EFFECT OF RHYTHM OF PATHOGENIC ACTION ON CELL RESPONSE OF THE LIVER STROMA

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The speed of the cell response of the mouse liver stroma, its stereotype, and its cyclic pattern were found to depend on the rhythm of action and the total dose of CCl_4 used as a hepatotropic poison. The rhythm of the stromal response showed quantitative changes in the infiltrating cells. During the action of CCl_4 on the liver morphological evidence of humoral (fixation of γ-globulin and of complement-reactive groups) and cellular (infiltration with macrophages and lymphocytes, signs of cellular aggression in the form of "piecemeal" necrosis) immunity were observed in the liver. With a more intensive rhythm of CCl_4 administration the adaptive responses of the stroma and parenchyma were more marked, and this resulted in more complete regeneration.

KEY WORDS: carbon tetrachloride; infiltration; rhythm of poisoning; stromal response.

Changes in the rhythm of metabolic processes in the cells under the influence of the rhythm of external stimulation have been known for a long time [2, 3, 5, 7-12]. However, the morphological basis of the rhythms of activity of organs and tissues under normal and pathological conditions has been inadequately studied. Conditions under which the dose of a pathogenic agent (CCl_4, for example) remains constant but the rhythm (frequency) of its administration is varied are very convenient for the study of variations in adaptive processes formed by a combination of simultaneous changes in the parenchyma and stroma. Variations in the activity of repair processes in the parenchyma of the liver associated with different rhythms of pathogenic action serve as the morphological characteristics of biological rhythms. Meanwhile the role of the stromal cells in the mechanism of adequate adaptation of the organ to changing environmental conditions has been inadequately studied.

The object of this investigation was to study the principles governing cellular responses of the liver stroma to various rhythms of administration of CCl_4 and correlation between these responses and the synthetic activity of the hepatocytes.

EXPERIMENTAL

Two series of experiments were carried out on noninbred mice receiving subcutaneous injections of CCl_4. The sessional dose remained constant: 0.2 ml of a 40% solution in peach oil. In series I the pathogenic agent was injected twice a week, in series II once. The animals were killed after six injections in the course of 30 days, two experimental mice and one control mouse each day. The remaining animals received CCl_4 at the frequency indicated above for each series. To determine the number of infiltrating cells synthesizing DNA, the animals received an intraperitoneal injection of thymidine-H^3 daily 70 min before sacrifice. Pieces of liver were fixed in Carnoy's fluid, embedded in paraffin wax, and stained with hematoxylin-eosin and with azure II-eosin; the histochemical reactions of Perles and Brachet and a reaction with toluidine blue were carried out; in series II the fixation of γ-globulin and of complement-reactive groups was studied in the cells infiltrating the stroma by the direct Coons' method. The total number of

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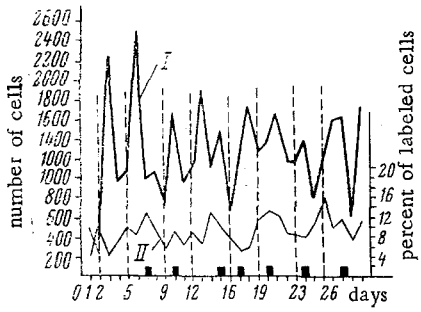

Fig. 1

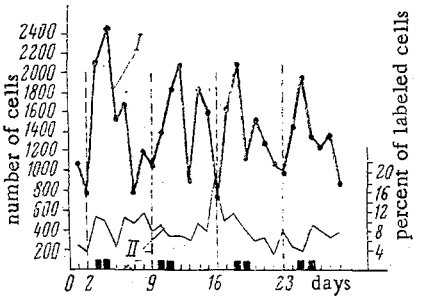

Fig. 2

Fig. 1. Cellular responses of mouse liver stroma in experiments of series I. I) total number of infiltrating cells; II) percentage of labeled cells. Black squares denote days of appearance of large foci of acidophilic necrosis in hepatic lobules; broken lines mark times of injection of CCl_4.

Fig. 2. Cellular responses of mouse liver stroma in experiments of series II. Legend as in Fig. 1.

TABLE 1. Cell Composition of Foci of Infiltration of the Liver Stroma Depending on Frequency of Administration of the Pathogenic Agent (CCl_4)

Cells infiltrating stroma	Series I		Series II		Control	
	CCl_4 twice a week		CCl_4 once a week			
	total number of cells	number labeled	total number of cells	number labeled	total number cells	number labeled
	(in %)					
So-called reticulum cells	41,5—71	3,7—23	29,9—67,8	4,—23	55—70	2,13—8
Lymphocytes:						
medium	8,26	2,3—11	11,4—40	1—26,8	6—22	—
small	3—10,6	—	2,4—11,2	—	2,5—9	—
Polymorphs	8—20	—	2,4—16	—	3—16	—
Monocytes	0,6—4	25—40	2,3—11,7	21—35	0—2	—
Plasma cells	0,1—0,9	—	0,2—2,4	—	—	—
Histiocytes	0,3—5	4—20	2,6—13,6	2,4—9,3	0—4	—
Fibroblasts	3,2—10	6—16	1,6—9,4	4—10	5—9	—

cells in 10 foci of infiltration in each preparation was counted (the mean value was obtained in two experimental mice), the infiltrating cells were identified, and the number of labeled cells among the various infiltrating forms was counted.

