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REVIEW

Evolutive phases of experimental prehepatic portal hypertension

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Abstract

Partial portal vein ligation is the experimental model most frequently used to study prehepatic portal hypertension. Different systemic and splanchnic biochemical and histological alterations in short-term (28–45 days) and long-term (12–14 months) evolutive phases which has been described in this experimental model suggest the existence of different pathophysiological mechanisms involved in their production. The enteropathy produced could develop in three phases: an early or acute phase with vasomotor hemodynamic alterations (ischemia-reperfusion associated with intestinal hyperemia, edema and oxidative stress); an intermediate phase with immunological alterations (mesenteric lymphadenopathy, increased mucosal infiltration by mast cells and the hepato-intestinal release of pro- and anti-inflammatory mediators); and a late or chronic phase with intestinal remodeling (vascular and epithelial). The alterations which are produced in these three evolutive phases make it possible to propose an inflammatory etiopathogeny for hypertensive portal enteropathy.

Introduction

Portal hypertension is one of the most serious complications of chronic liver disease. It manifests clinically as ascites, portosystemic encephalopathy and variceal hemorrhage, and often leads to death. In order to control or prevent these complications, it is important, but difficult, to understand the mechanisms involved in the development and maintenance of portal hypertension.[1]

Partial portal vein ligation (PVL) in various animals, but particularly in the rat, has been widely used for portal hypertension studies.[2–5] However, it is considered that PVL does not produce liver damage. There is some question about the relationship between prehepatic portal hypertension and the hemodynamic abnormalities associated with liver cirrhosis, such as those found in over 90% of humans with portal hypertension.[6]

It has also been suggested that the rat model of graded portal vein stenosis is much more homogeneous than human portal vein obstruction. This is because of its narrow range of portal hypertension, degree of portosystemic shunts and hepatic atrophy.[7]

However, the evolution of PVL rats is far from uniform. They can present wide variability in both hepatic weight (degree of liver atrophy)[8] and in the type and degree of portosystemic collateral circulation which develops.[8,9] Furthermore, the variability of this experimental model of prehepatic portal hypertension is not only observed in the short term evolution (14–28 days), where it is studied most[3,5], but also in the chronic evolutive stages (6–14 months).[10]

Thus, it is perhaps necessary to consider the arguments against the validity of this experimental model to perform the evolutive study of portal hypertension. All of the variations presented by the animals after PVL, rather than invalidating the experimental model and thus disappointing the researcher, probably add complexity. Even more importantly, they can pose problems that may be regarded as tempting challenges for the researcher. It is also possible that a greater knowledge of the etiopathogenic mechanisms involved in the evolutive variability of this experimental model will help to elucidate the evolutive characteristics of human portal hypertension.

Evolution of experimental prehepatic portal hypertension

The mechanisms which contribute to the development and maintenance of portal hypertension change with time in the PVL rat.[9,11]

In the first few days after portal stenosis, hypertension is attributed to the sharp increase in the resistance to the flow caused by portal stenosis. However, 4 days after portal stenosis, partial development of portosystemic collaterals reduces portal venous

resistance, and portal hypertension is maintained by a rise in the splanchnic venous flow, which is secondary to the intestinal hyperdynamic circulation established completely at 8 days of evolution.[11]

At 2 weeks postoperatively, the animals develop splanchnic and systemic hyperdynamic circulation, with derivation of 90% of the portal blood flow through the portosystemic collaterals, which means that there is a decrease in the portal flow that reaches the liver.[3,12,13] The portal pressure in this evolutive stage is about 15 mmHg, corresponding to an approximate increase of 6 mmHg in relation to its value in control rats.[11] Portal pressure can be measured directly or indirectly. The first approach involves cannulation of the mesenteric vein through the ileocecal vein or a small ileal vein with a PE-50 catheter, placing the tip of the catheter in the distal part of the superior mesenteric vein.[3,7,12,14] Indirect measurement of portal pressure is performed by determining the splenic pulp pressure by intrasplenic puncture, inserting a fluid-filled 20-gauge needle into the splenic parenchyma.[11] An excellent correlation has been shown between splenic pulp pressure and portal pressure.[11,13]

