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Phagocytic activity of Kupffer cells in splenectomized rats

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Summary. Carbonized polyethylene microspheres of 3.53 μm diameter were injected intravenously into splenectomized, laparotomized, and untreated rats. The number of Kupffer cells which phagocytosed microspheres and the number of microspheres phagocytosed by single Kupffer cells were counted. The number of Kupffer cells which phagocytosed microspheres was 1.51 times greater in splenectomized rats than in untreated rats, and 1.29 times greater in splenectomized rats than in laparotomized rats, both values being statistically significant. Although this same value was 1.18 times greater in laparotomized rats than in untreated rats, this was not statistically significant. The number of microspheres phagocytosed by single Kupffer cells also increased significantly in splenectomized rats. The intra-acinar distribution of the phagocytosing Kupffer cells showed the number in the periportal area to be 8–9 times greater than in the central area in all 3 groups. No significant difference in this intra-acinar distribution was observed between the groups. In addition, there was no difference in the phagocytic activity of Kupffer cells and the distribution of phagocytosing Kupffer cells between the hepatic lobes.

Key words: Kupffer cells – Phagocytic activity – Splenectomy

Introduction

Splenectomy is performed for various pathological conditions such as traumatic rupture, malignant diseases including tumour metastasis, and some haematological diseases. A previous study has shown that splenectomy has no influence on the ultrastructure of Kupffer cells (Wisse 1974), however, the cells have been reported to increase in number in splenectomized mice under experimental conditions (Naito et al. 1988). It has been postulated that the phagocytic activity of Kupffer cells compensates for the loss of splenic phagocytosis after removal of the spleen (Bogart et al. 1972) but, the evidence is not conclusive and has been debated by other investigators.

Using intravenous injection of carbonized polyethylene microspheres followed by morphometrical analysis, this report presents evidence of increased phagocytic activity of Kupffer cells after splenectomy. In addition, the intra-acinar distribution of phagocytosing Kupffer cells was also investigated.

Materials and methods

Experimental rats were of the male Wistar strain, weighing 280 ± 10 g. The rat weight was strictly prescribed to match to the microsphere dose which is described later. Thirteen rats were splenectomized (splenectomized rat group), 10 underwent a sham operation, a simple laparotomy without further procedure (laparotomized rat group), and 10 were used as controls without treatment, exclusive of intravenous injection of the microspheres (untreated rat group).

All rats received an injection of a suspension of microspheres into the dorsal vein of the penis under ether anesthesia. The microspheres (Lot No. NRF 003–019, 3M Co., St. Paul, MN, USA) were made of carbonized polyethylene, and ranged in diameter from 2.43 to 4.64 μm with a mean diameter of 3.53 μm and a standard deviation of 0.51 μm. The specific gravity was 1.3. One mg of the microspheres contained 6×10^7 spheres, and was suspended in 1 ml of Aq. dest. to which was

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added 10% dextran and 0.05% Tween 80. One ml of this suspension was intravenously injected into each rat 1 week after splenectomy or laparotomy. The untreated rats also received the same injection.

Twenty four h after injection of the microspheres, the livers were perfused through the portal vein under a pressure of 20 cm H_2O with 30 ml of saline solution to which was added 120 IU of heparin, followed by perfusion of 100 ml of 2.8% glutaraldehyde solution. Next, the livers were removed and fixed again in 10% neutralized formalin solution for 24 h. Two tissue pieces were obtained from each of the left median, left lateral and caudate lobes. The tissue pieces were dehydrated in a graded alcohol series, embedded in paraffin, and cut at 2 μm in thickness. Finally, the sections were stained with haematoxylin and eosin.

