Morphological Characteristics of Lymphocyte-Hepatocyte Contacts in Various Periods of Liver Regeneration in Mice

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The histo- and ultrastructure of regenerating murine liver is studied after excision of 2/3 of its tissue. Counts of leukocytes and lymphocytes are found to be increased in the intertrabecular spaces after the operation. Lymphocytes come in close contact with hepatocytes and reticuloendotheliocytes forming microtunnels at the sites of contact.

Key Words: *regeneration; cell-cell contacts; microtunnels*

Lymphocytes are known to acquire in the process of regeneration, e.g. of the liver, a capacity to stimulate or inhibit hepatocyte proliferation depending on the stage of the regeneration process [1-3]. The mechanism of action of morphogenetically active lymphocytes, as regeneration-activated lymphocytes have been arbitrarily termed, is still unknown. The morphological characteristics of intercellular (lymphocyte-hepatocyte) interrelations and the kinetics thereof are understood least of all. At the same time, according to available data, regeneration processes in the liver and kidneys are associated with an increase of lymphoid cell tropy to the tissue of these organs [1] even if it is intact [3]. Whether the increased lymphocyte flow into the contralateral kidney after unilateral nephrectomy or the intact lobes of the liver after removal of two of its lobes is a condition in which lymphoid cells may realize their morphogenetic function by interacting with regenerating organ epithelium is a problem still to be researched.

The aim of our study was to elucidate whether lymphocytes come in contact with regenerating hepatocytes and reticuloendotheliocytes, at what interval after the operation in relation to the proliferation wave this occurs, and whether there is morphological evidence that conditions exist for information exchange between these cells.

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MATERIALS AND METHODS

The histo- and ultrastructure of the liver of CBA mice was studied at various times after the removal of 2/3 of its volume according to routine procedures [1]. The animals were sacrificed by dislocation of cervical vertebrae 4, 17, 36, 48, and 72 h and 7 and 10 days after the operation. Intact and sham-operated mice were the controls. The times of the operations varied so that all the animals in the experimental and control groups were sacrificed on the same day within one hour (from 11:00 to 12:00 h). Each group consisted of 5 animals. A total of 75 male mice weighing 18 to 20 g were used. Material for light optic microscopy was fixed in Carnoy's fluid, for electron microscopy in 2.5% glutaraldehyde solution with postfixation in tetroxide, pH 7.3. The material was embedded in vestopal. For assessment of mitotic activity paraffin slices 4 μ thick were stained with hematoxylin and eosin. The mitotic index was estimated per 4000-5000 hepatic nuclei in a 7×7 mm square field under immersion and expressed in promille, that is, 1000 nuc-