In this evolutive phase, portal hypertension is a consequence of a pathological increase in the portal venous inflow ('forward' hypothesis) and resistance ('backward' hypothesis).[9,11,13] The increase in blood flow in the portal venous system is established through splanchnic arteriolar vasodilation that produces hyperdynamic splanchnic circulation or splanchnic hyperemia.[11] In turn, the increase in vascular resistance to the portal blood flow is found in the presinusoidal (partial portal vein ligation) and sinuosidal (hepatic atrophy) hepatic circulation, as well as in the portal collateral circulation (enhanced portal collateral resistance).[9,11,13,15] Therefore, normalization of elevated portal pressure can only be achieved by attempting to correct both elevated portal blood flow and elevated portal resistance.[14] (Fig. 1).

Hyperdynamic circulation in short-term PVL rats has been principally attributed to two mechanisms: (i) increased circulating vasodilators; and (ii) decreased response to vasoconstrictors.[6,15,16] Nitric oxide (NO),[17–20] carbon monoxide (CO),[21] tumor necrosis factor-alpha (TNF-α)[22] and glucagon[23–25] are well known factors that cause vasodilation. In turn, hyporeactivity to vasoconstrictors, that is, to endogenous (norepinephrine endothelin, vasopressin) or exogenous (alpha agonists) ones, reflects an impaired vasoconstrictor response, which contributes to vasodilation.[26,27] There could also possibly be other mediators, like prostacylin, endothelium-derived hyperpolarizing factor, endocannabinoids, adrenomedullin or hydrogen sulfide,[27] underlying the hyporeactivity to vasoconstrictors in portal hypertension.[21] Such is the importance of vasodilation in the development of hyperdynamic circulation that Iwakiri and Groszmann have recently proposed the term 'progressive vasodilatory syndrome' to name this hemodynamic change.[27]

In this evolutive phase of experimental prehepatic portal hypertension, there are two main types of portosystemic collateral circulation; splenorenal and paraesophageal[28,29] (Fig. 2). Although esophageal varices are not developed, the existence of hypertensive gastropathy[30–32] with more sustained and pronounced congestion of the stomach has been described when splenic vein ligation is associated.[31] It has also been reported that left perirenal devascularization, by preventing the development of splenorenal collateral circulation, increases the paraesophageal collateral

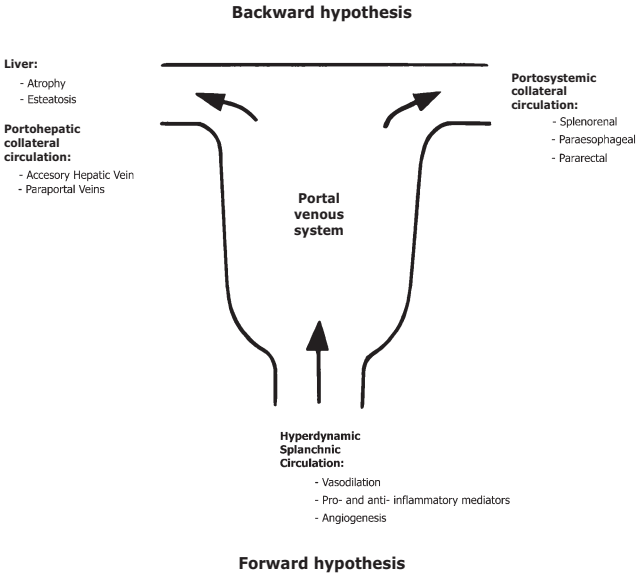

Figure 1 Mechanisms underlying the pathophysiology of short-term prehepatic portal hypertension in the rat. The splanchnic hyperdynamic circulation ('forward' hypothesis) and the increase in the portal blood flow ('backward' hypothesis) can be caused by different mechanisms (hemodynamic, immunological and remodeling by angiogenesis) during the evolution of experimental prehepatic portal hypertension.

circulation, as well as causing dilation of the submucosal veins of the distal portion of the esophagus.[29]

