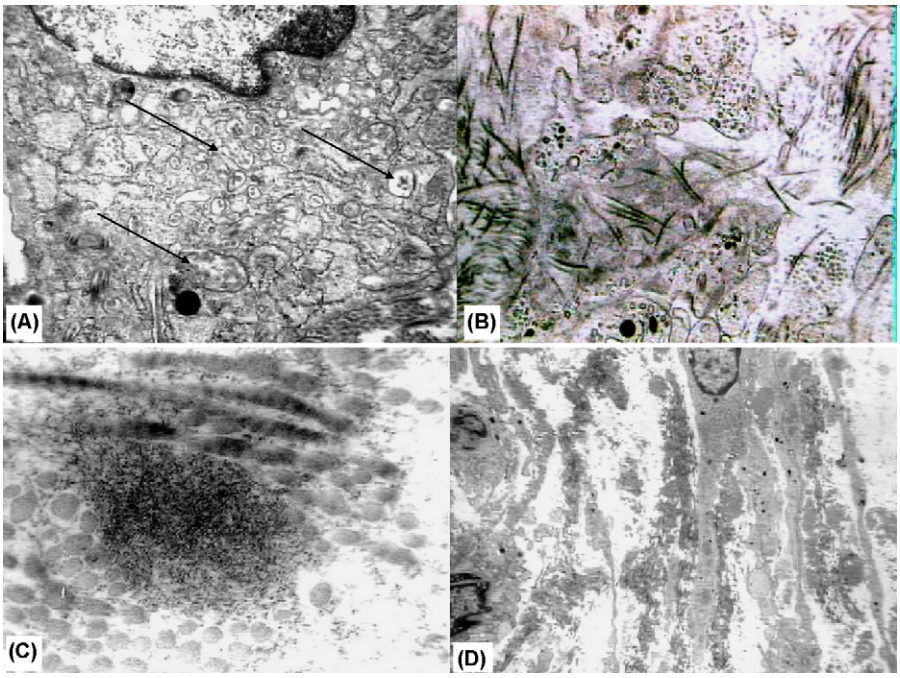

Fig. 1. Ultra-structural morphology of collagen degradation in treated schistosomiasis of the mouse. (A and B) Degradation of early fibrosis: (A) internalization of collagen fragments (arrows), 17,000×; (B) extracellular collagen breakdown, 7000×. Degradation of late fibrosis: (C) electron-dense change, focal accumulation of dark granules in between collagen fibrils, 52,000×; (D) lytic change, empty focal areas separating dense, fragmented collagen fibers, 7000×.

granulomas are at first stabilized by reducible cross-links and not by pyrodinoline.

Still intrigued by the problem of the relative irreversibility of hepatic fibrosis during late experimental infection, Andrade and Grimaud (1988) decided to observe the effect of treatment upon chronic schistosomiasis during a more prolonged time than hitherto attempted. The results were clear. The advanced hepatic fibrosis of the mouse, even when mimicking the "pipestem" type of portal fibrosis seen in advanced schistosomiasis of man, was also susceptible to degradation and resorption after curative treatment of the parasitic infection, once a sufficient time of 4 or more months was allowed to elapse. Instead of exhibiting a rapid and complete regression, as seen in early infection, the pattern of hepatic fibrosis degradation in late infection was slow and gradual, always leaving small pigmented scars around fragments of the egg shells. Extracellular matrix degradation, as seen in post-chemotherapy of early and late infections, revealed marked differences under the electron microscope. At the peak of the treatment-induced matrix degradation occurring in early infection, the ultrastructural picture was dominated by two main changes: extra-cellular collagen breakdown, and internalization of collagen fragments within mesenchymal cell cytoplasm (Andrade and Grimaud, 1986). Those ultra-structural changes are the very same main changes observed when the classical models of collagen degradation, such as the rat post-partum uterus (Henell et al., 1983) and the carrageenin granuloma (Perez-Tamayo, 1970) are used (Fig. 1A and B). On the other hand, two other ultrastructural changes were instead seen during late chronic schistosomal infection involution, both in the mouse (Andrade and Grimaud, 1988) as in man (Andrade and Peixoto, 1992): a *lytic change*, represented by multifocal areas of collagen dissolution; and an *electro-dense change*, when collagen and elastic fibers appeared as small dark focal areas of a finely granular material (Fig. 1C and D). The presence of these two ultra-structural changes has also been registered in other examples of chronic degradation affecting the extracellular matrix: in advanced periportal schistosomiasis of man (Andrade, 1992), in the capsule of the Seyle's pouch in rats (Freitas et al., 1992) and in experimental CCl_4-induced cirrhosis in rats (Cavalcanti et al., 2002). It is not known whether the same factors involved in "acute" collagen degradation are also responsible for the process of "chronic" degradation. One attempt to investigate this matter was made with liver biopsy material obtained from human schistosomiasis. The expression of metalloproteinases (MM-1, MM-2 and MM-9) and their inihibitors (TIMP-1 and TIMP-2) were searched in periportal areas presenting with the microscopical signs of "chronic" collagen degradation (Gomez et al., 1999). Results were negative. When a fresh periovular granuloma was occasionally present within these same areas, the presence of both collagenases and TIMP was strongly expressed. Of course, the possibility of those classical collagen degradation factors being present during chronic collagen degradation has not been ruled-out. Since the process is so slow, it is possible that only a minimal amount of collagenolytic factors is required, not susceptible to be revealed by routine immunohistochemistry. The important fact is that the mechanism of degradation of long standing fibrosis has not been adequately elucidated. The current experimental models used to study the factors involved in collagen degradation are all examples of "acute" fibrosis reversion. When dealing with "chronic" fibrosis regression, one assumes that the same factors, collagenases and TIMP, are at play, but such still needs to be demonstrated. At present, schistosomiasis is teaching us that the slow process of matrix degradation in long-standing fibrosis differs from that present in the rapid process of early fibrosis regression, not only in time, but in its ultra structural and histochemical features. Such differences suggest that they may be also under different patho-physiological mechanisms, a matter that still needs to be clarified (Fig. 2).

