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Altered tissue-specific opsonic activities and opsono-recognition of liposomes in tumour-bearing rats

S.M. Moghimi *, H.M. Patel [1]

Department of Biochemistry, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, UK

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Abstract

Reticuloendothelial phagocytic and serum opsonic activity was evaluated at terminal stages of tumour growth in rats transplanted subcutaneously with chondrosarcoma in an attempt to evaluate the role of opsonic protein(s) in governing liposome recognition and clearance by the macrophage system. The liver of the tumour-bearing animals manifested a decline in the uptake of multilamellar vesicles composed of egg phosphatidylcholine: cholesterol: dicetyl phosphate (mole ratio 7:2:1) from the blood when compared to healthy animals. In contrast, an increase in splenic clearance of liposomes was encountered in tumour-bearing rats. Studies with isolated liver non-parenchymal cells suggested that liposome recognition in both health and at terminal stages of cancer growth is influenced by a serum opsonin, which can be precipitated by 35–50% ammonium sulphate, as well as the concentration of calcium levels in serum. Serum of healthy animals equally enhanced liposome recognition by the hepatic macrophages of both normal and tumour-bearing rats. In contrast, both cell populations manifested poor liposome recognition in the presence of serum pooled from tumour-bearing animals and the results were comparable to the corresponding liposome-cell interaction in the absence of serum. The opsonic activity of serum derived from tumour-bearing rats could be demonstrated either by prior dialysis of serum against de-ionized water or by addition of EGTA. Liver phagocytes of healthy animals recognized more liposome in the presence of dialysed or EGTA-chelated tumour-serum than that of liver cells derived from tumour transplanted rats. A significant increase in serum calcium concentration was found in all tumour-bearing rats. When the concentration of calcium in the serum of normal animals was increased to the level that is encountered in tumour-bearing rats, a sharp drop in liposome recognition by liver phagocytes was observed. This drop in opsonic activity was not related to changes in the ionic strength of serum. The ammonium sulphate precipitated opsonin was also calcium-sensitive and its opsonic activity was abolished in the presence of calcium. Studies with isolated splenic phagocytes suggested that an increase in the opsonic activity of serum, but not the elevated calcium level, was responsible for hyperphagocytosis of liposomes by the splenic phagocytes of tumour-transplanted animals. The opsonic molecule which enhanced liposome recognition by liver non-parenchymal cells

* Corresponding author. Present address: Consultant in Drug Carrier Systems, Micro-Tek System, 28 Clumber Court, Nottingham NG7 1EE, UK. Fax: +44 (115) 941 1604.

[1] Present address: Sandol, 1 Bowls Close, Stanmore, Middlesex HA7 3LF, UK.

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failed to enhance liposome clearance by the splenic phagocytes. These findings suggest that the alteration in macrophage clearance of liposomes during the terminal growth of cancer may be mediated in part by changes in the opsonic capacity of serum.

Keywords: Calcium; Chondrosarcoma; Kupffer cell; Liposome; Opsonin; Reticuloendothelial system; Splenic macrophage; (Rat)

1. Introduction

The mechanisms responsible for the recognition and clearance of liposomes by phagocytic cells of the reticuloendothelial system from the blood are presently not well understood. In rats, it is believed that intravenously administered liposomes of different size, different lipid composition, and differing surface characteristics attract different arrays of blood components, the content and conformation of which may account for the different pattern in the rate and site of vesicle clearance from the circulation [1–10]. The rate and the site of particle clearance from the blood is also governed by pathophysiological alterations in the blood opsonic activity as well as changes in macrophage function and responsiveness (e.g., changes at the expression of plasma membrane receptors and antigens, receptor mobility, macrophage recruitment) [11–13]. Earlier we demonstrated that a heat-stable proteinaceous factor from rat serum can mediate the binding of multilamellar vesicles, composed of egg phosphatidylcholine (egg PC): cholesterol (Chol): dicetyl phosphate (DCP), in a mole ratio of 7:2:1, to freshly isolated rat liver phagocytes [6,14]. The activity of this factor in serum (designated here as the liver-specific opsonic molecule) is apparently regulated by calcium [14,15]. For instance, prior dialysis of serum against de-ionized water or the chelation of serum with EGTA increased its opsonic activity by 2- to 3-fold, whereas re-addition of calcium to dialyzed or EGTA-chelated serum suppressed the elevated opsonic activity as measured on the interaction of multilamellar liposomes with isolated rat liver macrophages [15]. Furthermore, the opsonic activity of serum on liposome recognition by liver non-parenchymal cells is abolished at calcium levels above the physiological concentration.

