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Experimental Model for Liver Metastasis Formation Using Lewis Lung Tumor

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Summary. A new experimental model is introduced for liver metastases using intrasplenically injected Lewis lung tumor cells. The appearance of liver metastases was studied in the presence and after the removal of primary tumor. The tumorous foci in the liver proved to be natural metastases and increased in number blocking the activity of the Kupffer cells by carragheenan. This model provides a useful tool to study different aspects of liver metastases.

Key words: Liver metastasis – Lewis lung tumor – Kupffer cells – Carragheenan

Introduction

The liver is one of the most important filters in the body for tumor cells (Walther 1948). Thus it is the organ most frequently involved in metastasis formation. The appearance of the liver metastases is a very bad prognostic sign for patient. Although there are some difficulties to take advantage of the experimental results gained from animal models in the clinical practice, the need for tumor systems producing liver metastases regularly seems to be essential. This paper is concerned with such a system using intrasplenically injected Lewis lung carcinoma cells.

The effect of a macrophage-blocking agent, carragheenan, on the behavior of the liver metastases was also studied since the macrophages play an important role in tumor formation and progression (Hibbs et al. 1978). This experiment was also supported by the fact that although Kupffer cells constitute about 2% of the liver on the basis of volumetric composition (Blouin 1977), they represent approximately 80–90% of the functional mass of the body's reticuloendothelial system (Salky et al. 1964). We used carragheenan to establish the role of Kupffer cells in the progression of Lewis lung tumor in the liver, since carragheenan was able to stimulate the growth of different experimental tumors inhibiting the activity of macrophages (Keller 1976; Lotzova and Richie 1977; Thomson and Fowler 1977; Kopper et al. 1980).

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Materials and Methods

Inbred male C 57 Bl mice (from the colony of the 1st Institute of Pathology and Experimental Cancer Research, Budapest) were used. The mice were housed under conventional circumstances and fed with normal mouse pellet and tap water ad libitum. Lewis lung tumor (LLT) obtained from the Institute of Cancer Research, Sutton (England), was maintained by i.m. implantation of finely chopped tumor pieces. For experiments single cell suspension was made by filtering the chopped tumor pieces through a four fold gauze. Viability of cells was estimated by 1‰ eosine in tyrode solution.

Throughout the study ether was used for anethesia. For intrasplenic injection the skin and peritoneum was opened. The required number of viable tumor cells were implanted in 0.01 ml of Parker TC 199 medium into the spleen. The injection site was covered by a small piece of Gelaspon (Jenapharm, Jena, GDR), and the skin was closed with Michel clips.

Splenectomy was performed according to Houghen et al. 1977 at different intervals after the intrasplenic injection.

In some experiments the activity of macrophages was inhibited by carragheenan (Carragheenan Type II, No. C-1138, Sigma Chemical Co., USA). The drug was dissolved and applied according to Lotzova and Richie 1977. A dose of 5 mg/mouse in 0.5 mg was used for i.p. administration, while 2 mg and 1 mg/mouse (in 0.2 ml and 0.1 ml, respectively) were administered i.v.

Experimental groups consisted of 6–12 mice.

Results

At the beginning most of the problems arose from the hemorrhage following the intrasplenic transplantation and the spread of tumor cells in the peritoneal cavity. Both could be avoided with some practice and all results presented here refer strictly to mice only with spleen and/or liver tumors.

Different numbers of the tumor cells were injected intrasplenically to find out the minimum of cells required for the appearance of macroscopic liver metastases within 2 weeks. Metastases were observed in some mice after the injection of 3.3×10^4 cells, but in all mice using 10^5 tumor cells. For further experiments, 10^6

Table 1. Development of liver metastases from intrasplenically growing LLT

Number of tumor cells[a]	Splenectomy[b]	Day of evaluation[c]	Liver metastases		
			Mean	± SD	Range
10^4	–	14	–		
3.3×10^4	–	14	1.3	1.6	0– 5
10^5	–	14	21.6	13.2	6– 44
10^6	–	14	78.3	17.4	51– 98
10^6	–	8	73.0	24.0	32– 91
10^6	–	10	96.3	13.5	80–115
10^6	–	14	97.1	10.7	92–118
10^6	1	14	–		
10^6	3	14	21.6	10.5	10– 36
10^6	6	14	62.3	12.1	51– 75
10^6	8	14	94.5	10.0	80–110
10^6	3	10	27.0	7.3	18– 35
10^6	3	14	34.6	6.4	28– 42