Kupffer cells which phagocytosed microspheres were counted in each section made from each tissue piece. The areas of the sections were measured by an image analyzer (IBAS 2000, Zeiss, FRG) and the number of phagocytosing Kupffer cells per unit area (1 cm^2) was calculated. The microspheres phagocytosed by single Kupffer cells were also counted. Next, Kupffer cells which phagocytosed microspheres in either the periportal or central area, which were paired and belonged to the same acinus, were counted. In counting the Kupffer cells in these areas, the rectangular frame was set in the eye-piece of the microscope. The frame corresponded to 235 μm × 355 μm actual size. Either the portal tract or the central vein was positioned in the center of the frame. The phagocytosing Kupffer cells within the frame were first counted. The area of the portal tract or the central vein was subtracted from that of the frame, then the number of Kupffer cells per unit area was calculated. The Kupffer cells were counted in 30 pairs of the periportal and central areas in each rat, i.e. in 10 pairs in each lobe. The ratio of the number of phagocytosing Kupffer cells in the periportal area to that in the central area was then calculated.

Statistically, the results were bilaterally tested using the Student's *t*-test at a probability of 0.05.

In addition to the control rats, 5 other untreated rats also received intravenous injections of 6×10^7 microspheres. The purpose of this part of the experiment was to discover the number of microspheres retained in the spleen and to determine the number of microspheres necessary for injection into the splectomized rats. The spleens were removed under anesthesia and immersed in 50% KOH solution in order to dissolve the tissue. The microspheres were separated by centrifugation and elutriation, then counted in a Türk chamber. The microspheres ranged in number from 904000 to 1710000 per spleen with a mean of 1273500 and a standard deviation of 288310. This number corresponded to 1.5–2.9% of the total microspheres injected, small enough to be within the error range of the actual number of microspheres in suspension. Therefore, the splenectomized, laparotomized and untreated rats all received the same number of microspheres.

Results

The microspheres were black and readily recognizable in the sections (Fig. 1). Those phagocytosed by Kupffer cells were easily found while those which remained free within the sinusoids were not. In the 6 sections of each liver, the number of Kupffer cells which phagocytosed microspheres

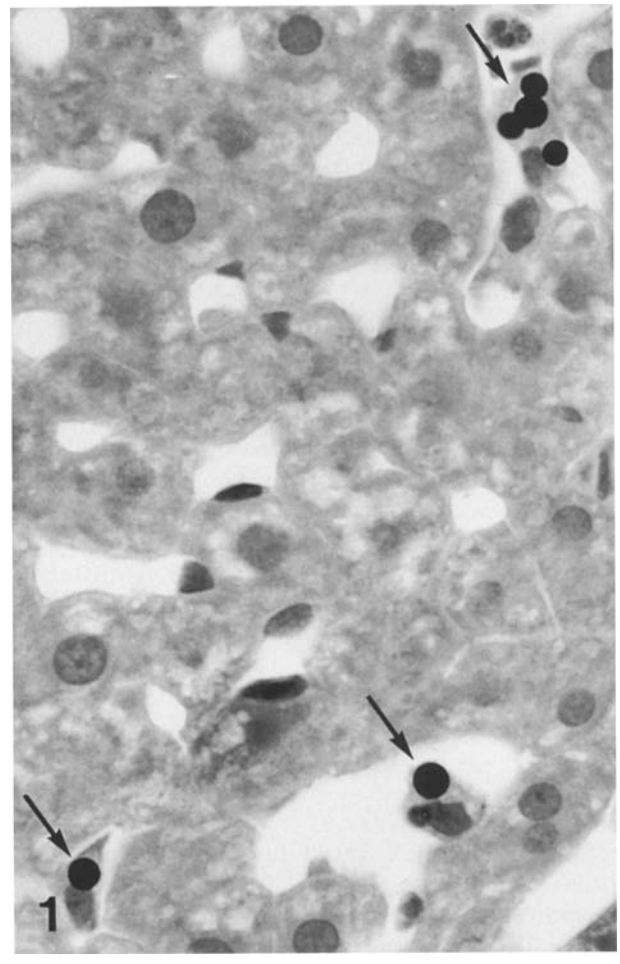

Fig. 1. Three Kupffer cells (*arrows*) have phagocytosed one and four microspheres, respectively. HE, × 1000