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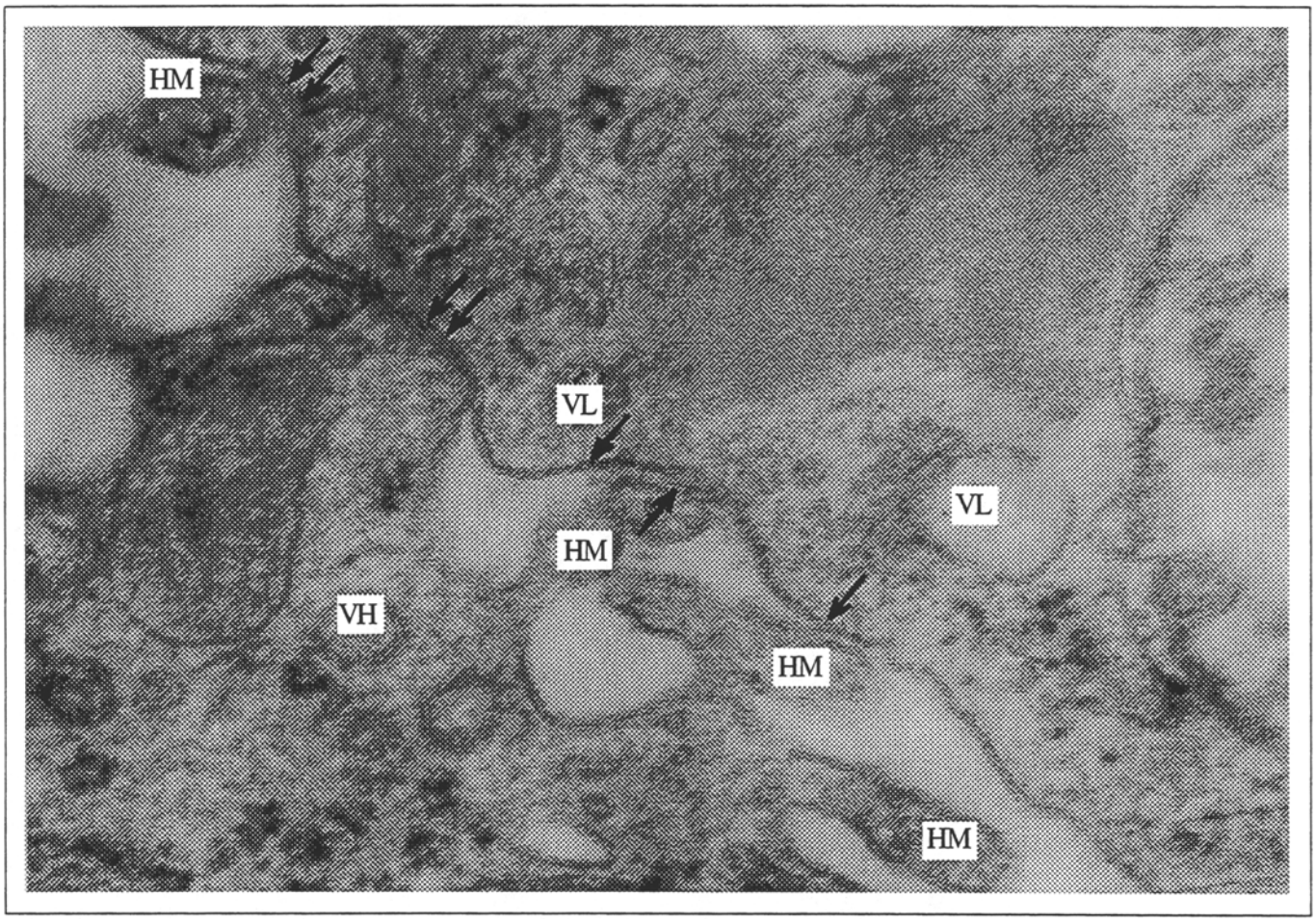

Fig. 1. Contact between hepatocyte and lymphocyte plasmalemmas: microtunnels (shown with double arrows). Fragmentation of their plasmalemmas along their whole length (shown with single arrows); hepatocyte microvilli (*HM*). Vesicles from smooth endoplasmic reticulum in the cytoplasm of both cells (*VH, VL*) 4 h postoperation (×115,000).

lei. Electron microscopic slices were stained after Reynolds.

RESULTS

Histological analysis showed that 4 and, especially, 17 h after the operation a marked increase of leukocyte leakage from vessels was observed, these cells inhabiting the spaces between hepatocytes and forming small focal infiltrates in some sections. A proliferation wave is not yet expressed in the regenerating liver either 4 or 17 h postoperation. In fact, a marked inhibition of proliferation is observed 17 h after surgery (Table 1). According to published data and our findings, no signs of entry of hepatocyte nuclei into the mitotic phase S phase are detectable in this period [1,5]. On the other hand, lymphocytes in the periods tested are characterized by morphogenetic or, to be more precise, mitogenetic activity [1]. As early as 4 h postoperation, and even more intensively 17 h postoperation, the lymphocytes, according to electron-microscopic data, come in close contact with hepatocytes, with their glycocalyces fusing first and then their membranes fragmenting and fusing (Figs. 1 and 2), forming microtunnels at some sites. Some authorities believe that cells coming in contact exchange substances through these microtunnels [3].

TABLE 1. Mitotic Activity of Hepatocytes (‰) at Various Times after Removal of 2/3 of the Liver and Sham Operation

Time postoperation	Group of animals	
	partially hepatectomized	sham operated
0 (intact control)	1.18±0.38	1.18±0.38
4 h	0.29±0.28	0.10±0.006
17 h	0	0
36 h	20.0±4.8	0
48 h12.9±3.7	0.12±0.07	
72 h	7.97±2.33	0
7 days	2.48±1.04	0.4±0.18
10 days	2.35±2.25	0.12±0.11