[a] Number of tumor cells injected intrasplenically

[b] Days after transplantation

[c] Days after transplantation when the mice were killed

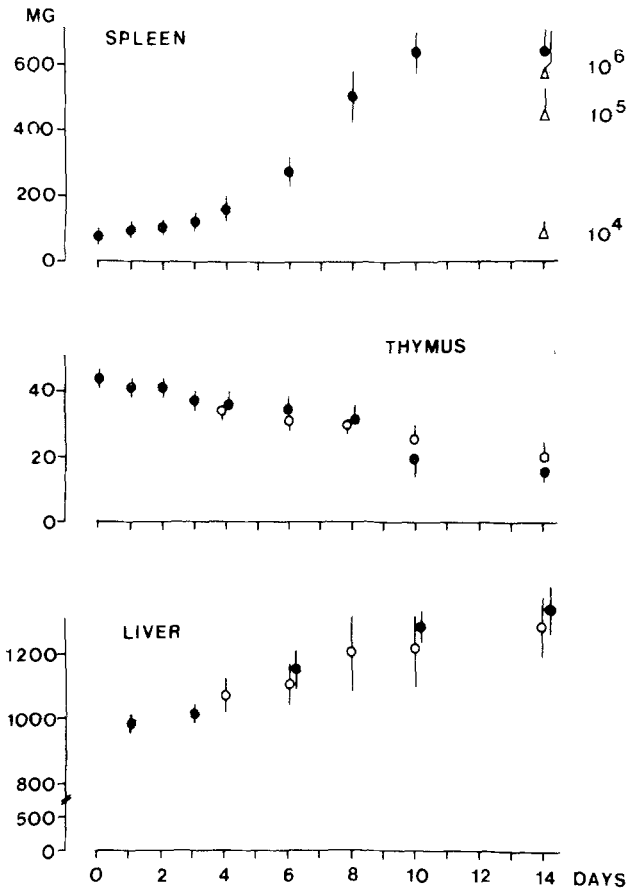

Fig. 1. Changes in the weight of the spleen, thymus and liver vs. days after transplantation in the presence of intrasplenically growing LLT (●) and in mice after splenectomy performed 3 days after transplantation (○). Triangules represent separate experiment injecting different number of tumor cells into the spleen. The vertical bars show the standard errors in groups of at least six mice

cells were chosen because at this level the number and size of metastases provided an easy counting. Only tumor nodules appearing on the surface of the liver were counted (Table 1).

The weight of the spleen increased after the implantation of 10^6 tumor cells. The increase was rather steep from the 4th day reaching an almost ten fold weight by the 10th day. The weight of the liver also increased whereas the weight of the thymus decreased (Fig. 1). Histologically, only few tumor cells were recognized in the spleen on the 1st day, but with rapid progression a well circumscribed tumor developed on the 4th day (Fig. 2). The first tumor cells could be observed mainly in the sinusoids, but with time tumorous foci of different size appeared randomly in the liver. Macroscopic metastases were present from the 6th to the 8th day reaching a countable size at 12–14 days after implantation (Fig. 3).

Next we studied the appearance of liver metastases after the removal of the primary spleen tumor. If it was removed 1 day after transplantation there was no

