Decreased Reticuloendothelial Phagocytic Capacity in Cirrhotic and Portacaval Shunt Rats

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Abstract. In cirrhosis, the phagocytic function of the reticuloendothelial system (RES) is decreased. In order to investigate the mechanisms of the hepatic reduced phagocytic activity present in cirrhosis, the hepatic and splenic uptake of $^{51}$Cr sheep red blood cells (SRBC) and of colloidal carbon was measured in three groups of Sprague-Dawley rats. Group 1 consisted of 42 control rats, group 2 of 36 rats with end-to-side portacaval shunt and group 3 of 24 rats with carbon tetrachloride-induced cirrhosis. The hepatic uptake of $^{51}$Cr SRBC and of colloidal carbon was significantly ($p < 0.001$) reduced in cirrhotic rats (group 3). Conversely, in rats with a portacaval shunt and a noncirrhotic liver (group 2), the hepatic uptake of $^{51}$Cr SRBC was moderately reduced, whereas the colloidal carbon hepatic uptake was not found to be decreased. These results suggest that the decreased RES phagocytic activity observed in cirrhotic rats is only partially due to portacaval shunt and that an intrinsic defective activity of hepatic phagocytic cells is probably present.

Impairment of the phagocytic function of the reticuloendothelial system (RES) occurs in cirrhosis [3, 7, 11] and plays a major role in predisposing cirrhotic patients to develop severe infections [14]. This impairment is mainly due to a depression of the hepatic RES which amounts to 80–90% of total RES activity [17].

The precise mechanisms of the hepatic reduced phagocytic activity present in cirrhosis are unknown. It can be postulated that this reduced phagocytic activity might be due either to a defective activity of the hepatic phagocytic cells or to an intra- or extrahepatic portacaval shunting of the blood flow allowing microorganisms, nonbacterial particulates or antigens to escape from the phagocytic activity of nonparenchymal hepatic cells. The purpose of this study was to investigate the role of portacaval shunting by
studying the hepatic uptake of particulate antigens in rats with cirrhosis and in rats with a noncirrhotic liver and a portacaval shunt.

Material and Methods

Animals
Male Sprague-Dawley rats, weighing 300-350 g, were purchased from Charles River Lab., France. They were kept in a temperature- and light-controlled room and received food and water ad libitum.

They were divided into 3 groups: in group 1, 42 rats served as controls, in group 2, 36 rats underwent an end-to-side portacaval shunt, and group 3 comprised 24 cirrhotic rats.

Portacaval Shunt Rats
End-to-side portacaval shunts (PCS) were performed as previously described [2]. Surgery was performed under light ether anesthesia using a clean but nonsterile technique. One to two months after surgery, the hepatic and splenic uptake of particulate antigens were studied in these animals. At this time, the rats weighed between 330 and 380 g.

Cirrhotic Rats
Cirrhosis was induced by subcutaneous injections of carbon tetrachloride (0.1 ml plus an equal volume of mineral oil per 100 g body weight) twice weekly during 5 months. The presence of cirrhosis was confirmed at laparotomy on all animals included in the study.

Control Rats
Twenty-one rats had a simple laparotomy (sham animals) and were studied 1–2 months after surgery. Twenty-one other animals received a subcutaneous injection of 0.1 ml of mineral oil per 100 g body weight twice daily during 5 months.

Assessment of the RES Phagocytic Activity
RES phagocytic activity was tested measuring the hepatic and splenic uptake of either colloidal carbon or 51Cr-labeled erythrocytes.

Carbon Uptake. Rats were intravenously injected with 30 mg of colloidal carbon (Pelikan AG, Hanover, FRG) and sacrificed 1 h and 30 min later. The liver and spleen were removed, weighed, washed in saline buffer and hydrolysed in 10 ml 4 N NaOH/g tissue for 20 min at 90°C. The hydrolysed mixture was submitted to ultrasonic waves for 1 min and the optical density was then determined at 730 nm. The results were expressed as percentage of carbon injected, either for the whole organ or for 1 g of the organ.

51Cr Erythrocyte Uptake. Rats were intravenously injected with $5 \times 10^8$ sheep red blood cells (SRBC) prepared as follows: 5 ml of SRBC were washed twice with Hanks’ solution using centrifugation at 2,500 rpm for 10 min. The globular pellet was resuspended in 5 ml of Hanks’ solution and 200 µCi of 51Cr were added to the suspension. The mixture was incubated at 37°C for 45 min. The pellet was washed 3 times again with Hanks’ solution in order to eliminate unfixed radioactivity. The pellet was diluted with Hanks’ solution in order to obtain a cellular suspension containing $10^9$ 51Cr erythrocytes/ml. Each rat received 500 µl of this suspension. Three hours later, the rats were sacrificed, and the liver and spleen were removed and weighed. Radioactivity of each organ was measured using a gamma counter. Control measurement of the radioactivity injected was made by counting the radioactivity of 500 µl of 51Cr erythrocytes. Results were expressed as percentage of control, either for the whole organ or for 1 g of the organ.

Statistical Analysis
Results were expressed as means ± SEM. Comparison of the means was performed using Student’s t-test.