3. Schistosomal periportal "Pipestem" fibrosis

The large majority of people infected with *Schistosoma mansoni* in endemic areas of Brazil exhibit an asymptomatic form of the infection, while others may complain of mild non-specific

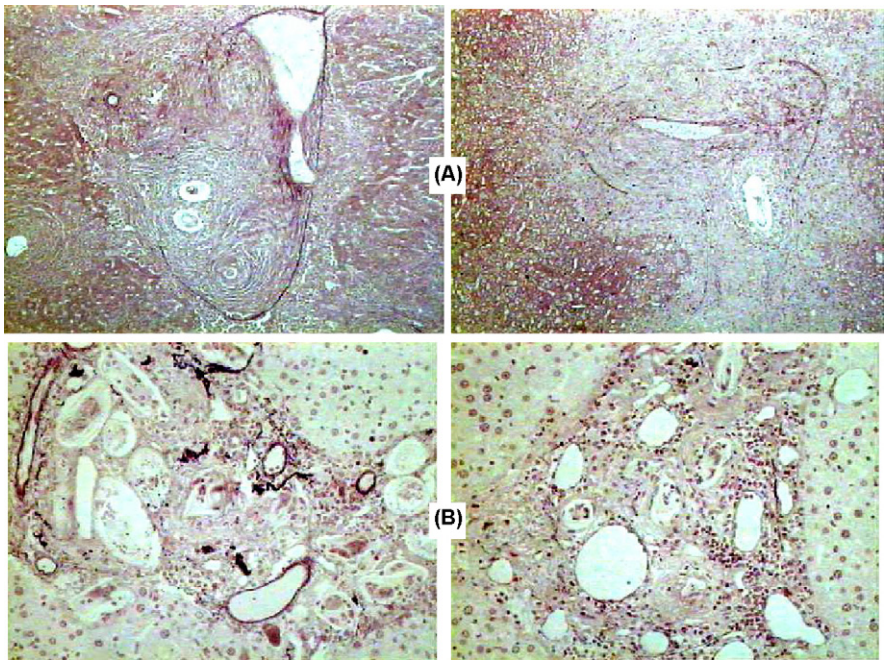

Fig. 2. Effect of specific treatment on chronic hepatic schistosomiasis of the mouse. (A) Biopsy sample showing portal fibrosis and severe obstructive portal lesions in the liver of a mouse with a 4-month-old infection. (B) Liver from the same animal seen above, taken 4 months after curative treatment of schistosomisis. Portal obstructive lesions are now replaced by vascular dilatation and fragmentation of elastic tissue. All the pictures with elastic tissue stain, 400×.