There are multiple factors involved in the development and enlargement of portosystemic collaterals, which tend to regulate the collateral flow.[20] Development of the portal collateral venous system is not only due to the opening of pre-existing vessels, but also to new vessel formation, which is a very active process.[19] Although portal-systemic collaterals have been considered to develop as a consequence of increased portal pressure, this is not essential to ameliorate the collateral circulation.[5,20] Moreover, collateralization and portosystemic shunting do not appear to be exclusively dependent on portal venous inflow either.[11,20]

PVL in the rat produces hepatic atrophy with loss of the hepatic sinusoidal bed and is the cause of an increased resistance to portal blood flow.[14] However, the degree of hepatic atrophy at 45 days post-PVL is not homogeneous and there are even some cases in which the hepatic weight increases compared to control rats.[8] In rats with prehepatic portal hypertension at 2 months of evolution, Van Thiel *et al.*[33] also described an increase in their liver weight, although its production mechanism was not known. The different evolution in hepatic weight in rats with short-term prehepatic portal hypertension is an interesting finding, because it demonstrates the existence of a heterogeneous hepatic response in this experimental model.

Although the resistance induced by ligature around the portal vein is fixed,[11] portoportal shunts that bridge the portal stenosis can be developed. Portohepatic collateral circulation, represented by the accessory hepatic vein, is more frequent in PVL rats with hepatic atrophy.[10] This vein accompanies the artery of the same

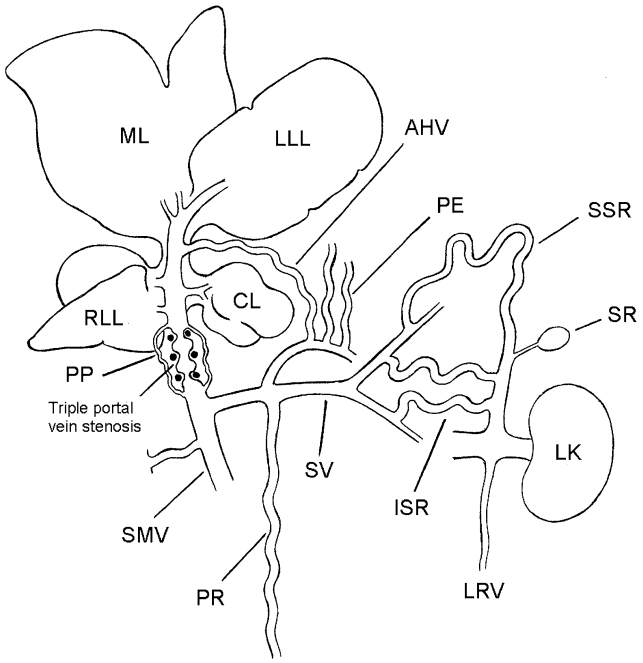

Figure 2 Types of portohepatic and portosystemic collateral circulation in prehepatic portal hypertension in the rat. RLL, hepatic right lateral lobe; ML, hepatic middle lobe; LLL, hepatic left lateral lobe; CL, hepatic caudate lobe; AHV, accessory hepatic vein; PE, paraesophageal veins; SSR, superior splenorenal collateral; SR, suprarenal gland; LK, left kidney; LRV, left renal vein; ISR, inferior splenorenal collateral; SR, splenic vein; PR, pararectal collateral; MVS, mesenteric vein; PP, paraportal collaterals.

name.[34] This collateral vein originates in the left gastric vein, follows an ascending paraesophageal pathway and enters the left lateral and caudate lobes, producing portal revascularization of the liver when the vein reaches the hilum[10] (Fig. 2). Although these animals also present bridging paraportal veins, the sizes of these are always too small and too few to be considered hepatic revascularization pathways. On the contrary, sometimes, the size of the accessory hepatic vein suggests that the portal revascularization through it is effective.[10]

