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RES Function and Tumour Take and Tumour Growth in the Liver and in the Kidney—An Experimental Study in Rats

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Abstract—*This experimental study in rats examines tumour take and growth after RES modulation in an organ rich in macrophages—the liver—vs. an organ poor in macrophages—the kidney. A control group of* 16 *rats had* 1.0×10^6 *transplantable adenocarcinoma cells inoculated in the liver and the same number in the left kidney. They were compared with a RE-stimulated group of* 16 *rats treated i.v. with Zymosan* (3 mg/100 g *for* 3 *days) and a RE-depressed group of* 16 *rats treated with i.v. methylpalmitate* (100 mg/100 g *for* 3 *days) before tumour inoculation. Tumour size was measured on days* 7 *and* 14. *The animals were killed on day* 14. *Mortality was significantly higher in methylpalmitate-treated rats than in control groups. Tumour take in the kidney was not affected by RES stimulation or depression. In the liver, RES stimulation caused significantly less tumour take. Depression of RES with methylpalmitate did not increase tumour take or tumour growth in the liver, which was very high in the control group.*

INTRODUCTION

THE reticuloendothelial system (RES) participates in host defence against infections and malignant tumours by means of various mechanisms, among them a phagocytic function and immunomodulating capacity. Variations in RES function in individuals with growing malignant tumours reflect antineoplastic defence reactions of the RES or damage to the RES resulting from the cancer. Malnutrition, infections and various forms of therapy also influence reticuloendothelial function in the cancer host [1–3]. Decreased phagocytic function has been registered after surgery [4]. The influence of chemotherapy on macrophage function has been explored to a limited extent [5]. No single factor in the RES is responsible for its effect on cancer. In general, damage to the RES promotes and stimulation of the RES inhibits cancer take and cancer growth [6].

Several pharmacological agents can enhance RES function [7, 8]. Zymosan, well-accepted for RES stimulation, is a yeast cell wall preparation, which has been demonstrated to produce marked hyperplasia and increased phagocytic function of the RES. The active RE stimulant component of Zymosan is glucan, an insoluble polysaccharide, which constitutes over 50% of the dry weight of Zymosan. Increased phagocytic function is registered 2 h after injection and maintained for at least 72 h. In mice a maximum phagocytic activity has been registered 10 days after injection [8–10]. Depression of the RES phagocytosis can be achieved using methylpalmitate. Phagocytic function measured as impairment of intravascular clearance of colloidal carbon was significantly depressed up to 17 days with maximal depression after 2 days. RE depression induced by methylpalmitate has not been seen to be followed by a phase of hyperactivity. There is also an increased growth rate in subcutaneous and liver tumours after methylpalmitate administration [3, 11, 12].

The growth rate of experimental tumours varies in different organs. In normal rats an experimental sarcoma and hepatoma grew somewhat faster in the kidney, but a syngeneic nitrosoguanidine induced transplantable colonic adenocarcinoma had similar growth rate in liver and kidney [13].

Tissue macrophages constitute the local reticuloendothelial system. Resident macrophages (Kupffer cells) are numerous in the liver. In the kidney resident macrophages are sparse, although macrophage properties have been ascribed to mesangial cells located close to the glomeruli. More

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than 80% of the phagocytic capacity of the body is in the liver [14, 15].

The aim of this study was to investigate if RES stimulation or depression influenced tumour growth and tumour take in the macrophage rich liver and macrophage poor kidney. RES macrophage stimulation was induced by 3 days administration of Zymosan and depression by 3 days administration of methylpalmitate. On the third day the liver and the kidney were challenged with a suspension of adenocarcinoma cells.

MATERIALS AND METHODS

Forty-eight Wistar/FU inbred rats, fed on water and pellets, maintained on a normal day and night cycle were used. They were challenged with a nitrosoguanidine (NGW)-induced transplantable adenocarcinoma of the colon. This is a syngeneic adenocarcinoma maintained viable by deep-freezing and then, after thawing, by passage transplantation every 14th day [16]. The first tumour generation in this study was number 10 and the last was number 22.

Four experiments were done each consisting of 12 rats divided into three groups. All i.v. injections were given in the tail vein.

Experimental groups

Control group. Sixteen rats were injected i.v. with 0.5 ml of 0.9% NaCl and two drops of Tween 20 on 3 consecutive days.

Zymosan group. Sixteen rats were injected i.v. with 3 mg Zymosan per 100 g body wt on 3 consecutive days.

Methylpalmitate group. Sixteen rats were injected i.v. with 100 mg methylpalmitate per 100 g body wt on 3 consecutive days. Methylpalmitate was dissolved in 0.5 ml 0.9% NaCl with two drops of Tween 20.