Although calcium at physiological levels is a prerequisite for the process of phagocytosis [15,16], Ryder et al. [18] have demonstrated that the elevation of serum calcium levels above normal can also inhibit opsono-phagocytosis of lipid emulsions by Kupffer cells. Previous analysis of serum ionic composition from experimental and clinically encountered neoplasms have interestingly reported a frequent pronounced hypercalcaemia during tumour growth [18–22]. Upon chemotherapy or surgical resection of the tumour, the calcium levels have been reported to return to normal, while tumour recurrence is accompanied by a reappearance of hypercalcaemia [19]. Furthermore, studies of Saba and Antikatzides [23] on the functional state of the reticuloendothelial system during tumour growth have revealed that the terminal stages of tumour growth and spread (a hypercalcaemic serum state) are associated with pronounced serum opsonic dysfunction and associated depression in hepatic Kupffer cells clearance of particles from the blood.

The above findings when placed in relationship to the temporal change of calcium levels with tumour growth suggest a potential effect of serum calcium concentration on reticuloendothelial function. Since calcium can regulate opsono-recognition of multilamellar liposomes composed of egg PC: Chol: DCP (mole ratio 7:2:1) by liver non-parenchymal cells [14,15], we have now extended our studies to determine the role of calcium and serum factors on liposome recognition and clearance by macrophages of the reticuloendothelial system during the terminal stages of an experimentally induced non-metastatic tumour model in rats.

2. Materials and methods

2.1. Liposome preparation

Multilamellar liposomes (300–380 nm average diameter) containing [125-I]-labelled poly(vinylpyrrolidone) (Amersham, UK), [125-I PVP], were prepared from a mixture of egg PC (Lipid Products, UK), Chol, and DCP (Sigma Chemicals, UK) in a molar ratio of 7:2:1 and 7:7:1 for Chol-poor and Chol-rich vesicles, respectively as described earlier [1,5].

2.2. Tumour transplantation

Male CFY rats, approx. 6 weeks of age, were used in all experiments as tumour recipients. Chondrosarcoma [24] (a gift from Prof. R. Mason, Department of Biochemistry, Charing Cross Hospital, London) was routinely maintained by serial transplant every 4–6 weeks. Transplantation was accomplished by subcutaneous administration of 2.5×10^4 viable cells in sterile Hanks' balanced salt solution, HBSS (Gibco, Scotland), in an injection volume of 0.5 ml at each flank. Control rats of the same age received only HBSS. Full tumour growth occurred in 5–6 weeks following transplantation (the total tumour weight removed from both flanks was 90 ± 24 g). In accordance with previous observations [24] there was no spontaneous regression or sign of metastases in the host. Transplanted animals did not show any sign of discomfort throughout the experimental period.

2.3. Liposome administration and biodistribution studies

Groups of 3–4 control and tumour-bearing rats (at 5–6 weeks post transplantation) were injected intravenously via the tail vein with 15 mg (total lipid) of radiolabelled chol-poor liposomes. Rats were killed at 1 h post-administration of liposomes and the radioactivity associated with the blood and organs of the reticuloendothelial system was measured. To determine the amount of liposomes in the blood, we assumed a total blood volume per rat of 7.5% body weight [1]. Correction factors for the blood content in tissues were determined according to the established procedures [1,25].