In PVL rats at 6 months of evolution, the splanchnic hyperdynamic circulation has been described to disappear, although portal hypertension persists (12.3 ± 0.4 mmHg). This is due, among other factors, to the development of an increased resistance of the portosystemic collateral to the portal venous inflow.[9] These animals also present a high degree of portosystemic shunting and it has been suggested that the increase in portocollateral resistance could be attributed to the structural or functional changes induced by the chronically increased blood flow through the splanchnic circulation in previous stages.[9] It has also been proposed that the spleen has a modulating function on the portal pressure because ligature of the splenic artery, but not splenectomy, reduces portal pressure.[35,36] This protective effect of the spleen in portal hypertension has been attributed to the preservation of the splenorenal collaterals that facilitates drainage of the splanchnic venous blood.[35] In this evolutive stage (6 months), the most frequent and developed types of portosystemic collateral circulation are the splenorenal and paraesophagaeal ones, in detriment to the pararectal type.[10]

PVL rats of 6 months evolution also present a wide variability in hepatic weight, although this cannot be attributed to the existence of different degrees of hepatic atrophy because they evolve with hepatomegaly or their hepatic weight is similar to that of control animals.[10] The accessory hepatic vein, a bridging portohepatic collateral, is also frequent in this evolutive stage of experimental prehepatic portal hypertension.[10] Although these rats develop portohepatic collaterals, the possibility that the vasoactive substances secreted into the portal system will undergo hepatic metabolism, which could prevent their systemic effects, has been rejected. One of the reasons for supporting this supposition is the decrease in indocyanine green clearance in long-term PVL rats compared to control animals. Clearance of this dye, which is largely extracted by the liver and not by other tissues, is directly related to hepatic blood flow.[9]

Portal hypertension also persists at 12 months of evolution in an experimental rat model using an ameroid constrictor, in which the gradual portal venous occlusion is associated with hepatic lymphatic ligation. However, in this evolutive period the earliest splenorenal shunts developed regress, while the large bridging portohepatic collaterals and the paraesophageal and intraesophageal veins persist.[28] However, PVL rats at 12 months of evolution show a great variability in both types of collateral circulation developed and also in liver weight.[37] Regarding portosystemic collateral circulation, the paraesophageal (94.1%), splenorenal (82.3%) and pararectal (64.7%) types[37] tend to predominate, showing that the splenorenal shunt does not regress in this experimental model. The liver weight of rats with portal hypertension is also similar to or greater than that of the control animals in this evolutive period. Especially in cases with hepatomegaly, the incidence of portohepatic collateral circulation, represented by the accessory hepatic vein, decreases.[10,37]

Finally, at 14 months of PVL in the rat, the most frequent types of portosystemic collateral circulation also correspond to splenorenal and paraesophageal ones. Moreover, no animal developed hepatic atrophy and, in cases with hepatomegaly, the increase in hepatic weight occurred at the expense of excessive growth of the posterior hepatic lobes, that is, the right lateral and caudate lobes.[38] Furthermore, the significant hypertrophy of the posterior hepatic lobes is associated with atrophy of the anterior lobes, that is, the middle and left lateral lobes.[38]

Evolutive types of experimental prehepatic portal hypertension

Owing to the different mechanisms that contribute to the development of prehepatic portal hypertension in the rat, attributing different evolutive phases to this disease is possible.[11,13,35] Therefore, the study of the late evolutive phases could be considered of greater interest because the mechanisms involved in its production as well as the disorders that it causes would be more similar to those described in human clinical features, because they are secondary to the chronicity of the portal hypertension, among other factors.[10,38]

The wide variation in hepatic weight presented by the PLV rats in both early as well as late evolutive phases suggests that the liver could be one of the factors that determines the evolutive

heterogeneity of this experimental model.[8,10] If the animals are distributed according to their hepatic weight in each evolutive phase, from more to less, into three groups called A, B and C, a cluster analysis shows that in early evolutive phases (45 days) of the experimental prehepatic portal hypertension, the percentage of animals with less hepatic weight is greater (group C). On the contrary, in the late evolutive phases (6, 12 and 14 months post-PVL) the percentage of animals with greater hepatic weight (group A) increases progressively.[10] It could therefore be considered that the hepatic atrophy (group C) that characterizes the early evolutive stages of prehepatic portal hypertension in the rat[11,39] may be a reversible alteration in the long term.[10] It is significant that the animals belonging to the A group, although characterized by an increase in hepatic weight, also present portosystemic collateral circulation. Thus, this type of evolution (groups A) does not invalidate the experimental model of prehepatic portal hypertension in the rat in either the short or long term.[10,37]