On the 3rd day, laparotomy was performed under ether anaesthesia through a midline abdominal incision and 1.0×10^6 tumour cells were inoculated into the central liver lobe and the left kidney. Seven days later, all rats were subjected to a second laparotomy and tumour take and tumour size were registered. This procedure was uneventful in all cases. Tumour size was measured with vernier calipers in two perpendicular diameters (a and b). Tumour volume (V) was estimated using the formula [17]

$$V = \frac{a \times b^2}{2}.$$

Seven days later, the animals were killed and the tumour size, spleen weight and tumour weight were registered. The weight of tumours in the kidney was determined by substracting the weight of the normal right kidney from that of the tumour-bearing left kidney.

Table 1. Survival 7 and 14 days after inoculation of 1.0×10^6 *NGW tumour cells in the liver and in the kidney*

	n	Day 7	Day 14
Control	16	16	13
Zymosan	16	13	10
Methylpalmitate	16	12*	8*

*Indicates statistically significant difference ($P < 0.05$) from control.

Statistical analyses

Survival and tumour take frequences were compared using Student's two-tailed t-test, tumour volumes and weights were compared using Fisher's exact non-parametric permutation test. Factorial analyses on the influence of organ or RES function on tumour volume were done using a Kruskal–Wallis non-parametric multiple variance test [18].

RESULTS

Mortality

The mortality was higher in the methylpalmitate-treated group 7 and 14 days after inoculation than in the control group ($P < 0.05$) (Table 1).

Tumour take

Comparing tumour take in the two investigated organs, there were 15 takes in the liver and nine takes in the kiney on day 7 ($P < 0.05$) in the control group, but no significant differences in tumour takes could be detected between the liver and the kidney in the two treatment groups.

In the kidney there was no difference in tumour take between the three different RES function groups.

In the liver the Zymosan-treated RES-stimulated group five out of 13 animals had live tumours on day 7 and six out of 10 animals on day 14, which was significantly fewer than in the control group ($P < 0.01$ and $P < 0.05$, respectively) (Table 2).

Tumour volume

The volume did not differ significantly between all the liver and all the kidney tumours (Fig. 1).

There was no difference in tumour volume of the kidney tumours on either day 7 or day 14 between the three RES function groups.

There was no statistically significant difference in tumour volume of the visible tumours in the liver between the three RES function groups. Factorial

Table 2. Tumour take after inoculation of 1.0×10^6 *NGW tumour cells in the liver and in the kidney*

	Day 7		Day 14	
	Liver	Kidney	Liver	Kidney
Control	15/16	9/16‡	13/13	13/13
Zymosan	5/13†	8/13	6/10*	8/10
Methylpalmitate	9/12	9/12	8/8	8/8

*Indicates statistically significant difference ($P < 0.05$) and †($P < 0.01$) from control.
‡Indicates statistically significant difference ($P < 0.05$) from liver.

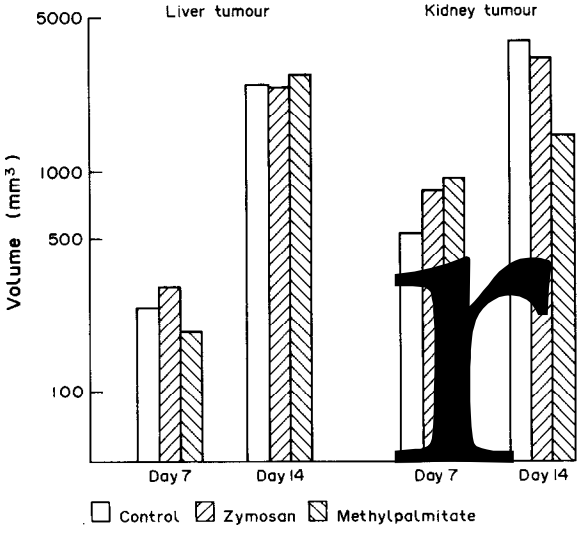

Fig. 1. Mean tumour volume in liver and kidney 7 and 14 days after inoculation of 1.0×10^6 *NGW cells in the liver and in the kidney.*

Spleen weight

The spleen was significantly heavier in the Zymosan-treated group than in the control group and the methylpalmitate-treated group ($P < 0.05$) (Table 3).

DISCUSSION

The host defence against cancer depends on many factors. The local macrophage activity is controversial. Several studies have indicated that activated macrophages are tumouricidal to cancer cell lines [19, 20]. Other studies have indicated that host inflammatory response encourages tumour growth through angiogenesis and growth factors [21–23]. This experimental study has investigated tumour take, this is the ability of tumour cells to grow in the kidney and the liver after inoculation. About 5% of the liver cell mass is Kupffer cells, which are the major part (80%) of the immobile macrophage monocyte system [14]. The macrophage content of the kidney is low although mesangial cells close to glomeruli have been proposed to have macrophage-like properties [15].