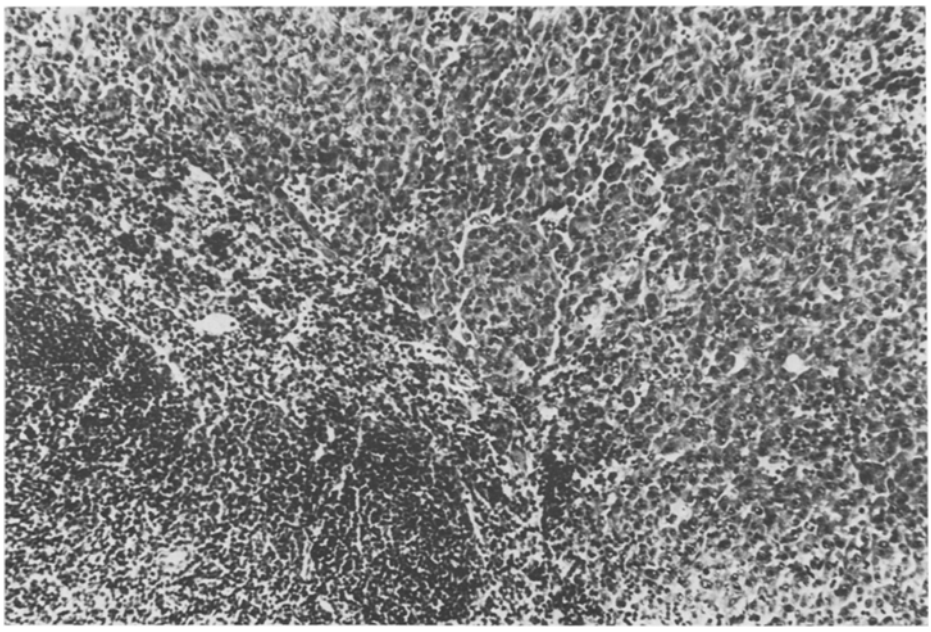

Fig. 2. Histology of Lewis lung tumor growing in the spleen 4 days after transplantation. ×75

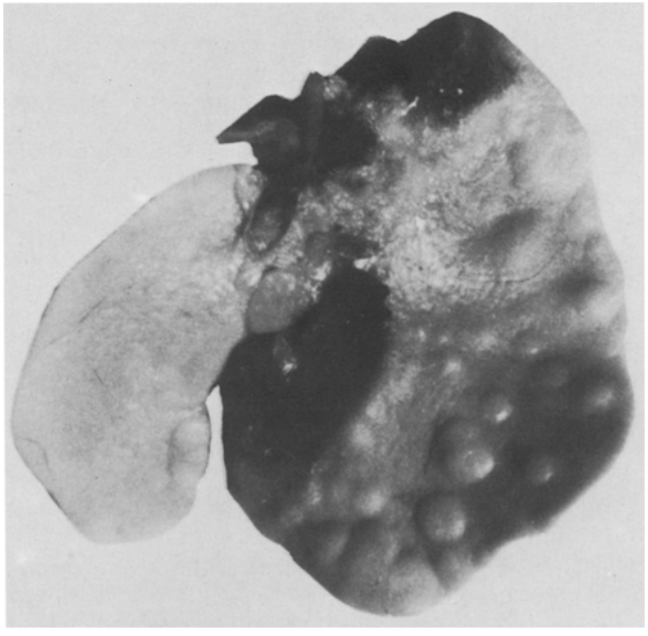

Fig. 3. Liver metastases 14 days after intranplenic transplantation of LLT

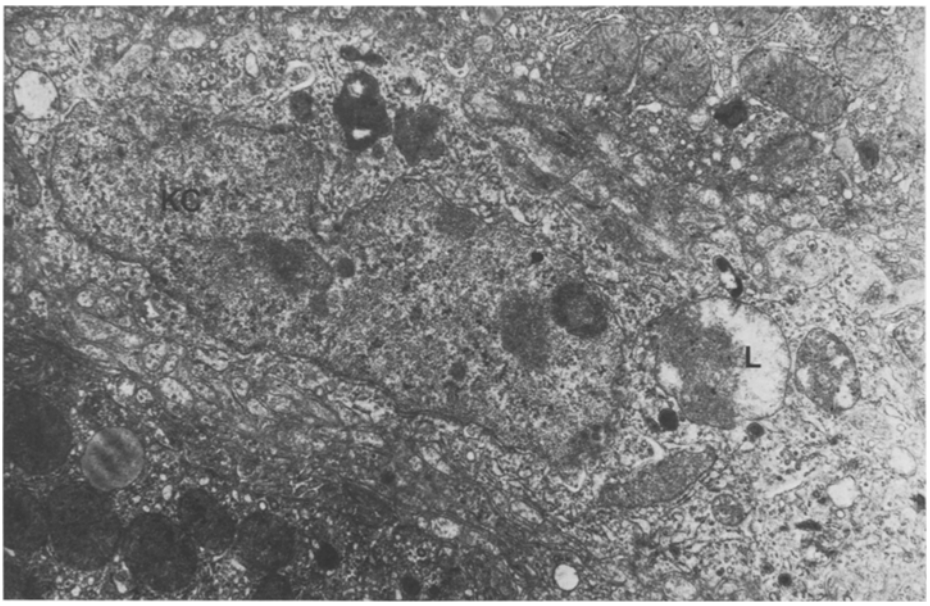

Fig. 4. Kupffer cell *KC* 24 h after treatment with carragheenan (1 mg/mouse i.v.). Note the dilated lysosomes *L*. ×9,790 (×140,986)

metastasis in the liver 14 days later. The number of metastases increased with the length of the interval between implantation and splenectomy (Table 1). For further experiments, the splenectomy primary tumor removal was performed on the 3rd day, especially for technical reasons. (With a longer interval the antihemorrhagic material, Gelaspon, "sticked" to the surroundings and the splenectomy became increasingly difficult). If the primary tumor was removed on the 3rd day the change in the weight of the liver and thymus was similar to that observed in the presence of the primary tumor (Fig. 1).