The different evolution of hepatic weight in rats with prehepatic portal hypertension is an interesting finding, because it demonstrates the existence of a heterogeneous hepatic response in this experimental model. A histological study of the liver, performed to verify if the existence of a liver pathology could justify this wide spectrum of liver weight, has demonstrated that hepatocytic fatty infiltration exists in PVL rats.[40] It has also been verified in this study that the fat accumulation in the hepatocytes is progressive from a short-term (1 month) to a long-term (1 year) evolutive stage of portal hypertension and hence the possible persistence of the etiopathogenic mechanisms involved in its production.[40] Besides, liver steatosis and hyperplasia are accompanied by the presence of megamitochondria in the hepatocytes, as shown in rats with portal hypertension after 6 weeks of evolution.[41]

Liver steatosis could also cause the hepatomegaly which characterizes PVL rats belonging to the A group. If so, it could be considered that partial portal vein ligation does not only produce an experimental model of portal hypertension but also a steatosis model.[42]

Experimental prehepatic portal hypertension and splanchnic inflammatory response

Portal hypertension is essentially a type of vascular pathology resulting from the chronic action of mechanical energy in splanchnic venous circulation. This kind of energy can stimulate the endothelium which, owing to its strategic position, plays an exceedingly important role in regulating the vascular system. It does so by integrating diverse mechanical and biochemical signals and by responding to them by releasing vasoactive substances, cytokines, growth factors and hormones.[43–46]

Mechanical energy may also act in the vascular endothelium as a stress stimulus generating an inflammatory response.[47] In the case of portal hypertension, there is an endothelial inflammatory response induced by mechanical energy that affects the splanchnic venous circulation and, by extension, the organs into which its blood drains. There could therefore be a common etiopathogeny that integrates the pathophysiological alterations presented by these organs.

Several of the early as well as late morphological and functional disorders presented by the splanchnic organs in experimental prehepatic portal hypertension seem to suggest that inflammatory mechanisms participate in its etiopathogeny. Portal hypertensive rats at 1 month of evolution present liver production of both proinflammatory (TNF-α, IL-1β and NO) and anti-inflammatory (CO) mediators and ileum release of anti-inflammatory IL-10. This gut-liver inflammation relationship could mediate the regulation of portal pressure and its complications.[48]

Furthermore, in the early evolutive stages of this experimental model, several substances have been proposed as mediators of the hyperdynamic circulatory state,[16] especially the proinflammatory cytokine TNF-α.[22] It is hypothesized that TNF-α causes vasodilation by both prostaglandin and NO pathways, where the NO-dependent mechanism is dominant.[49,50] If so, treatment of PVL rats with specific anti-TNF polyclonal antibodies[22] and inhibition of TNF-α with thalidomide[51] hinder the development of the hyperdynamic circulatory syndrome and reduce portal pressure. The plasma increase in TNF-α and NO concentrations in short-term PVL rats not only contributes to splanchnic and systemic hyperdynamic circulation development, but also suggests that they could mediate the hormonal alterations that are produced; for example, the increased plasma levels of corticosterone and prolactin and the decreased levels of T_3 and T_4.[50] In this case, it could be hypothesized that splanchnic hyperemia represents a regional inflammatory response and the associated systemic hyperkinetic circulation could be its systemic consequence. If so, stimulation of the hypothalamic-pituitary-adrenal axis by TNF-α with a serum concentration increase in corticosterone, and inhibition of the hypothalamic-pituitary-thyroid axis, with a serum decrease in T_3 and T_4, could be components of the neuroendocrine response to the inflammatory splanchnic stress caused by PVL in the rat.[52]