Table 1. The number of Kupffer cells which phagocytosed microspheres

Rats	n	Range in cm^2	$\bar{X}$ in cm^2	SD	Confidence limits for $\bar{X}$	$\bar{F}$	$\bar{t}$
Splenectomized	13	712–1549	1178	251	1026–1330	2.22 (F=3.87)	2.84 (t=2.08)
Laparotomized	10	666–1186	916	160	795–1037	1.75 (F=4.03)	1.56 (t=2.10)
Untreated	10	432–1247	778	222	618– 938		

n, number of rats; $\bar{X}$, mean; SD, standard deviation; $\bar{F}$, computed F-value; F, critical F-value; $\bar{t}$, computed t-value; t, critical t-value

Table 2. Phagocytotic activity of single Kupffer cells

Number of microspheres in single Kupffer cells	Number of Kupffer cells		
	Splenectomized	Laparotomized	Untreated
1	1055 /cm^2	835 /cm^2	702 /cm^2
2	105 /cm^2	70 /cm^2	67 /cm^2
3	16 /cm^2	10 /cm^2	9 /cm^2
4	2.3/cm^2	1.5/cm^2	1.0/cm^2
5	0.3/cm^2	0.1/cm^2	0.1/cm^2
6	none	1 in 10 rats	none
7	none	none	1 in 10 rats
8	1 in 13 rats	none	none

ranged from 1962 to 6127 in total, the means being 1178 per cm^2 in splenectomized rats, 916 in laparotomized rats and 778 in untreated rats (Table 1). The phagocytosing Kupffer cells per unit area significantly increased in splenectomized rats when compared with that of laparotomized, as well as in laparotomized rats when compared with that of untreated. The number of microspheres phagocytosed by a single Kupffer cell ranged from 1 to 8 (Table 2, Fig. 1). Kupffer cells which phagocytosed 1 to 3 microspheres were significantly more in number in splenectomized rats than in laparotomized, and significantly more in laparotomized

