EFFECT OF STIMULATION OF MONONUCLEAR PHAGOCYTE SYSTEM BY YEAST POLYSACCHARIDES ON ENDOCYTOSIS BY LIVER CELLS

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Yeast polysaccharides (YP) occupy an important place in the rapidly growing group of immunomodulators [4, 6, 8]. YP exert their action by a mechanism of general detoxication [1], their effect being mediated through macrophagal lysosomes, whose function is closely connected with endocytosis.

Endocytosis (phagocytosis, humoral and receptor-induced endocytosis – RE) by liver cells and activity of lysosomal enzymes were studied under the influence of YP rhodexman and cryelan. For comparison, a single injection of zymosan, a stimulator of the mononuclear phagocyte system (MPS), was used [3, 4].

EXPERIMENTAL METHOD

Experiments were carried out on 180 male Wistar rats weighing 180-200 g and 84 male CBA mice weighing 18-20 g. The animals were given rhodexman (mol. wt. 10^6-10^9) and cryelan (mol. wt. $5 \cdot 10^5$ daltons, produced at the Leningrad Chemopharmaceutic Institute) in the form of a solution in 0.9% NaCl in a dose of 5 mg/100 g intravenously and intraperitoneally. The animals were decapitated 2 h and 1, 2, 4, 5, 6, 13, and 21 days later, when granuloma formation was observed morphologically in the liver [3]. Animals receiving 0.9% NaCl solution by intravenous injection served as the control. Zymosan (Olaine Chemical Reagents Factory, "Bioreaktiv" Research and Production Combine) was given as a single intravenous injection in a dose of 100 mg/kg.

The total ingestive capacity of the liver cells (phagocytosis) was determined on the basis of their ability to clear the blood of intravenously injected particles of Gunther–Wagner colloidal carbon in a dose of 0.2 ml/100 g body weight, with calculation of the K index [10].

Humoral endocytosis was assessed with the aid of labeled polyvinylpyrrolidone (PVP), with mol. wt. of $24 \cdot 10^3$ daltons (from "Ferak," Germany) [9], specific radioactivity 20 MBq/ml, as marker. Considering the kinetics and the slow rate of the process of humoral endocytosis, elimination of ^{125}I-PVP from the blood stream and its seizure by liver cells were assessed at intervals of 5 days. The results were expressed in percent of the injected dose. Cryelan was injected three days after ^{125}I-PVP. RE by hepatocytes was studied after injection of the marker, asialofetuin (ASF) [11, 12] (from "Sigma," USA), generously provided by Professor T. Berg, Norway, specific radioactivity 50 MBq/ml, into the retro-orbital sinus of the mice. The marker was injected into the animals simultaneously with the preparation into the caudal vein under superficial ether anesthesia, in a dose of 0.28 mg ^{125}I-PVP/100 g body weight. To assess RE, the preparation containing 0.002-0.003 mg ^{125}I-ASF in 0.1 ml physiological saline was injected into a mouse weighing 20 g, and the radioactivity of the liver homogenate, precipitated with TCA on "Synpor" No. 3 membrane filters ("Chemapol," Czechoslovakia), was counted in a γ-radiometer ("Tesla," Czechoslovakia). The results were expressed as percentages of the injected dose of the compound.

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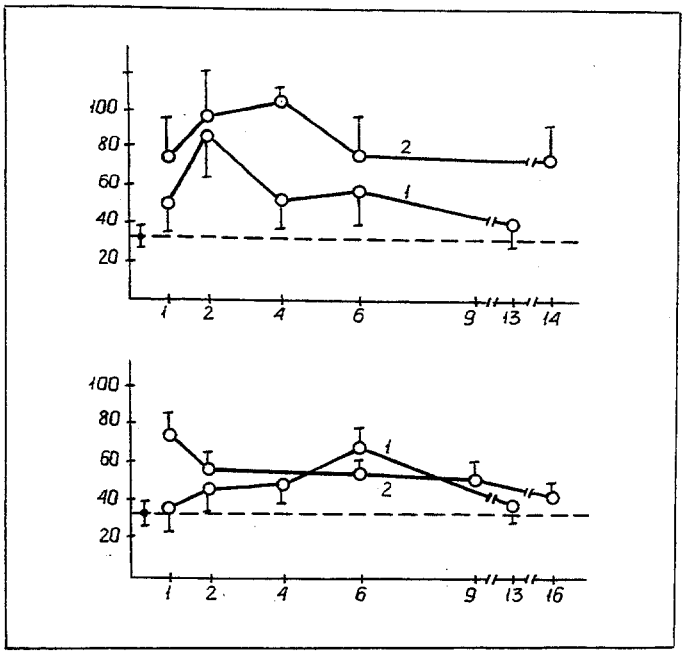