RESULTS

The investigation showed an increase in the number of infiltrating cells in the liver stroma of the animals of series I and II compared with the control; increases in the number of cells (by about 2-3 times) occurred in peaks that corresponded strictly to the times of injection of CCl_4. In series I (Fig. 1) the peaks appeared on the first day, in series II (Fig. 2) they appeared on the second day after injection of the pathogenic agent. The significance of the peaks was confirmed statistically by the Kolmogorov–Smirnov method ($\lambda^2 = 2.9$ for series I, $\lambda^2 = 2.7$ for series II, $\lambda^2 > \lambda^2_{01} = 2.65$, the negative result disregarded). In series I the peaks of increase in the number of cells preceded or appeared simultaneously with destruction of the hepatocytes in foci of acidophilic necrosis. In series II the peaks were delayed and appeared immediately after the development of large foci of acidophilic necrosis, regularly visible on the second day after each injection and persisting for 2 days. In the intervals between injections of the pathogenic agent, a second peak of increase in the number of cells appeared, on account of the presence of these foci of necrosis. The increase in the number of foci associated with the more frequent injection of the pathogenic agent and its greater total dose reflects the intensification of regenerative processes in the organ and the appearance of the most efficient adaptation [5]. Stromal responses, which differed in series I and II, probably play a definite role in the formation of this new rhythm of organ regeneration.

Comparison of the changes in the number of stromal cells in the liver with the synthetic activity of the hepatocytes, the peaks of increase in which appeared strictly on the second day after injection of the pathogenic agent, revealed that the peaks of increase in the number of infiltrating stromal cells in series I preceded the peaks of increase in the synthetic activity of the hepatocytes, but in series II they coincided. This difference in the rate of the cellular responses with differences in the frequency of injection of the pathogenic agent is evidence of an active role of the stroma in the formation of adequate adaptation of the organ to changing environmental conditions. The appearance of peaks of increase in the number of infiltrating cells at times corresponding strictly to the times of injection of CCl_4 reflects the rhythm of the stromal response; each series was characterized by its own rhythm, corresponding strictly to the frequency of injection of the poison. Morphologically the rhythm of the stromal response was expressed as a change in the total number of infiltrating cells. During the identification of the cells infiltrating the stroma (Table 1) no quantitative variations were discovered in particular types of cells depending on the rhythm of CCl_4 injection. This stable qualitative composition of the focus of infiltration regardless of the rhythm of injection of the pathogenic agent is evidence of the stereotyped response of the hepatic stroma, in which the macrophagal processes of immunity proceed. The slight difference in the number of monocytes, medium lymphocytes, and histiocytes in the stromal focus of infiltration in the liver of the animals of series I and II reflects a difference in the intensity of the various processes taking place in the organ. Cells synthesizing DNA were demonstrated autoradiographically. The total percentage of labeled cells increased in series I to 16 and in series II to 18 from the control level of 8. No cyclic pattern of increase in the number of labeled cells corresponding with the times of injection of the pathogenic agent could be detected. This fact can be explained most probably on the grounds that not all the infiltrating cells synthesized DNA simultaneously; some cells, hematogenous in origin, did not start to divide immediately. The increase in the number of infiltrating cells was largely due to cells of hematogenous origin [6].

Results in support of the immunologic nature of the stromal cell reactions were obtained. Fixation of γ-globulin and complement-reactive groups occurred in the infiltrating cells. Areas in which one or more hepatocytes were surrounded by lymphocytes were constantly found, and sometimes the lymphocyte penetrated into their cytoplasm. The nuclei of the hepatocytes were picnotic or absent and their cytoplasm was fragmented. These changes, known as piecemeal necrosis, have been described by many workers in infectious virus diseases and after exposure to certain hepatotropic poisons. They are among the morphological features of reactions of hypersensitivity of the delayed type [1, 4].

LITERATURE CITED

1. B. K. Besprozvannyi, P. P. Doronin, M. E. Semendyaeva, et al., Arkh. Pat., No. 10, 51 (1972).
2. A. P. Golikov and P. P. Golikov, Seasonal Biorhythms in Physiology and Pathology [in Russian], Moscow (1973).
3. D. D. Lebedev, Vestn. Akad. Med. Nauk SSSR, No. 7, 68 (1971).
4. G. S. Levin, Sh. I Rasulov, V. M. Mal'tsev, et al., Arkh. Pat., No. 8, 32 (1969).
5. D. S. Sarkisov, L. D. Krymskii, K. V. Botsmanov, et al., Byull. Éksperim. Biol. i Med., No. 5, 113 (1972).
6. N. G. Khrushchov, Arkh. Pat., No. 4, 7 (1973).
7. J. Aschoff, in: Biological Clocks [Russian translation], Moscow (1964), p. 25.
8. D. J. Barlow, in: Biological Clocks [Russian translation], Moscow (1964), p. 194.
9. E. Bünning, Rhythms of Physiological Processes [Russian translation], Moscow (1961).
10. E. Bünning, Biological Clocks [Russian translation], Moscow (1964), p. 409.
11. F. Halberg, in: Biological Clocks [Russian translation], Moscow (1964), p. 203.
12. J. Harker, in: Biological Clocks [Russian translation], Moscow (1964), p. 475.