2.4. Cell isolation and incubation procedures

Hepatic non-parenchymal cells, containing predominantly endothelial and Kupffer cells, and splenic white cells were prepared from both control and tumour-bearing rats (at the terminal stages of tumour growth) as described in detail elsewhere [5,6]. Details of incubation procedures and uptake assessment are presented in full elsewhere [5,6]. Briefly, splenic cells (10^8 cells/ml) were incubated at 37°C for 1 h with liposomes (0.6 mg total lipid) in the absence and presence of 25% (v/v) of fresh rat serum (details regarding serum preparation and its treatment is reported elsewhere [15]) in a total volume adjusted to 2.0 ml with 10 mM oxygenated phosphate-buffered saline (pH 7.4) containing 5.0 mM glucose and 1.0% bovine serum albumin. The incubation media for hepatic cells (10^7 cells/ml) was the same as that described for splenic cells except that phosphate-saline buffer was replaced with calcium/magnesium free HBSS. The uptake of radiolabelled PVP was measured in the cells at the end of the incubation. Since free PVP is not degradable and not adsorbed onto or taken up by the cells (pinocytic uptake of free PVP was not more than 0.2% of the dose for both the liver and the spleen phagocytes), the radioactivity associated with cells indicates the presence of liposomes [1,5,6]. The leakage of PVP from liposomes was minimum in the presence of 25% (v/v) control and tumour-derived sera as well as the cells. Each incubation was performed in triplicate (otherwise stated) and each experiment was repeated at least three times. Because of variation in opsonic activity from animal to animal, the mean results of a typical experiment ± the standard error is reported.

2.5. Serum fractionation

Serum from normal and tumour-bearing rats was subjected to ammonium sulphate fractionation in an attempt to obtain a fraction containing the liver-specific opsonin responsible for enhancing liposome recognition by hepatic phagocytes [14]. Regardless of their source, the same quantity of serum (in terms of mg protein) was used for fractionation. Protein determination was made by the Lowry method [26]. Initially, serum (pH 7.0) was brought to 35% saturation by slow addition, while gently stirring, with solid ammonium sulphate at 4°C (pH 7.0–7.2). After the complete addition of ammonium sulphate, the suspension was stirred for 45 min at 4°C and the precipitated proteins were removed by centrifugation at $12000 \times g$ for 15 min, 4°C. To the supernatant from the previous step, ammonium sulphate was added slowly over 20 min to bring the final saturation to 50% at 4°C. The mixture was allowed to stir gently for an additional hour and the precipitate was removed by centrifugation as described above. The precipitate was dissolved in 10.0 mM Tris–0.15 M sodium chloride (pH 7.0) at 4°C and dialyzed exten-

sively against the same buffer at 4°C. Finally, 15.0 mg of the final product was passed through a sephadex G-200 column (0.7 × 50 cm) equilibrated with 10.0 mM Tris–0.15 M sodium chloride (pH 7.0) at 4°C. The collected fractions were monitored for protein by measuring the absorbance at 280 nm. The protein-containing fractions were tested for opsonic activity on liposome recognition by freshly isolated Kupffer cells and splenic macrophages and the results are expressed as percentage-specific recognition mediated by fractionated serum proteins. Percentage-specific recognition is defined as (A − B)/(mg of fractionated serum protein added), where A and B are percentage of initial liposomal radioactivity associated with liver cells in the presence and in the absence of fractionated serum proteins, respectively.

3. Results

3.1. Tissue distribution of liposomes

We have found no significant changes in the weight of liver between control (9.5 ± 1.2 g) and tumour-bearing animals (10.1 ± 1.0 g) at terminal stage of tumour growth. In contrast, the spleen weights from tumour-bearing animals (0.71 ± 0.01 g) were significantly ($P < 0.02$) higher than the control rats (0.56 ± 0.01).