Fig. 1. Effect of single injection of rhodexman and cryelan (5 mg/100 g body weight) into rats on phagocytosis of colloidal carbon. Abscissa, time after injection of preparations (in days); ordinate, index (in per cent). a: curve 1) cryelan 5 mg/100 g, intraperitoneally; 2) cryelan 5 mg/100 g, intravenous injection. b: curve 1) rhodexman 5 mg/100 g, intraperitoneally; 2) rhodexman 5 mg/100 g, intravenous injection into rats. Broken line denotes control with 0.9% physiological saline injected intravenously.

The preparative and analytical procedures were carried out as described in [2].

Activity of cathepsin B and L was determined by a fluorometric method [7], using carbobenzoxy-arginyl-arginine-N-methylcoumarylamide and carbobenzoxy-phenylalanyl-arginine-N-methylcoumarylamide as substrates respectively. The results were subjected to statistical analysis by the parallel series method and Student's t test.

For electron-microscopic investigation the liver was perfused with 1.5% glutaraldehyde solution, postfixed with osmium, and treated by the usual methods [5]; ultrathin sections were stained with uranyl acetate and lead citrate and examined under the IEM-100B electron microscope.

EXPERIMENTAL RESULTS

Like zymosan, a single intravenous injection of which into rats in a dose of 10 mg/100 g body weight is accompanied by a more than twofold increase in phagocytic activity on the 5th day [3], intravenous injection of cryelan caused marked stimulation of phagocytic activity of the MPS after 1-14 days (Fig. 1a). Intraperitoneal injection of cryelan in the same dose was accompanied by a weaker effect (2-6 days). Intravenous injection of rhodexman also stimulated the phagocytic activity of MPS (1-9 days) and a similar effect was observed 4-6 days after its intraperitoneal injection (Fig. 1b). Compared with rhodexman, injection of cryelan into rats in single dose caused a more marked effect, however it was injected. A similar result was obtained in CBA mice after a single injection of cryelan in the same dose (control 0.046 ± 0.002, n = 10; experiment, 2 days, 0.127 ± 0.022, n = 5).

Investigations of humoral endocytosis showed that zymosan in a dose of 10 mg/100 g delays elimination of I-PVP from the serum on the 2nd and 5th days (control, 2 days, 1.40 ± 0.075, and experiment, 1.95 ± 0.045; control, 5 days 0.19 ± 0.040 and experiment, 0.49 ± 0.048). A similar inhibitory effect was observed two days after intraperitoneal injection of cryelan (control, 0.18 ± 0.040 and experiment, 0.043 ± 0.045), and 5 days after injection of PVP.

