EFFECT OF STIMULATION OF HEPATIC MACROPHAGES ON THE DEVELOPMENT OF CIRRHOSIS OF THE LIVER IN RATS

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KEY WORDS: cirrhosis of the liver; hepatic macrophages; granulomas; bacterial polysaccharide.

The writers' previous investigations showed that the course of acute toxic hepatitis depends essentially on the functional state of the hepatic macrophages (Kupffer cells) at the time of administration of the hepatotropic poison [3]. Hence the need to explain the influence of Kupffer cell activity on the course of experimental cirrhosis of the liver in rats. There is evidence that fixed tissue-specific macrophages and the motile macrophages from inflammatory foci (granulomas) under certain conditions begin to secrete into the medium factors which stimulate synthesis of collagen precursors in fibroblasts [5] and growth and multiplication of these cells [6]. In the later stages of repair macrophages secreting collagenase accumulate [7]. In all probability, the course of fibrosis depends to a large degree on the functional properties of the macrophages and the character of their interaction with fibroblasts.

The object of the investigation described below was to compare the development of fibrosis of the liver in normal rats and in rats stimulated by bacterial polysaccharide. Polysaccharide from *Bacterium prodigiosum*, known as "prodigiosan" [1], was used as stimulator of the macrophages.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were used. Twice a week for 4 months all the animals were given subcutaneous injections of 0.25 ml/100 g body weight of a 10% solution of CCl_4 in vegetable oil in the dorsal region. During poisoning the rats of the experimental group were additionally stimulated with the bacterial polysaccharide prodigiosan. Instead of prodigiosan, rats of the control group received the same volume of 0.85% NaCl solution. Stimulation began 1 day before the first injection of CCl_4, after which prodigiosan continued to be given at intervals of 10 days during the development of fibrosis in the liver in a dose of 25 μg/100 g body weight. The liver was fixed in Carnoy's mixture and paraffin sections, 6 μ thick, were stained with hematoxylin and eosin, with Schiff's reagent, and by Van Gieson's method. Fixed cryostat sections of the liver were stained by Gomori's method for acid phosphatase (AP). ^{3}H-thymidine with specific radioactivity of 10 mCi/ml was injected intraperitoneally 1 h before sacrifice in a dose of 1 μCi/g body weight. Liver sections were coated with type M emulsion (Photographic Chemical Research Institute) and incubated at 4°C for 15 days. The percentage of connective-tissue cells incorporating ^{3}H-thymidine was determined on autoradiographs of the liver sections. The hydroxyproline concentration in whole liver homogenates was determined by the method of Neuman and Logan [8] with some modifications. The ingestive capacity of the Kupffer cells was assessed from the rate of elimination of ^{198}Au from the peripheral blood [9].

EXPERIMENTAL RESULTS

During repeated injections of CCl_4 characteristic injuries to the hepatocytes developed, in the form of balloon degeneration and necrosis. Injury to the parenchyma began in the centrilobular zones, after which it became generalized in character.