In early evolutive periods, PVL rats present vasodilation, edema, atrophy of the villi, dilation of the crypts and angiogenesis in the small bowel.[51] As the above-mentioned alterations can be explained by the increased levels of mast cell mediators, it was suggested that these cells could be involved in the intestinal inflammatory response to portal hypertension. Later demonstration of an increased infiltration by mast cells in the small intestine of 6-week long evolution PVL rats sustains this hypothesis. The mast cell density progressively increases distally along the small bowel. Thus, it is greater in the ileum than in the jejunum and is lowest in the duodenum. The mast cell number also increases in the mesenteric lymph node complex probably because these cells, after stimulation in the intestinal mucosa, migrate via the lymphatic pathway and participate in the production of mesenteric lymph nodes.[53,54]

The pattern of electrophoretic alterations of serum proteins in rats with 1 month prehepatic portal hypertension are similar to those described in chronic hepatic failure[55,56] because albumin and α_1-globulins concentrations decrease, γ-globulins concentrations increase and the albumin/globulin ratio decreases.[57] Therefore, the inflammation hypothesis proposed to explain the alterations related to experimental portal hypertensive enteropathy would involve the possibility of episodes with a sudden release of inflammation mediators from mast cells that would cause plasma exudation response and hypoalbuminemia. The inflamed gut mucosa could also cause plasma protein to be lost in the gut, or protein-losing gastroenteropathy, and the resulting hypoalbuminemia would be a predisposing factor to the development of intestinal edema. The rise in plasma TNF-α in this experimental model as

well as hypercortisolemia stimulate hepatic secretion of acute phase proteins and thus changes in α_2 and β globulins. Hyper-γ-globulinemia in these rats could be due to humoral antibody production after intestinal antigenic and bacterial stimuli. The existence of intestinal bacterial translocation to mesenteric lymph nodes which, at the same time, show an increased infiltration by mast cells, has been demonstrated in short-term PVL rats.[58] It is possible that the alteration in the permeability of the intestinal mucosal barrier, which has an inflammatory etiology,[51,52] favors the intestinal translocation of bacteria and bacterial antigens into both the lymph node and portal venous system, reaching the systemic circulation by passing the liver and providing a stimulus to γ-globulin production.[57]

The villosities in the chronic (6–14 months) PVL rats were widened, with an increase in lymphocytes and plasma cells (Fig. 3) and mesenteric lymph nodes showed xantomatous macrophages in node sinuses, with no evidence of hemosiderin. Furthermore, in chronic portal hypertensive rats the number of macrophages in splenic sinuses clearly outnumbered those found in control rats and hemosiderin incrustation of connective tissue fibers and groups of macrophages could be seen in the wall and in the vicinity of large hilar vessels. The partial atrophy of villosities presented by these animals suggests the existence of malabsorption.

Because the hyperdynamic circulation disappears while the portal hypertension and portosystemic circulation persist in experimental chronic portal hypertension,[40,59] it has been suggested that the increased portocollateral resistance is involved in maintaining the elevated pressure in the splanchnic venous system.[40] It may be true therefore that chronically increased blood flow through the splanchnic circulation will cause an increase in portocollateral resistance by inducing structural or functional changes in the portocollateral circulation.[40,60] However, by applying the inflammatory hypothesis of experimental portal hypertension to its chronic evolution, it could be suggested that the alterations produced in these late phases occur as a consequence of an epithelial and vascular remodeling process.[61] These alterations consist in increased density of mucosal goblet cells and dilatation of the distal branches (third and fourth order) of the superior mesenteric vein, respectively.[61] This macroscopic vascular change is associated with an increase in the number and diameter of intestinal submucosal vessels.[53]

The splanchnic alterations produced in the experimental model of prehepatic portal hypertension along its evolution could therefore be divided into three successive phases. The first or early phase is predominated by vasomotor disorders with an intestinal ischemia-revascularization phenomenon. Splanchnic vasodilation would cause intestinal hyperemia, mucosal ischemia, oxidative stress and edema. In the second or intermediate phase immunological mechanisms would be prominent: mast cell infiltration of the small bowel mucosa and submucosa,[53] mesenteric lymphadenopathy, splenomegaly and hepato-intestinal release of pro- and anti-inflammatory mediators.