Results

Uptake of Colloidal Carbon
There was no difference in the hepatic uptake of colloidal carbon between normal rats (81.0 ± 12.8%) and PCS rats (71.03 ± 13.67%). On the other hand, the hepatic uptake in cirrhotic rats (25.0 ± 10.6%) was significantly decreased ($p < 0.001$). The splenic uptake of colloidal carbon increased from 4.67 ± 2.48% in normal rats to 18.89 ± 12.28% in cirrhotic rats ($p < 0.05$) (fig. 1).
Fig. 1. Hepatic and splenic uptake of colloidal carbon in normal, PCS and cirrhotic rats.

Fig. 2. Hepatic and splenic uptake of colloidal carbon in normal, PCS and cirrhotic rats. Results are expressed as percentage of colloidal carbon injected per gram of tissue.
When the results were expressed per gram of tissue in order to take into account liver atrophy of PCS rats and liver hypertrophy of cirrhotic rats, the decrease in hepatic colloidal carbon uptake was significant (p < 0.001) in cirrhotic rats. No decrease was observed in the PCS rats and no increased splenic uptake was observed due to the hypertrophy of the spleen in cirrhotic rats (fig. 2).

**Uptake of $^{51}$Cr SRBC**

The normal rat retained 63.98 ± 12.42% of injected SRBC in the liver. The hepatic uptake of SRBC was significantly reduced to 40.92 ± 9.40% in PCS rats (p < 0.001) and to 16.35 ± 12.54% in cirrhotic rats (p < 0.001). The splenic uptake of SRBC increased from 4.17 ± 1.24% in normal rats to 9.19 ± 1.62% in PCS rats and to 30.20 ± 27.40% in cirrhotic rats (p < 0.001) (fig. 3). When the results were expressed per gram of tissue, the decrease in hepatic SRBC uptake in PCS rats was moderate: 5.85 ± 1.52% in normal rats versus 4.94 ± 0.91% in PCS rats (p < 0.02), but the decrease in hepatic SRBC uptake in cirrhotic rats was significant: 5.85 ± 1.52% in normal rats versus 1.14 ± 0.69% in cirrhotic rats (p < 0.001) (fig. 4).
Discussion

In this study, we have observed a marked decrease in the hepatic uptake of particulate antigens in cirrhotic rats, a finding which contrasts with the moderate reduction in PCS rats.

In cirrhotic rats, the hepatic uptake of radiolabeled erythrocytes and of colloidal carbon was significantly (p < 0.001) reduced. The splenic uptake was increased, but not enough to compensate the marked decrease of the hepatic uptake. Conversely, in PCS rats, the hepatic uptake of $^{51}$Cr erythrocytes was moderately reduced, whereas, the colloidal carbon hepatic uptake was not found to be decreased.

A reduced hepatic RES activity has been described in cirrhotic patients [3, 7, 11] and animals [15, 18]. The mechanisms of impaired RES activity in cirrhosis are not well known; responsible factors are either a reduced blood flow to RES hepatic cells or a defective activity of RES hepatic cells. The impaired RES function in cirrhosis may be related to intra- or extrahepatic shunting of blood escaping from the phagocytic action of the hepatic reticuloendothelial cells [14]. Due to the reduced phagocytic activity of the liver, gut-derived antigens might lead to an increased stimulation of spleen and other lymphoid tissues, resulting in elevated immunoglobulin titers [18, 19]. In patients with chronic liver disease; the serum antibody titers and immunoglobulin levels correlate with the amount of portosystemic shunting [5]. Furthermore, rats with a noncirrhotic liver subjected to PCS [10] or a portacaval

Fig. 4. Hepatic and splenic uptake of $^{51}$Cr SRBC in normal, PCS and cirrhotic rats. Results are expressed as percentage of radioactivity injected per gram of tissue.
transposition [1] develop elevated immunoglobulin levels. However, humans with a noncirrhotic liver and extrahepatic portal venous obstruction leading to chronic portosystemic shunting have normal immunoglobulin titers [21]. In our study, rats with PCS and a noncirrhotic liver had only a moderately reduced phagocytic activity. This result contrasts with the significant decrease observed in cirrhotic rats. Blood flow to the liver is probably greater in cirrhosis than after PCS [9], and intrahepatic disturbance of blood flow is common in cirrhosis but does not occur after PCS. These observations imply that the decrease in phagocytic activity observed in cirrhotic rats cannot be attributed solely to PCS and that a defective activity of nonparenchymal liver cells is also responsible. Several studies have shown an impaired function of peripheral monocytes in liver cirrhosis [4, 6, 8]. Although some discrepancies may exist between peripheral monocytes and hepatic Kupffer cells [16], this may suggest an intrinsic defective activity of hepatic mononuclear phagocytic cells. Whether the reduced hepatic phagocytic activity observed in cirrhotic rats is related to an intrinsic functional defect of RES cells or to a decrease in serum opsonin (complement [13] or fibronectin [12]) concentrations remains to be determined.

In conclusion, the hepatic uptake of particulate antigens was markedly reduced in cirrhotic rats. On the contrary, a moderate reduction was observed in rats with a noncirrhotic liver and PCS. These findings suggest that the decreased RES phagocytic activity observed in cirrhotic rats is only partially due to PCS and that an intrinsic defective activity of hepatic phagocytic cells and/or an intrahepatic shunting of blood from the RES cells are probably present.

References

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Effects of Thrombogeni
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Abstract. In a model of portal vein occlusion with this grafts, a failure has been described at graft insertion on the routine distension and perfusion sc, which grafts were identified as immunologically depressed. Ramos et al. and Gundry et al. Changes in the morphological face of hepatic venous bed.  

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