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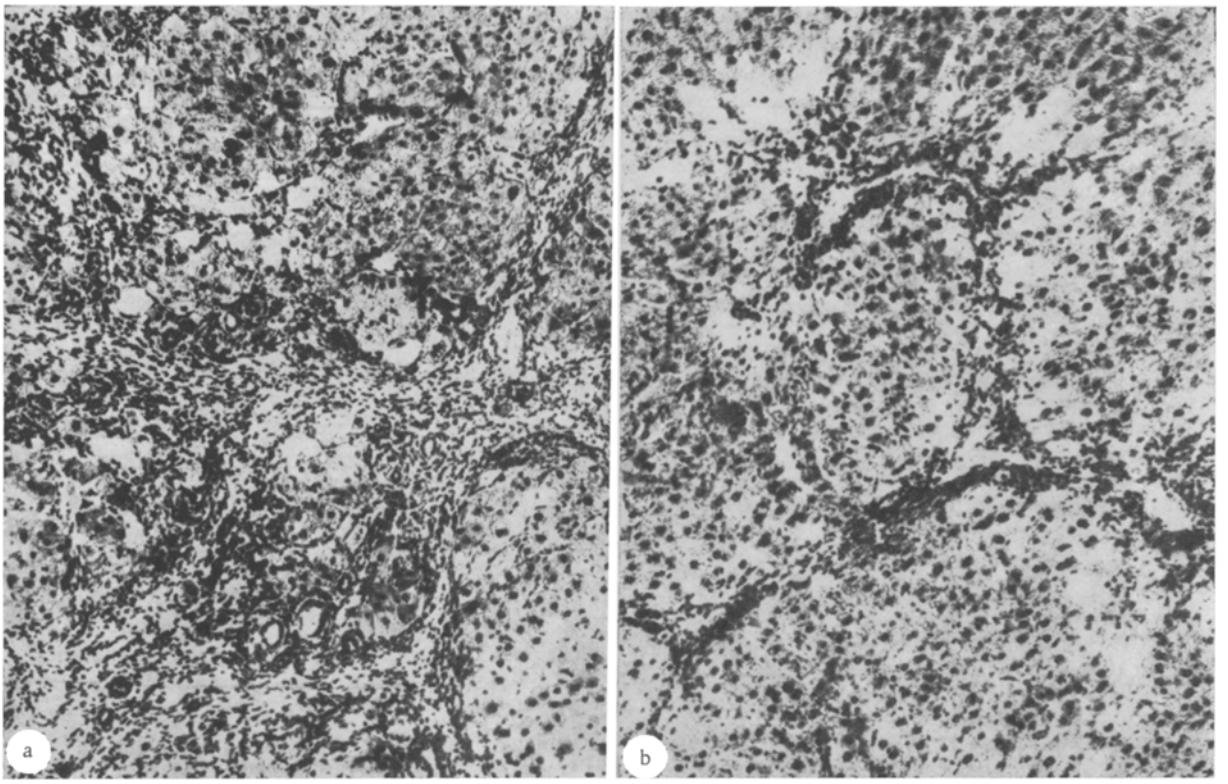

Fig. 1. Rat liver 10 weeks after beginning of CCl_4 poisoning: a) control; b) stimulation by prodigiosan. Hematoxylin and eosin, 160 ×.

Attention was concentrated on reactive changes in the liver stroma in response to damage to the hepatocytes by CCl_4. Accordingly the whole process was conventionally divided into three phases: proliferative, proliferative-fibrotic, and fibrotic proper, with transition to cirrhosis. The first phase was completed by the end of the 2nd week after the beginning of poisoning. Reactive changes of the stroma in this phase consisted mainly of an increase in the number of Kupffer cells in relatively intact areas of parenchyma. This was particularly clearly manifested in rats stimulated with prodigiosan (Table 1). Furthermore, compared with the control animals, in these rats AP-positive Kupffer cells accumulated in higher concentration in the areas of destruction of their parenchyma. The other reactive response of the stroma was infiltration of the liver by monocytes. Solitary polynuclear cells were included in the foci of infiltration only at the very beginning of the process. Larger and denser foci of monocytic infiltration, containing a larger number of AP-positive cells and cells labeled with ^{3}H-thymidine, were formed in rats stimulated with prodigiosan (Table 1).

The second phase of the process lasted from the 2nd until the 6th-8th week. During this period the heterogeneity of the Kupffer cells increased sharply. At least three morphological types of cells, which could not be strictly identified as Kupffer macrophages, appeared in the liver sinusoids. The liver stroma was transformed into a mixture of granulation of fibrous tissue. Bands of collagen fibers were initially rich in cells: fibroblast-like cells together with a few cells of the infiltrative pool. AP-positive macrophages were distributed mainly along the course of the connective-tissue bands. Fibroblast-like cells in the bands incorporated significantly less ^{3}H-thymidine in the experimental material than in the control (Table 1). Later the increased collagenization of the liver stroma was accompanied by gradual exhaustion of the infiltrative component of inflammation.

The transition from the infiltrative-fibrotic phase to true fibrosis took place gradually. In the period of permanent fibrotic changes very few cells remained in the bands of connective tissue, mainly AP-negative fibroblasts with low proliferative activity. In this period the architectonics of the liver was disturbed and characteristic changes of cirrhosis appeared: pseudolobules and nodules of regeneration of hepatocytes. Characteristically in rats stimulated with prodigiosan fibrogenesis was retarded. This was reflected 10-12 weeks after the beginning of CCl_4 poisoning as the weaker development of fibrous bands and a

TABLE 1. Morphological Characteristics of Liver of Rats with Experimental Cirrhosis