The tumour take in the present study comparing tumour growth in liver vs. kidney revealed no difference using a multiple variance factorial analysis. However, tumour take in the kidney was significantly lower than in the liver in the control group.

In a previous experimental study with the same experimental adenocarcinoma tumour take was 100% after 1.0×10^6 as well as after 0.1×10^6 tumour cells. Tumour weight was not significantly different between liver and kidney with 1.0×10^6 tumour cells challenge [13]. In the present study

Table 3. Mean tumour weight in grams ± S.D. 14 days after inoculation of 1.0×10^6 *NGW cells in the liver and in the kidney and spleen weight*

	Liver tumour	Kidney tumour	Spleen
Control	4.1 ± 2.8 (13)	4.8 ± 4.0 (13)	1.3 ± 0.3
Zymosan	2.9 ± 1.4 (6)	5.5 ± 3.8† (8)	1.9 ± 0.3*
Methylpalmitate	3.6 ± 3.3 (8)	2.7 ± 1.8 (8)	1.2 ± 0.3

*Indicates statistically significant difference ($P < 0.05$) from control.
†Indicates statistically significant difference ($P < 0.05$) from liver.

analyses with multiple variance technique showed a statistically significant influence of RES function on liver tumour volume.

Tumour weight

There was no significant difference between the weight of the kidney tumours and that of the liver tumours.

In the Zymosan-treated group tumour weight was significantly lower in the liver than in the kidney ($P < 0.05$) (Table 3).

the tumour cell number was chosen for similar growing properties in liver and kidney in order to identify differences in patterns of growth following modulation of RES function. The lower tumour take in the kidney in the control group, although it might be a chance variation, only strengthens the importance of RES function modulation on tumour growth in the liver.

In animals which were RES stimulated with Zymosan the tumour take in the liver was significantly lower than in controls, both on day 7 and on

day 14 after tumour inoculation. These findings confirm the influence of tissue macrophage function on tumour take. Stimulation with Zymosan did not influence the tumour take in the kidney nor did it influence the volume or weight of kidney tumours. Macrophage activation has little or no influence on an organ poor in tissue macrophages. Macrophage activation was also registered through increased weight of the spleen in the Zymosan-treated group. Zymosan in a single dose of 3 mg per 100 g rat increased RES phagocytic function by about 30% calculated as the uptake rate of an albumin colloid after 1 day. This increase continued for at least a week [24]. In another study the lysosomal enzymes calculated as betahexosaminidase activity were increased in the blood after Zymosan injection [25].

Methylpalmitate did not enhance the tumour take compared to the control group. The tumour take was, however, so high in the control group that an enhanced tumour take could not be expected to be recognized. Survival was significantly lower in the methylpalmitate treated group. This might be explained by a combination of depressed RES function and tumour-induced death since methylpalmitate in the same dose did not cause any mortality on sham operated rats [26].

In a previous study, in which methylpalmitate was administered in a higher dose (200 mg per 100 g for 2 days), there was a reduced rate of uptake of sulphur colloid in the liver as a sign of depressed RES function. No mortality was registered in 7 days but 60% mortality in 14 days. An enhancement of tumour growth was registered but no difference in tumour take [3]. That study was, however, only performed on five rats. Similar results have been found in mice with a subcutaneous adenocarcinoma [27].

The volume and weight of the kidney tumours were not significantly influenced by stimulation or depression of the RES, in accordance with the hypothesis that RES modulation has no major influence on tumours in the kidney.

The volume and weight of the visible liver tumours did not differ significantly between the two treatment groups and control group.

The well-known immunosuppressive effects of anaesthesia and surgery should not to any extent influence the results in this study since all animals were subjected to identical procedures.

This study indicates that RES stimulation with Zymosan can protect against tumour progression in the liver in an early phase. This effect seems to be transient since once liver tumours are established, they seem to grow as fast as tumours in control animals or other organs.

In conclusion, reticuloendothelial function of local tissue macrophages is a factor of importance in host defence against cancer. These cells need stimulation or activation to suppress tumour growth.