Carragheenan, a macrophage toxic agent, was used to study whether the damage of Kupffer cells has any effect on the appearance of liver metastases. Intravenous application of caragheenan was preferable because after i.p. injection inflammatory reaction developed in the peritoneal cavity, and this environment seemed to increase the possible complications of splenectomy. A dose of 1 mg carragheenan per mouse applied i.v. was tolerable. In this case, profound morphological alterations were evident only in Kupffer cells showing mainly the damage of lysosomes (Fig. 4). Pretreatment with carragheenan drug was injected 1 day before transplantation was able to enhance the number of metastases (Table 2). This effect was more obvious if the primary tumor was removed from the pretreated mice on the 3rd or 4th day after implantation (Table 2).

Discussion

Intrasplenically injected LL tumor cells produced regular liver – and only liver – metastases. This system seems suitable to approach different aspects of liver

Table 2. Effect of carragheenan on the development of liver metastases[a]

Carragheenan[b]	Splenectomy[c]	Day of evaluation[d]	Liver metastases		
			Mean	± SD	Range
–	–	12	76.1	5.7	69– 84
5 mg/mouse i.p.	–	12	105.1	22.0	90–135
–	–	12	74.2	12.4	60– 95
2 mg/mouse i.v.	–	12	123.3	14.0	110–145
–	–	12	72.1	13.6	52– 85
1 mg/mouse i.v.	–	12	82.8	13.7	70–102
–	3	12	22.8	10.1	14– 38
–	4	12	29.3	10.1	18– 40
1 mg/mouse i.v.	3	12	74.6	27.6	56–120
1 mg/mouse i.v.	4	12	74.1	23.7	44–105
–	3	13	29.0	8.2	18– 42
1 mg/mouse i.v.	3	13	64.0	16.5	46– 85

[a] 10^6 LLT cells were used for intrasplenic transplantation
[b] Carragheenan treatment was performed 1 day before transplantation
[c] Days after transplantation
[d] Days after transplantation when the mice were killed

metastases, e.g. therapeutic sensitivity, effect of different cells, and modulatory substances on the settlement and progression of tumor cells in the liver.

It should be decided whether the tumor cells reached the liver promptly after the injection as a result of mechanical forces (artefícial metastases) or it is a matter of real (natural) metastases arising from cells deriving from the growing primary spleen tumor via the portal tract. The latter seems to be predominant since, if the primary tumor was removed on the day after transplantation, metastases could not be observed. This result does not exclude the possibility that tumor cells are present in the liver shortly after the injection, but it demonstrates the insufficiency of these cells – if they exist – to produce metastatic foci during the experiment.

Pretreatment with carragheenan increased the number of liver metastases in the presence or after the removal of the primary spleen tumor. The tumor growth-enhancing effect of carragheenan can be explained by its inhibitory effect on the phagocytotic capacity of Kupffer cells (Fowler and Thomson 1978) as a result of the damaged lysosomal system demonstrated mainly on peritoneal macrophages (Allison et al. 1966; Catanzaro et al. 1971). Ross and Dingemans (1977) found great differences in the liver metastasis formation by two adenocarcinoma tumor cell sublines depending on their "sensitivity" to phagocytosis. Only that subline produced profound metastatic lesions which did not undergo phagocytosis by Kupffer cells. According to our previous observations carragheenan, besides its effect on the lysosomal system, can alter the cell coat of peritoneal macrophages (Lapis et al. 1980). It may suggest that pretreatment with carragheenan damaged both the ability of Kupffer cells to "recognize" and "trap" the tumor cells and the phagocytotic process. The contribution of Kupffer cells to the elimination of circulating malignant cells was also proved by Di Luzio (1977) activating the macrophages by

glucan with a result of profound reduction in liver metastases. These data clearly indicate the significance of the Kupffer cells in the regulation and control of malignant cell population but it remains to be clarified whether these cells have a cytotoxic capacity as observed with other macrophage populations.