The results of tissue distribution of intravenously injected liposomes is given in Fig. 1. The liver of tumour-bearing animals manifests suppressed liposome uptake when compared to that of normal rats. This difference in hepatic uptake between the two groups is significantly clear when the results are expressed as percentage of injected dose per gram tissue. Thus, the corresponding values are 2.4 ± 0.1 and 4.0 ± 0.1 for tumour-bearing and control rats, respectively. In contrast, splenic uptake of liposomes was considerably higher in tumour-bearing rats (28.3 ± 3.0% per gram spleen) than in normal rats (16.2 ± 3.3% per gram spleen, $P < 0.05$). Similar to the liver, the uptake of liposomes in lung is also suppressed in tumour-bearing rats.

3.2. Liposome-liver cell interaction

We have compared the opsonic activity of normal serum to that derived from tumour-transplanted rats on liposome interaction with hepatic phagocytes (Fig. 2). Normal serum equally enhanced liposome recognition by hepatic macrophages of both normal and tumour-bearing animals, whereas both cell populations manifested poor liposome recognition in the presence of serum from tumour-bearing animals (values were comparable to the corresponding incubations without the serum). Interestingly, the serum of tumour-bearing animals when chelated with EGTA or previously dialyzed against de-ionized water or saline (0.9% w/v) was able to enhance the interaction of liposomes with both cell populations. However, more liposomes were found in association with liver cells of normal animals than of tumour-bearing rats in the presence of the chelated or dialysed serum of tumour-transplanted rats (Fig. 2).

Earlier we demonstrated that dialysed serum can enhance the binding of liposomes to Kupffer cells: a process that could be due to removal of ionic calcium, or other divalent cations, on dialysis of serum [15]. The total concentration of calcium in serum, as measured by atomic absorption, was found to be significantly higher ($n = 15$, $P < 0.001$) in tumour-

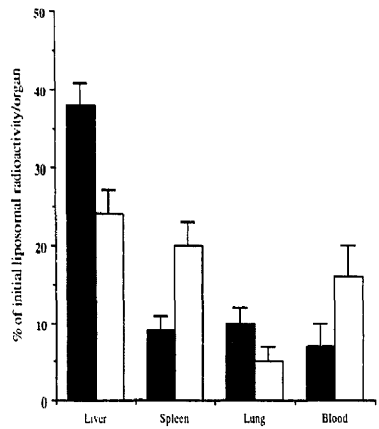

Fig. 1. Biodistribution of intravenously injected liposomes in normal and tumour-bearing animals at 1 h post-intravenous administration. Radioactivity associated with carcass, 20–30% of the administered dose in both groups, may represent liposomal radioactivity associated with bone marrow, vascular endothelium, skin, gut and to a certain extent the tumour tissue. Black columns: normal rats; open columns: tumour-bearing rats.

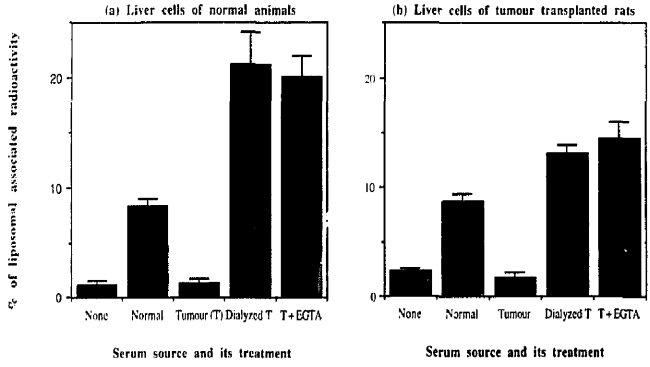

Fig. 2. Opsonic effect of serum, derived from both normal and tumour-bearing rats, and its treatment on liposome interaction with liver non-parenchymal cells of normal (a) and tumour-bearing (b) animals.

bearing animals (3.00 ± 0.08 mM at the terminal stages of tumour growth) than in the control animals of the same age (2.80 ± 0.03). Therefore, calcium concentration in normal serum was first titrated to the level that is found in the serum of tumour-bearing rats and then its opsonic activity was measured. When the concentration of calcium in serum was increased by 0.25 mM, liposome recognition by liver phagocytes decreased dramatically (Fig. 3a). The opsonic activity of serum was abolished when calcium concentration was increased by 0.75 mM (Fig. 3a). In order to determine whether this decrease in liposome opsono-recognition was due to increase in the ionic strength of medium, similar incubations were per-