PVL rats at 1 year of evolution present a plasmatic increase in IL-10 and CO, powerful anti-inflammatory cytokines of liver origin, which coexist with proinflammatory intestinal mediators (TNF-α, IL-1β and NO).[37,62] The serum increase in IL-10 in rats with chronic portal hypertension also supports the hypothesis of the evolutive nature of the pathophysiological mechanisms

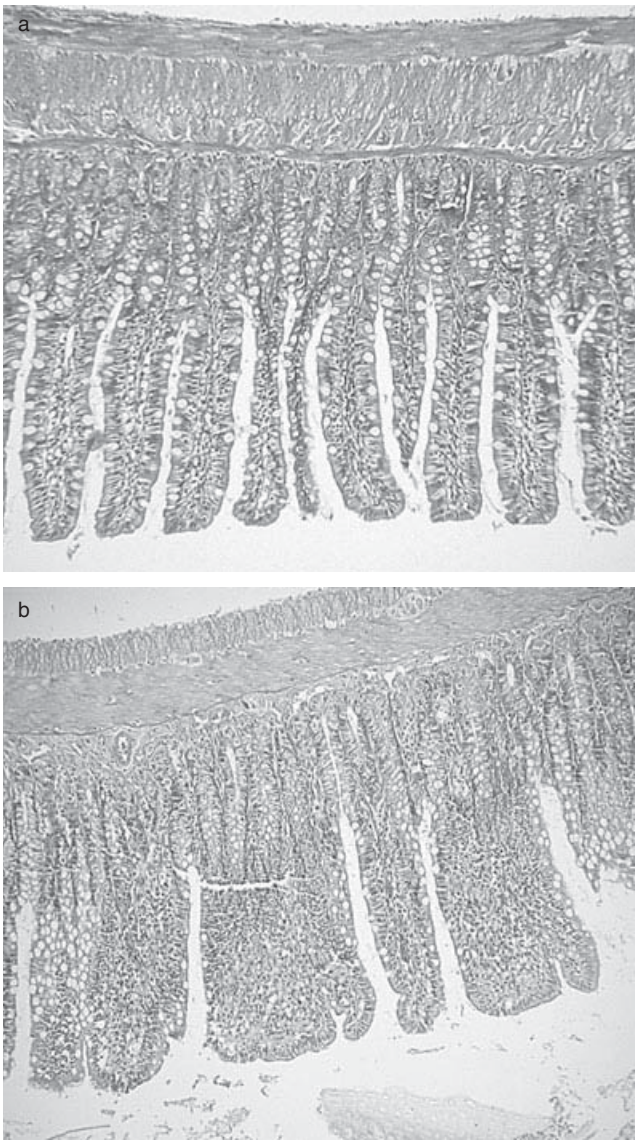

Figure 3 Jejunal vellosities of portal hypertensive rats are widened (b) compared to control rats (a) at 6 months of evolution. The widened vellosities are infiltrated by lymphocytes and plasma cells.

involved. Thus, the hyperproduction of anti-inflammatory cytokines, especially of IL-10, in the initial phase of portal hypertension[22,51,52] could be balanced by the hyperproduction of proinflammatory cytokines, such as TNF-α, in the late phases.[63] Because IL-10 reduces the production of IL-1, IL-6 and TNF-α,[64,65] its increase in rats with chronic portal hypertension could reflect the existence of a modulating anti-inflammatory response.[62,63]

Finally, a late or chronic phase occurs, in which vascular and epithelial splanchnic remodeling is developed. Epithelial remodeling consists of the substitution of absorptive epithelium in small bowel by secretory epithelium (goblet cells).[61] Vascular remodeling, in turn, is both macroscopic, with an increase in the distal branches of superior mesenteric vein (third and fourth order

branches)[61] and microscopic, with an increase in the number and diameter of the submucosal vessels in the small bowel.[53] Because this inflammatory response evolves from ischemia to angiogenesis, it could be considered to be similar to those described in acute local post-traumatic inflammation.[66]

It can be concluded that the study of experimental prehepatic portal hypertension in late evolutive stages could be more suitable to obtain results applicable to human clinical features. The reason for this is that the pathophysiological mechanisms involved in maintaining portal hypertension are different from those that characterize the early evolutive phases.

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