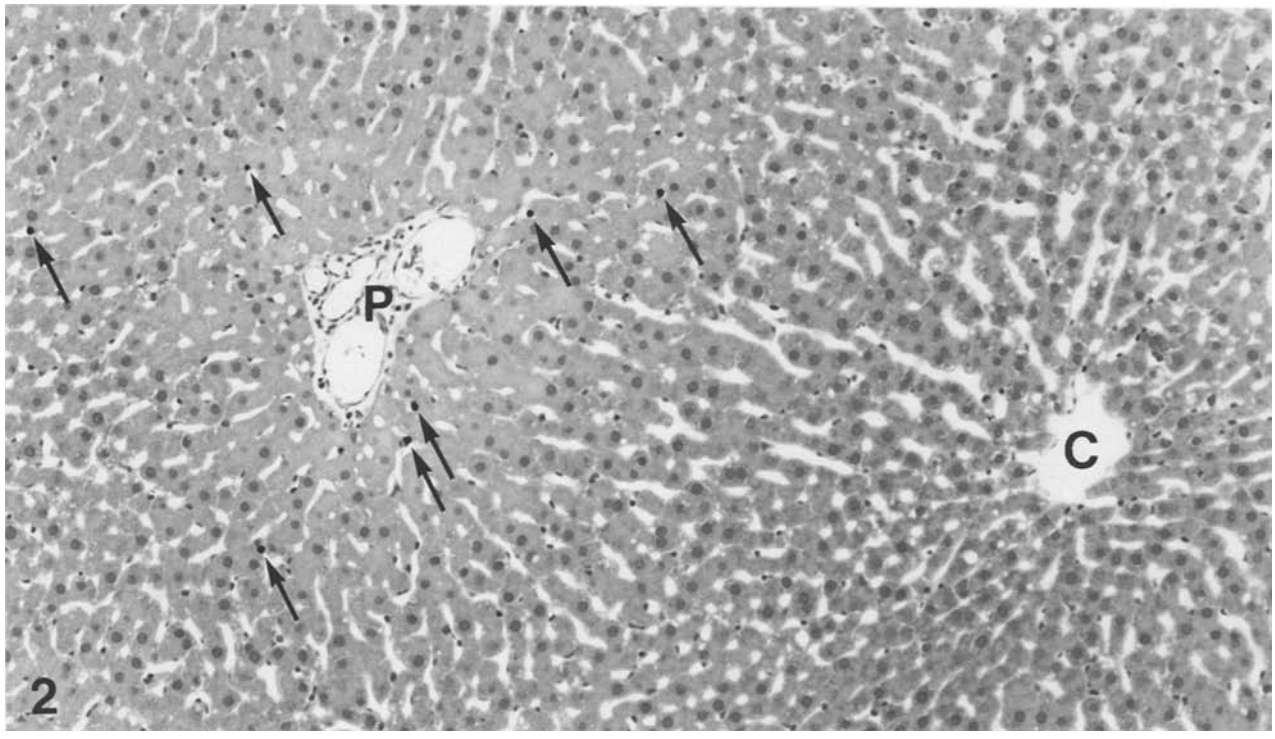

Fig. 2. The *arrows* indicate Kupffer cells which have phagocytosed microspheres. Phagocytosing Kupffer cells are greater in number in the periportal area than in the central area. C, central vein; and P, portal tract. HE, ×200

rats than in untreated. As for the number of Kupffer cells which phagocytosed 4 microspheres, a significant difference was observed between splenectomized and untreated rats, however, no significant difference was found between splenectomized and laparotomized rats, or between laparotomized and untreated rats. Kupffer cells which phagocytosed 5 or more microspheres were too few in number for statistical analysis.

A comparison of the distribution of phagocytosing Kupffer cells in the periportal and central areas is shown in Table 3 and Fig. 2. In each group of rats, the number of phagocytosing Kupffer cells in the periportal area was 8 to 9 times that in the central area. A comparison between groups of the number of the Kupffer cells in the periportal area showed them to be significantly more in number in splenectomized rats than in laparotomized and untreated rats, 1.23 times and 1.46 times, respectively (Table 3). No statistically significant difference, however, was found between laparotomized and untreated rats. As for the number of the Kupffer cells in the central area, no significant difference was found between the 3 groups although the number of phagocytosing Kupffer cells was greater in splenectomized rats than in laparotomized and in the laparotomized rats than in untreated.

A comparison of the number of phagocytosing Kupffer cells in the different hepatic lobes is shown in Table 4. There was no statistical difference between the 3 lobes in the number of the Kupffer cells. Similarly there was no difference between the lobes in the ratio of phagocytosing Kupffer cells in the periportal area to those in the central area.

Table 3. The number of Kupffer cells which phagocytosed microspheres in the periportal and central areas of the acini

Rats	n	Areas	Range in cm^2	$\bar{X}$ in cm^2	SD	Confidence limits for $\bar{X}$
Splenectomized	13	Periportal	1683–4050	2690	755	2215–3165
		Central	53– 634	291	174	181– 401
Laparotomized	10	Periportal	1855–2654	2194	252	2004–2388
		Central	161– 376	264	82	203– 326
Untreated	10	Periportal	1013–2999	1841	672	1333–2350
		Central	106– 270	197	56	155– 239

n, number of rats; $\bar{X}$, mean; SD, standard deviation. Kupffer cells which phagocytosed microspheres were counted in 30 pairs of the periportal and central areas of the acini in each rat

Discussion

The result of an experiment on clearance using radioisotope-labeled Type 2 pneumococci in rabbits showed that the overall uptake by the liver exceeded that of the spleen, although the spleen had a higher capacity for uptake per unit weight (Schulkind 1967). The clearance activities of the spleen and liver differ according to the type of injected substances. Non-opsonized substances are more actively taken up in the spleen than in the liver (Jandl and Kaplan 1960; Bogart et al. 1972). In contrast, opsonized substances are more actively taken up in the liver than in the spleen (Schulkind 1967). According to a report on the clearance of non-opsonized substances of ^{59}Fe-labeled pneumococci Type 3 by Bogart et al. (1972), the clearance in circulating blood was markedly decreased

Table 4. The number of Kupffer cells which phagocytosed microspheres in the left median, left lateral and caudate lobes