The progression of tumors in the spleen and liver was accompanied by a decrease in thymus weight. Similar changes had been observed after i.m. or. s.c. transplantation of LLT (Treves at el. 1976; Elbling et al. 1977). The thymus seems to be very sensitive to the growth of LLT. This change also occurred after splenectomy, i.e., after removal of the primary tumor and the presence of liver metastases only. From the point of tumor-macrophage-thymus relationship it is interesting that, using 5 mg carragheenan i.p. per mouse, Fowler and Thomson (1978) observed hepatosplenomegaly (temporary inflammation and edema in the liver) and thymus involution besides the blockade of the mononuclear-phagocytic system. Furthermore, we observed a decrease in the chemotactic capacity of peritoneal macrophages parallel to the growth of LLT (Tran Van Hanh et al. unpubl. data). It is well possible that the decrease in the thymus weight during the tumor progression is a consequence of the decrease in macrophage activity. These changes should be important elements in the immunosuppressive effect of LLT as described by Klykken and Munson (1979).

Finally, we would like to emphasize the advantage of the system introduced here. It offers possibilities to compare the characteristics of the metastatic lesions of different organs since LLT is able to produce lung metastases after i.m. or. s.c. transplantation. It also seems to be a useful tool to answer specific questions related to liver metastases, such as the importance of Kupffer cells an different agents modifying the metabolic state of the hepatocytes or other liver cells in tumor progression.

References

Allison AC, Harmington JS, Birbeck M (1966) An examination of the cytotoxic effects of silica on macrophages. J Exp Med 124:141–153

Blouin A (1977) Morphometry of liver sinusoidal cells. In: Wisse E, Knook DL (eds.) Kupffer cells and other liver sinusoidal cells. Elsevier/North-Holland, Amsterdam, p 61 (1977)

Catanzaro PJ, Schwartz HJ, Graham RC (1971) Spectrum and possible mechanism of carrageenan cytotoxicity. Am J Pathol 64:387–399

Di Luzio NR (1977) Influence of glucan on hepatic macrophage structure and function. In. Wisse E, Knook DL (eds) Kupffer cells and other liver sinusoidal cells. Elsevier/North-Holland, Amsterdam, p 397

Elbling L, Kurata T, Micksche M (1977) Lewis lung tumor system as a model for studying the immune function in syngeneic allogenic chimeras. Oncology 34:209–211

Fowler EF, Thomson AW (1978) Effect of carrageenan on activity of mononuclear phagocyte system in the mouse. Br J Exp Pathol 59:213–219

Hibbs JB, Chapman HA, Weinberg JB (1978) The macrophage as an antineoplastic surveillance cell: Biological perspectives. J Reticuloendothel Soc 24:549–570

Houghen HP, Hansen F, Jensen KE, Röpke C (1977) Kinetics of small lymphocytes in normal and nude mice after splenectomy. Scand J Haematol 18:256–266

Keller R (1976) Promotion of tumor growth in vivo by antimacrophage agents. J Natl Cancer Inst 57:1355–1361

Klykken P, Munson AE (1979) Immunosuppressive effects of the Lewis lung carcinoma. J Reticuloendothel Soc 26:623–633

Kopper L, Tran Van Hahn, Lapis K, Timár J (1980) Increased take rate of human tumor xenografts after carrageenan treatment. Eur J Cancer 16:671–678

Lapis K, Timár J, Bencsáth M, Tran Van Hanh, Kopper L (1980) Cytochemical study of peritoneal macrophages in carrageenan-treated immunosuppressed mice. Arch Geschwulstforsch 50:435–442

Lotzova É, Richie ER (1977) Promotion of incidence of Adenovirus type 12 transplantable tumor by carrageenan, a specific antimacrophage agent. J Natl Cancer Inst 58:1171–1172

Ross E, Dingemans KP (1977) Phagocytosis of tumor cells by murine Kupffer cells in vivo and in the perfused mouse liver. In: James K, McBride B, Stuart A (eds) The macrophage and cancer. Econoprint, Edinburgh p 218

Salky NK, DiLuzio NR, P'Pool DB, Sutherland AJ (1964) Evaluation of reticuloendothelial function in man. JAMA 187:168

Thomson AW, Fowler EF (1977) Potentiation of tumor growth by carrageenan. Transplantation 24:397–399

Treves AJ, Cohen IR, Feldman M (1976) A syngeneic metastatic tumor model in mice: The natural immune response of the host and its manipulation. Israel J Med Sci 12:369–383

Walther HE (1948) Krebsmetastasen. Schwabe, Basel

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