gastro-intestinal symptoms, probably in response to a low worm burden. A small proportion (2–8%), with heavy infection, usually present a serious hepatic condition, with hepatosplenomegaly and esophageal varices, that may lead to gastro-intestinal hemorrhage, hepatic insufficiency, and death. At autopsy, the classical gross picture is pathognomonic. There is systematized portal fibrosis, forming large plaque of hard white fibrous tissue which stands on a background of a normal-looking parenchyma (Andrade, 2004). Inside this periportal fibrous tissue there are fibrotic inflammatory lesions around schistosome eggs, and frequent obstructive vascular lesions, compromising the intra-hepatic branches of the portal vein. However, such advanced fibro-vascular lesions may sometimes almost completely regress. Historically, hepatosplenic schistosomisis can be considered as the first human disease in which widespread hepatic fibrosis was demonstrated to be susceptible to regression, both clinically (Bina and Prata, 1983; Dietze and Prata, 1986; Homeida et al., 1988; Mohamed-Ali et al., 1991; Richter, 2003) and pathologically (Andrade et al., 1992), following curative treatment of the parasitic disease. In case the extinction of parasitism spontaneously occurs, as suggested by some reports, the consequences upon the hepatic lesions are apparently the same as that of post-curative treatment. The report by Katz and Brener (1966) was impressive on this regard. They visited an endemic isolated area in the interior of Brazil and classified the clinical forms presented by those infected. Ten years later they returned to that same area and were able to re-examine 112 patients. None of them had been treated for schistosomiasis. Amongst 91 of them seen with mild infection at the first visit, seven had evolved to hepatosplenic disease. Amongst the 21 seen with hepatosplenic schistosomiasis, in eight of them splenomegaly had spontaneously disappeared, indicating that they regressed to a milder form of the disease. These were the first cases of spontaneous regression of hepatosplenic schistosomiasis to be reported in the literature. When new curative anti-schistosome drugs were introduced in therapy, the observation of regression of hepatosplenic schistosomiasis was frequently reported. It usually took 2 or more years for the beneficial effects of curative treatment to become evident, but this interval usually drops to months when the patients are young and present with a disease of more recent installation (Dietze and Prata, 1986). This was in keeping with the experimental observation that early fibrosis was more susceptible to degradation in comparison to the late one.

So far the attention on the possibility of fibrosis regression in hepatosplenic schistosomiasis has been focussed on fibrosis itself. However, although fibrosis reflects prognosis and severity of a chronic hepatic condition, being it schistosomiasis, hepatitis or cirrhosis, its role in pathogenesis is merely secondary. The vascular lesions within the fibrosis are really the main factor responsible for the physiologic derangement. Therefore, when a treated case of hepatosplenic schistosomiassis presents later on with hepatic fibrosis regression, accompanied by reduction of the spleen size and disappearance of esophageal varices, it means that not only fibrosis has been degraded and reduced, but that a considerable degree of intra-hepatic vascular remodeling had occurred. Therefore, besides the interplay of factors already pointed out by connective tissue research, such as metalloproteinases, TIMPS, cells and cytokines, on extracellular matrix degradation, there are even more complex factors involved in hepatic fibrosis regression. Recently it has been shown that treatment of schistosomiasis in mice with the "pipestem" type of schistosomiasis is followed 4 months later, not only by fibrosis degradation and resorption, but also by the repair of the intra-hepatic damaged portal vessels, disappearance of vascular occlusion, and the sprouting of numerous small blood vessels (Andrade et al., 2006). This last finding suggested that an important role is being played by angiogenesis in the repair process. As a matter of fact, angiogenesis has more recently been linked with fibrosis formation (Rosmorduc et al., 1999; Bataller and Brenner, 2005), rather than with fibrosis removal. Probably angiogenesis can play a two-way role in connective tissue metabolism. Although the presence of a rich vasculature in schistosomiasis treated mice could be explained by re-canalization of large obstructed portal vessels and pressure dilatation of the numerous small branches and capillaries,

the vascular neo-formation (angiogenesis) cannot be discarded. The mechanisms behind these changes seem to have a high degree of complexity. By seen such impressive vascular changes during the experimental regression of schistosomiasis in the mouse, one cannot help but speculate about the presence of stem-cells, resident or migrated from elsewhere, with potential to induce vascular remodeling, removal of the excess extracellular matrix, and angiogenesis (Theise, 2003). Recently, great emphasis has been given to angiogenesis in fibrosis formation (Rosmorduc et al., 1999; Souza et al., 2006). The evidences are so strong on this regard that a recent editorial suggests that new approaches to therapy of liver fibrosis may soon be derived from progress on angiogenesis management. (Lai and Adams, 2005). Now, the evidences of endothelial proliferation and the appearance of numerous vessels in periportal tissue following curative chemotherapy of schistosomiais bring about the question whether angiogenesis may act in a two-way mechanism, being important to form as well as to remove fibrosis (Andrade et al., 2006). The reason for angiogenesis being so prominent during the process of wound healing (granulation tissue) and in fibrogenesis in general, seems related to the presence of a special cell-type, which is present within the capillary walls—the pericyte. Actin-containing pericytes may be detached from the capillary walls during wound healing and undergo transformation into myofibroblasts, a key-cellular factor in fibrogenesis. Since pericytes are a kind of primitive cell, with a large potential to differentiate into other connective-tissue cell types, therefore their presence may also explain the role of angiogenesis in fibrosis degradation and tissue remodeling (Gerhardt and Betsholtz, 2003). Future research is expected to clarify this matter, probably with the schistosomiasis model being more and more utilized.

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