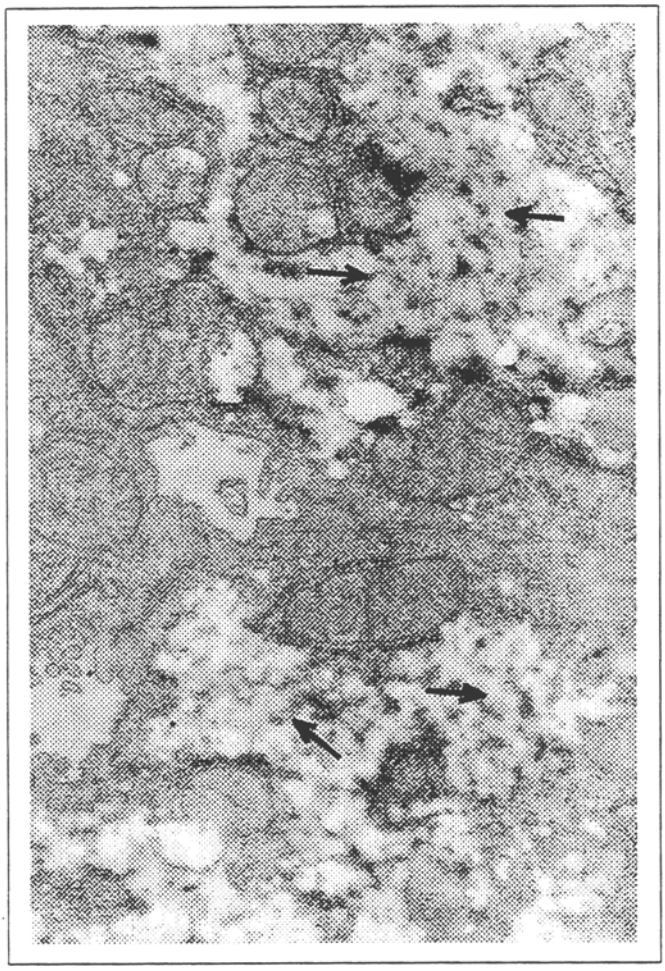

Fig. 2. Partial necrosis in hepatocytes 36 h postoperation (shown with arrows). ×42,000.

Partial cytoplasm necrosis is observed in some hepatocytes 17 h after the operation (Fig. 2). In the same period, and even more so 36 h postoperation, similar contacts of lymphocytes with reticuloendothelial cells are observed (Fig. 3). Later (36 and 48 h postoperation) the number of lymphocytes coming in close contact with hepatocytes increases. Mitogenetic (mitosis-stimulating) activity in this period is replaced by mitostatic (mitosis-inhibiting) activity [3]. A reliable increase of the mitotic index to 20 and 12.9%, respectively, is observed in the regenerating liver (Table 1). The number of cells with partial cytoplasm necrosis increases, this necrosis involving the major portion of the hepatocyte cytoplasm. However, lymphocytes, as a rule, do not come in contact with these cells or with immediately dividing cells. The total count of lymphoid cells in the intertrabecular space did not noticeably decrease in this period.

The count of proliferating cells reliably drops 72 h postoperation in comparison with 36 h postoperation (Table 1). The count of lymphoid cells in the liver parenchyma remains high, but only solitary small lymphocytes are in contact with hepatocytes. The area of their contact surface noticeably decreases as well. The cytostatic effect of lymphocytes of operated mice is no different from that in the control in this period.

Later, 7 and 10 days after the operation, the number of lymphocytes in close contact with hepatocytes is similarly low. In cells with partial cyto-