REFERENCES

1. Biozzi G, Stiffel C. The physiopathology of the reticuloendothelial cells of the liver and spleen. In: Popper H, Schaffner F, eds. *Progress in Liver Diseases*. New York, Grune & Stratton, 1965, 166–187.
2. Norman SJ, Schardt M, Sorkin E. Biphasic depression of macrophage function after tumour transplantation. *Int J Cancer* 1981, **28**, 185–189.
3. Bergqvist L, Hafström Lo, Hultberg B, Rydén S, Stenram U, Strand S-E. Influence of reticuloentothelial suppressing agent on liver tumour growth in the rat. *J Surg Oncol* 1984, **26**, 245–251.
4. Magarey CJ, Baum M. Reticulo-endothelial activity in humans with cancer. *Br J Surg* 1970, **57**, 748–752.
5. Domellöf L, Athlin L, Berghem L. Effects of long-term combination chemotherapy on the reticuloendothelial system. *Cancer* 1984, **53**, 2073–2078.
6. Stern K. *Control of Tumours by RES. The Reticuloendothelial System—A Comprehensive Treatise*. New York, Plenum Press, 1983, Vol. 5, 59–153.
7. Flemming KBP. *Stimulation and Depression of the RES by Pharmacological Agent. The Reticuloendothelial System—A Comprehensive Treatise*. New York, Plenum Press, 1985, Vol. 8, 247–284.
8. Riggi SJ, DiLuzzio NR. Identification of a reticuloendothelial stimulating agent in Zymosan. *Am J Physiol* 1961, **200**, 297–300.
9. Tennant RJ, Donald KJ. The ultrastructure of platelets and macrophages in particle clearance stimulated by Zymosan. *J Reticuloendothel Soc* 1976, **19**, 269–273.
10. Wooles WR, DiLuzzio NR. The phagocytic and proliferative response of the reticuloendothelial system following glucan administration. *J Reticuloendothel Soc* 1964, **1**, 160–165.
11. DiLuzzio NR, Blickens DA. Influence of intravenously administered lipids on reticuloendothelial function. *J Reticuloendothel Soc* 1966, **3**, 250–270.
12. Saba TM, DiLuzzio NR. Evaluation of humoral and cellular mechanisms of methylpalmitate induced reticuloendothelial depression. *Life Sci* 1968, **7**, 337–347.
13. Carlsson G, Hafström L. Influence of tumour implantation site on tumour take and tumour growth—an experimental study in rats. *J Exp Clin Cancer Res* 1985, **4**, 31–38.
14. Saba TM. Physiology and physiopathology of the reticuloendothelial system. *Rev Arch Intern Med* 1985, **126**, 277–291.
15. Holdsworth SR, Thomson NM, Glasgow EF, Dowling JP, Atkins RC. Tissue culture of

isolated glomeruli in experimental crescentic glomerulonephritis. *J Exp Med* 1978, **147**, 98–109.
16. Steele G Jr, Sjögren HO. Crossreacting tumour associated antigen(s) among chemically induced rat colon carcinomas. *Cancer Res* 1974, **34**, 1801–1807.
17. Carlsson G, Gullberg B, Hafström Lo. Estimation of liver tumour volume using different formulas—an experimental study in rats. *Cancer Res Clin Oncol* 1983, **105**, 20–23.
18. Bradley JV. *Distribution Free Statistical Tests*. London, Prentice Hall, 1968, 68–86.
19. Fogler WE, Fidler IJ. Non-selective destruction of murine neoplastic cells by syngenic tumouricidal macrophages. *Cancer Res* 1985, **45**, 14–18.
20. Nestel FP, Casson PR, Wiltrout RH, Kerbel RS. Alterations in sensitivity to non-specific call mediated lysis associated with tumour progression. Characterization of activated macrophage and natural killer cell resistant tumour variants. *J Natl Cancer Inst* 1984, **73**, 483–491.
21. Van der Brenk HAS, Stone M, Kelly H, Orton C, Shapington C. Promotion of growth of tumour cells in acutely inflamed tissues. *Br J Cancer* 1974, **30**, 246–260.
22. Argyris TS, Slaga TJ. Promotion of carcinomas by repeated abrasion in initiated skin of mice. *Cancer Res* 1981, **41**, 5193–5195.
23. Norman SJ. Macrophage infiltration and tumour progression. *Cancer Met Rev* 1985, **4**, 277–291.
24. Holmberg SB, Hafström Lo, Jacobsson L. Zymosan stimulated macrophage function and liver tumour growth—an experimental study in rats. In: Kirn A, Knock DL, Wisse E, eds. *Cells of the Hepatic Sinusoid*. Rijswijk, Netherlands, 1986, Vol. I.
25. Rydén S, Hultberg D, Hafström L, Isaksson A. Influence on plasma beta-hexosaminidase of reticuloendothelial stimulation and depression—an experimental study in rats. *J Clin Chem Clin Biochem* 1984, **22**, 219–222.
26. Holmberg SB. RES macrophage function. Experimental studies on tumour growth and MAb kinetics. Thesis, University of Göteborg.
27. DiLuzzio NR. Macrophage functional status and neoplasia. In: Alturo M, Saba T, eds. *Pathophysiology of the Reticuloendothelial System*. New York, Raven Press, 1981, No. 4, 209–224.