Rats	n	Hepatic lobes	Range in cm^2	$\bar{X}$ in cm^2	SD	Confidence limits for $\bar{X}$	$\bar{F}$	$\bar{t}$
Splenectomized	13	Left median lobe	729–1559	1235	244	1203–1267	1.51 (F=3.28)	0.89 (t=2.78)
		Left lateral lobe	620–1519	1135	300	946–1324		
		Caudate lobe	641–1924	1135	315	937–1333	1.10 (F=3.28)	0.00 (t=2.78)
Laparotomized	10	Left median lobe	506–1316	964	202	811–1116	1.06 (F=4.03)	1.08 (t=2.88)
		Left lateral lobe	480–1016	859	209	702–1016		
		Caudate lobe	547–1539	963	273	758–1169	1.71 (F=4.03)	0.91 (t=2.88)
Untreated	10	Left median lobe	551–1108	749	177	615– 883	2.06 (F=4.03)	0.35 (t=2.88)
		Left lateral lobe	386–1353	786	258	593–1353		
		Caudate lobe	337–1292	796	254	604– 988	1.03 (F=4.03)	0.08 (t=2.88)

n, number of rats; $\bar{X}$, mean; SD, standard deviation; $\bar{F}$, computed F-value; F, critical F-value; $\bar{t}$, computed t-value; t, critical t-value

after splenectomy. Using ^{99m}Tc sulfur colloid, however, a small compensatory increase was observed in hepatic reticuloendothelial activity in splenectomized rats (Malangoni et al. 1985). Clearances of the radioisotope-labeled lipid emulsion and bacterial suspension decreased immediately after splenectomy relative to those in sham-operated rats or mice, but it returned to normal 3 months later or was not significant (Bogart et al. 1972; Browder et al. 1983). Concerning the clearance of macromolecular substances in the liver, it has recently been confirmed that the endocytotic activity of sinusoidal endothelial cells is more important than that of Kupffer cells and, in addition, highly dependent on the nature of the substances used (Praaning-van Dalen et al. 1981; Praaning-van Dalen and Knook 1982).

The results of this study proved to be different from those of previous studies which used microorganisms, lipids or other macromolecules. In this study, the phagocytic activity of Kupfer cells was estimated from the number of Kupffer cells which phagocytosed microspheres as well as from the number of microspheres phagocytosed by Kupffer cells. It is clear that the number of phagocytosing Kupffer cells and the number of microspheres phagocytosed by single Kupffer cells both increase in splenectomized rats. While it is known that peritoneal irritation generally increases blood clearance (Soba 1975; Moustoukas et al. 1985), no significant difference between the phagocytic activity of Kupffer cells in laparotomized rats and that in untreated rats was observed. Sinusoidal endothelial cells take up various substances through endocytosis (Praaning-van Dalen et al. 1981; Praaning-van Dalen and Knook 1982). According to an experiment of Dan and Wake (1985) using latex microspheres of 0.33, 0.46 and 0.80 μm in size, microspheres were not taken up by endothelial cells in vivo, although these were incorporated into endothelial cells in the perfused liver. Praaning-van Dalen et al. (1982) reported that microspheres of and over 0.29 μm were not ingested by endothelial cells in vivo. The microspheres which we used ranged from 2.43 to 4.64 μm in diameter, so these microspheres were assumed not to be taken up by sinusoidal endothelial cells.

Concerning the intra-acinar distribution of Kupffer cells in rats, as estimated by an immunohistochemical method, the numerical ratio of Kupffer cells in the periportal, middle and central areas was 4:3:2 according to Sleyster and Knook (1982), and 3:2:2 according to Bouwens et al. (1986). In this study, the number of phagocytosing Kupffer cells in the periportal area was 8–9 times higher than that in the central area. According to a report by Bouwens et al. (1986), blood-borne foreign particles were first and mainly taken up by Kupffer cells located in the periportal area. They observed that 68% of the Kupffer cells which were located in the periportal area phagocytosed foreign particles. In contrast with this, only 32% of the Kupffer cells in the central area phagocytosed foreign particles. In addition, the Kupffer cells were larger in size in the periportal area than in the central area.

In conclusion, it seems that the phagocytic activity of Kupffer cells increases significantly after splenectomy, when carbonized polyethylene microspheres are the object of this activity. Kupffer cells are activated in the periportal areas of the acini, but not in the central areas.

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