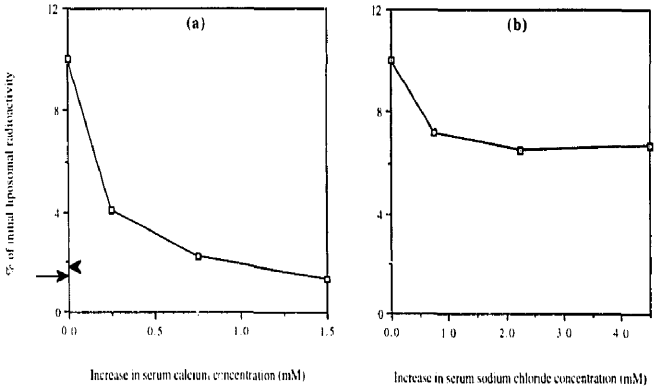

Fig. 3. Interaction of liposomes with hepatic non-parenchymal cells following experimental manipulation of ionic strength by calcium chloride (a) and sodium chloride (b). The arrowhead indicates liposome–cell interaction in the absence of both rat serum and calcium chloride, and the arrow indicates liposome–cell interaction in the absence of serum but in the presence of 3.0 mM calcium chloride. Because of charge differences, a sodium chloride solution three times as concentrated as calcium chloride solution has the same ionic strength. Liver cells are from control rats.

Table 1
Opsonic activity of serum proteins precipitated by ammonium sulphate on liposome recognition by liver non-parenchymal cells

Ammonium sulphate saturation level (%)	% specific recognition
I. Serum of tumour transplanted rats:	
35–50% precipitate	2.5 ± 0.5
35–50% supernatent	0.7 ± 0.05
II. Serum of healthy rats:	
35–50% precipitate	3.9 ± 0.1
35–50% supernatent	0.7 ± 0.1

Liver non-parenchymal cells were obtained from healthy rats. Incubations contained 5.0–7.0 mg serum protein.
For both sera, the materials precipitated in the range of 0–35% ammonium sulphate had no opsonic activity and failed to promote liposome recognition by liver cells.

formed but sodium chloride at a concentration three times higher than calcium chloride (equivalent to same ionic strength) replaced calcium chloride. Changes in liposome–cell interaction observed in the sodium chloride-supplemented samples were indeed minimum (Fig. 3b). We also studied the effect of increasing concentrations of calcium on the interaction of liposomes with liver cells, but in the absence of serum, and found no significant changes on liposome–cell interaction upon the addition of calcium (data not shown).

Serum from healthy and tumour-bearing rats was subjected to ammonium sulphate fractionation in an attempt to obtain a proteinaceous component capable of enhancing liposome recognition by hepatic phagocytes. In a series of ammonium sulph.t fractionation studies, we found that a serum prot.in factor, precipitable by 35–50% ammonium sulphate, can enhance liposome recognition by liver cells (Table 1). Liposome recognition by liver phagocytes was more enhanced in the presence of the precipitated factor from the control animals than that of tumour-bearing rats (Table 1). To further purify the opsonic molecule from both sera, the 35–50% ammonium sulphate-precipitated materials were passed through a sephadex G-200 column. Although the A280 profiles looked so different (Fig. 4), the opsonic activity was confined to the second peak in both sera (with optimum activity occurring in fraction 20 regardless of serum source).