Stage of process	Parameter	Control (n = 6)	Experiment (n = 6)	P
Proliferative	Injury to parenchyma, conventional units [3]	17 ± 0.84* ± 2.4†	10 ± 1.7 1.3 ± 0.5	<0.05 <0.05
	Reaction of stroma to injury			
	Mean number of Kupffer cells in areas of intact parenchyma (in 10 fields of vision, ×1000)‡	6.1 ± 0.7	16.0 ± 1.7	<0.05
	Dimensions of foci of monocytic infiltration	Number of cells in focus of infiltration ≤50 11 >50 12 ≥100 2	Number of foci of infiltration 7 7 11	
	ILN of monocytes in foci of infiltration, %	7.6 ± 0.8	10.2 ± 1.5	>0.05
Proliferative-fibrous	ILN of connective-tissue cells, %	9.0 ± 1.5 (n = 9)	0.75 ± 0.2 (n = 9)	<0.05

*Stained with hematoxylin and eosin.
†Stained by PAS method.
‡Conventional field of vision of intact liver contained 5.4 ± 0.4 cells.
Legend. ILN) Index of labeled nuclei.

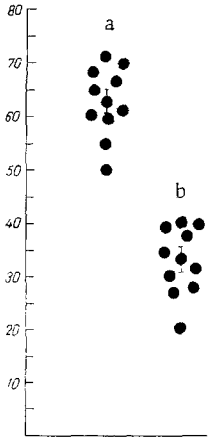

Fig. 2. Hydroxyproline concentration (in mg %) in rat liver homogenates 10-12 weeks after beginning of CCl_4 poisoning: a) control; b) experiment.

correspondingly less severe disturbance of the general structure of the liver (Fig. 1). In homogenates of the liver from rats stimulated by prodigiosan, however, the hydroxyproline content was on average only half that in the control animals (Fig. 2).

Consequently, during stimulation by the bacterial polysaccharide prodigiosan the development of experimental cirrhosis of the liver in rats was considerably retarded. On the one hand, during stimulation of the macrophagal phagocytic system by prodigiosan the degree

of injury to the parenchyma was reduced, possibly in connection with activation of Kupffer macrophages [3]. The results indicate that prodigiosan stimulates the ingestive function of the Kupffer cells, as shown by the twofold increase in the rate of elimination of colloidal ^{198}Au from the blood of rats stimulated by prodigiosan compared with that of unstimulated animals. Meanwhile, migration of precursors of Kupffer cells into the liver is intensified by prodigiosan [4]. This effect is probably linked with the much greater accumulation of Kupffer cells in the intact areas of the liver parenchyma in the stimulated rats during the stage of acute hepatitis. On the other hand, under the influence of bacterial polysaccharide, monocytic infiltration was stimulated in the period preceding collagenization of the stroma. The process of fibrogenesis and the development of cirrhosis as a whole were appreciably retarded. Is there a relationship of cause and effect between monocytic inflammatory infiltration in the period of acute reactive structural change in the stroma of the liver and its subsequent collagenization? The results of the present experiments are in favor of a positive answer, although the internal nature of this relationship is not yet clear. It can be tentatively suggested that the slower development of fibrosis during stimulation by bacterial polysaccharide is connected with the accumulation of cells delaying fibroblast proliferation in the zone of inflammatory infiltration and (or) the absence of cells potentiating their collagen-forming function. The possibility cannot be ruled out that activated hepatic macrophages resorb collagen more effectively and destroy the collagen fibers as a result of intensified secretion of collagenolytic enzymes [2].

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NUMBER OF CELL TYPES IN RAT PANCREATIC ISLETS

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Despite many investigations of the cell composition of the endocrine part of the mammalian and human pancreas, this problem is far from its final solution [4]. The apparent diversity of types of endocrine cells found by different workers in the pancreas can be basically reduced to four generally accepted ultrastructural types of endocrine cells: A, B, D, PP. However, in the literature reports have sometimes been published indicating that the pancreas of certain mammals (horse, dog, guinea pig, opossum) may contain other morphological types of endocrine cells, such as X, F, and G which, in the opinion of the authors cited, are independent types [2, 3, 5, 7]. Data on the presence of six types of endocrine cells, for which the hormonal profile has been determined [9], are particularly interesting in this connection. These data suggest that in other mammals types of insular cells may be more widely represented.

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