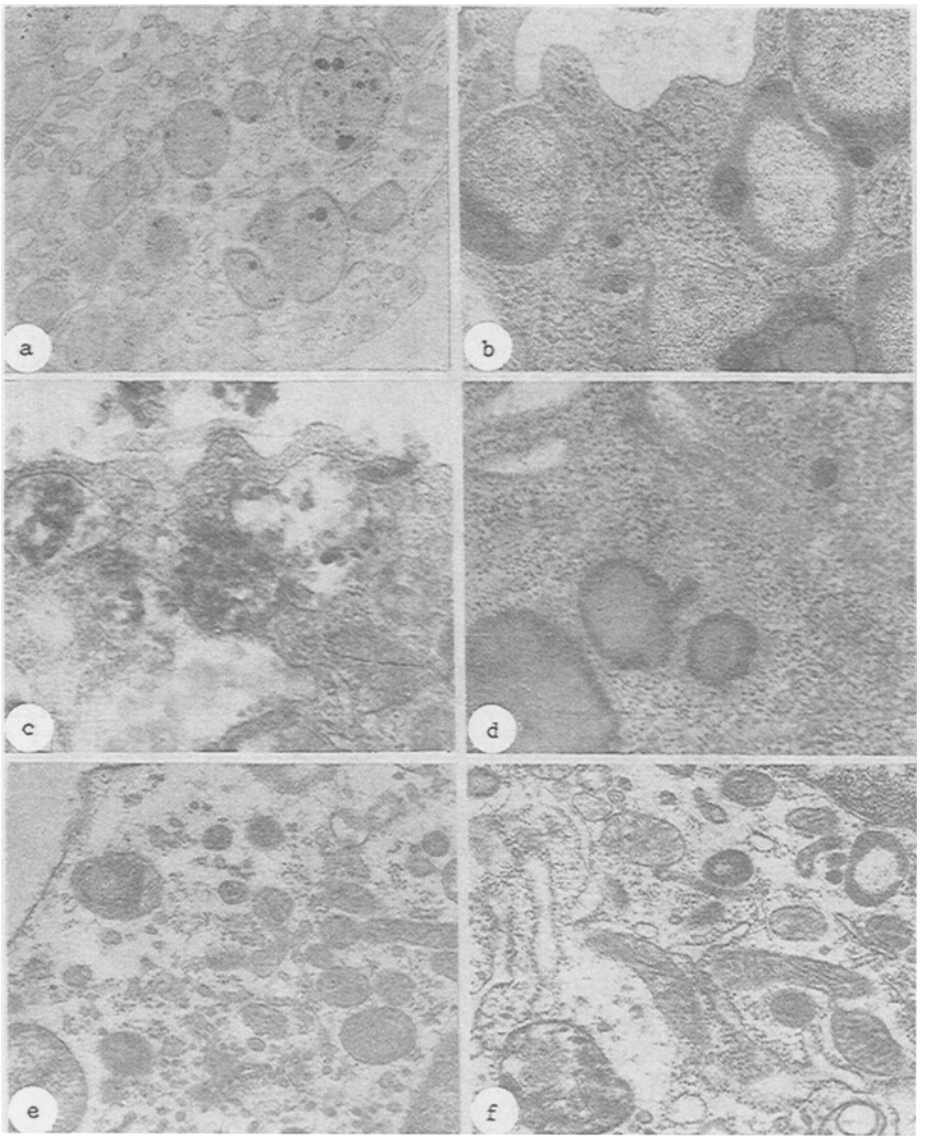

Fig. 2. Ultrastructure of mononuclear phagocytes of mice in response to stimulation by cryelan (5 mg/100 g, intravenously, 2 days): a) fragment of Kupffer cell with many primary lysosomes (60,000×), b) circular lysosomelike structures in cytoplasm of Kupffer cells (40,000×), c) colloidal carbon particles near surface and inside phagosomes of Kupffer macrophages (60,000×), d) lipid inclusions in cytoplasm of Kupffer cells (60,000×), e) primary lysosomes and phagosomes in a peritoneal macrophage (24,000×), f) circular lysosomelike structures in peritoneal macrophage (24,000×).

During evaluation of RE, seizure of I-ASF by hepatocytes of intact animals takes place very quickly (48.6% after 5 min), but the rate of its ingestion was unchanged by stimulation with zymosan and cryelan.

An increase in the number of small primary lysosomes was observed electron-microscopically in the Kupffer and endothelial cells of the hepatic sinusoids 48 h after injection of cryelan (Fig. 2a). The number of secondary lysosomes was greater in the Kupffer cells than normally. Lysosomelike structures with a narrow dark peripheral border and with a pale fine-grain matrix in the center were often visible in the Kupffer and endothelial cells (Fig. 2b). After injection of colloidal carbon large phagosomes with inclusion of colloidal particles were observed in the Kupffer cells (Fig. 2c). Associations of such particles were found near the surface of the Kupffer and endothelial cells. Multiple lipid droplets could be seen in individual Kupffer cells (Fig. 2b). The number of primary lysosomes and phagosomes also increased in the peritoneal macrophages under the influence of cryelan (Fig. 2e). In some cells

quite large lysosomelike structures with a dark peripheral border could be seen (Fig. 2f), and electron-translucent vacuoles increased in number.

Stimulation by yeast polysaccharides and, in particular, by cryelan and rhodexman, significantly accelerates phagocytosis by macrophages. The increase in content of lysosomal enzymes [8] and our discovery of cathepsin B, which is most characteristic of macrophages, correlate with activation of YP-induced activation of phagocytosis.

Stimulation of macrophages by the action of YP thus activates phagocytosis and inhibits humoral endocytosis. The investigation showed that stimulation of phagocytosis by YP depends on interaction between YP and macrophagal receptors. Intravenous injection is the most effective method, and of the preparations used, cryelan is the best for intensity of macrophagal stimulation.

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