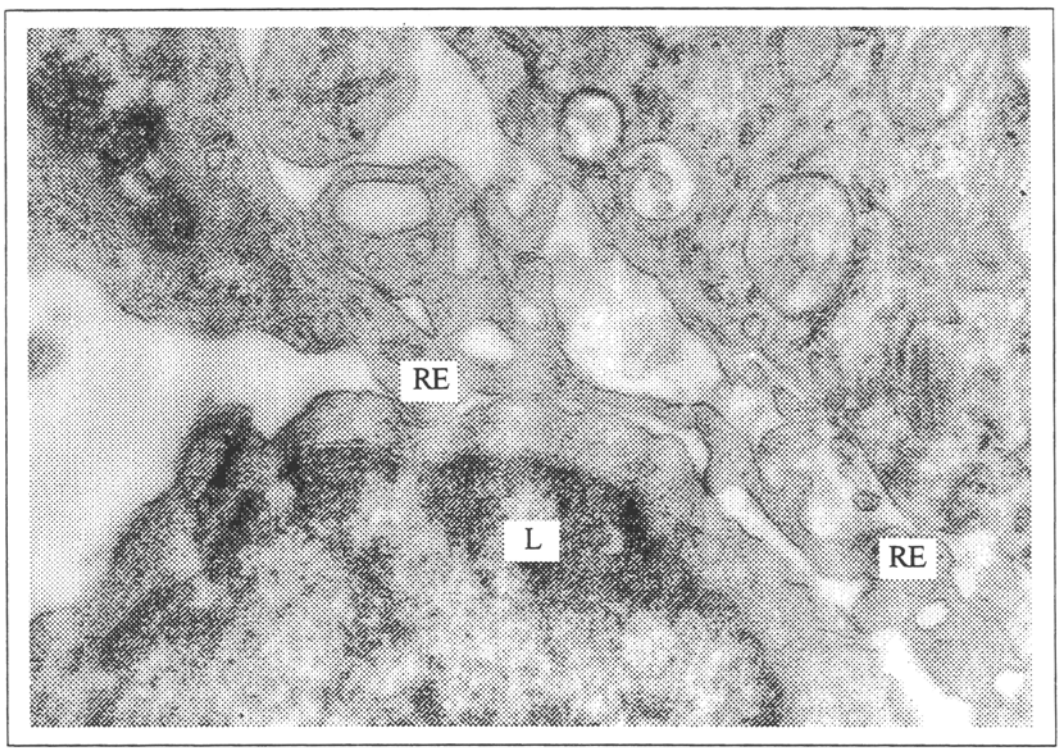

Fig. 3. Contact between lymphocyte (*L*) and reticuloendotheliocyte (*RE*) plasmalemmas. ×49,000.

plasm necrosis, fragmentation of granular and agranular cytoplasmic reticula is observed. Simultaneously they are fragmented and expanded, forming so-called small and large vesicles.

The proliferative activity of hepatocytes 7 and 10 days postoperation is virtually the same as that in intact controls but is reliably higher than in sham-operated animals in similar periods of follow-up.

Close contacts of lymphocytes with hepatocytes are extremely rare in sham-operated and intact animals.

Hence, lymphocyte flow into the liver is enhanced in the early periods of regeneration of this organ. Lymphocytes come in close contact with hepatocytes and reticuloendotheliocytes, forming microtunnels over the entire proliferative wave in the regenerating liver, starting from its genesis to the point where mitotic activity starts to wane (4-48 h postoperation). At the time of total decline of proliferative activity the number of contacts is no different from that in the control, that is, they are just solitary.

The number of close contacts between hepatocytes and lymphocytes in the course of the proliferation wave gives grounds for suggesting that both the mitogenetic function and mitostatic activity are realized similarly, the only difference being that different lymphocyte subpopulations may come in contact. It is, however, possible that the persistence of a high number of lymphocytes coming in contact forms a second proliferation wave which we failed to detect. The existence of substance exchange between contacting cells, and the trend of movement and nature of these substances are still to be elusidated.

REFERENCES

1. A. G. Babaeva, N. A. Kraskina, and L. D. Liozner, *Tsitologiya*, № 12, 1511-1516 (1969).
2. A. G. Babaeva, N. V. Yudina, and T. A. Druzhkova, *Ontogenez*, **19**, № 6, 573-581 (1988).
3. E. I. Gimmel'farb, *Morphofunctional Characteristics of the Immune System after Various Surgical Injuries of Murine Kidney. Author's Abstract of Candidate of Biological Sciences Dissertation*, Moscow (1990).
4. V. A. Shakhlamov, *Capillaries* [in Russian], Moscow (1971).
5. E. G. Bade, I. L. Sadnik, C. Pilgrim, and W. Maurer, *Exp. Cell Res.*, **44**, 676 (1966).

Lymph Circulation in the Febrile Reaction and Possible Antipyretic Correction

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The febrile reaction in dogs, as established experimentally, is accompanied by a significant intensification of the lymph flow. Metapyrin provides a strong boost to the lymph circulation, improving exchange processes between the blood and tissues.

Key Words: *febrile reaction; lymph circulation; Metapyrin*

The febrile reaction (FR) is known to be accompanied by marked changes in the function of organs and systems and, consequently, by disturbances in homeostasis. The nature of these disturbances varies widely depending on age. On the other hand, a key role is played by the lymphatic system in the maintenance of a constant internal environment due to its reliable resorptive and

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