In a separate series of experiments, we found that the uptake of liposomes by liver cells in the presence of the partially-purified opsonin (pooled from fraction 20 and 21, Fig. 4a) was temperature-dependent and could be inhibited at a low temperature (4°C) of incubation (data not shown). Furthermore, at 37°C and in the presence of the partially-purified opsonin, the phagocytic inhibitor cytochalasin B reduced the association of liposomal aqueous marker with liver cells by 40%. In accordance with the above observa-

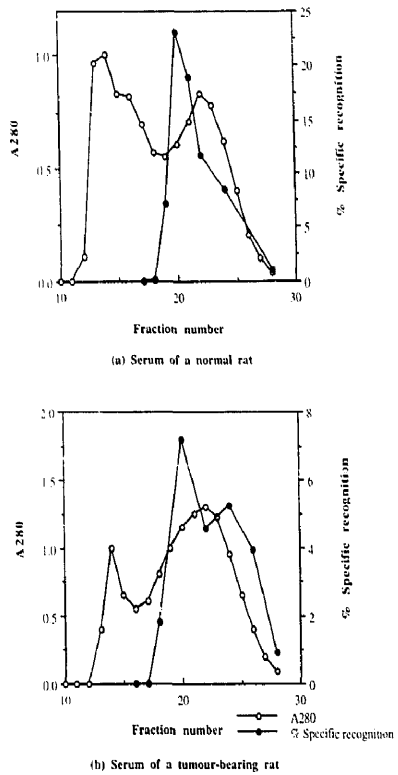

Fig. 4. Elution profiles of serum proteins precipitated by 35–50% ammonium sulphate on a sephadex G-200 column. Percentage specific recognition of liposomes by liver non-parenchymal cells is also indicated (the results are the mean of duplicate incubations; individual values were within 5% of the mean value. Incubations contained 0.3–0.6 mg serum protein. Duplicate incubations contained the same quantity of serum proteins). Liver non-parenchymal cells were derived from normal animals. For experimental details see Section 2.

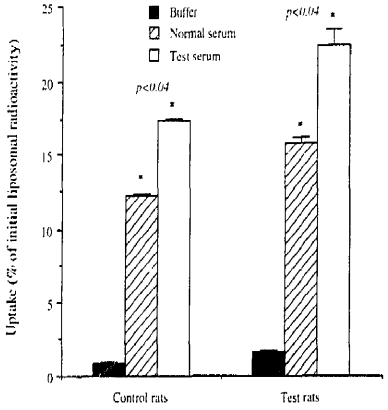

Fig. 5. Opsonic effect of normal and test (tumour-derived) sera on liposome interaction with splenic phagocytic cells of normal and tumour-bearing animals. Opsonic activity of serum varied from animal to animal but in all separate experiments the opsonic activity of serum derived from tumour-transplanted rats was significantly higher than that of healthy rats. The results presented are the mean of three incubations ± the standard error of one typical experiment (i.e., spleen cells and serum was from the same animal).

tions, the partially-purified opsonin was calcium-sensitive; its opsonic activity was reduced by approx. 40% in liver cell incubations supplemented with 0.5 mM calcium chloride. The presence of 1.0 mM calcium chloride, however, totally abolished the opsonic activity of the partially-purified protein.

3.3. Liposome-spleen cell interaction

Serum is known to stimulate the interaction of both chol-poor and chol-rich vesicles by the splenic phagocytes, but its effect is significantly greater on the uptake of chol-rich liposomes [5,6]. Hence, we tested the opsonic activity of both control and tumour-derived sera on interaction of chol-rich liposome with the splenic phagocytes. The results in Fig. 5 shows that both normal and tumour-derived sera can stimulate liposome interaction with spleen cells of both normal and tumour-bearing animals. The opsonic activity of serum from tumour-bearing animals is 40% higher than that of normal serum in promoting liposome clearance by both cell populations. Furthermore, the opsonic molecule which enhanced liposome recognition by Kupffer cells (i.e., fractions with the highest activity in sephadex G-200 column) failed to enhance liposome clearance (regardless of their cholesterol content) by the splenic phagocytes (data not shown).

4. Discussion

The current investigation demonstrates a good correlation between the in vivo results of liposome distribution at the terminal growth phase of chondrosarcoma and the in vitro cellular assay systems. The diminished clearance of liposomes by Kupffer cells during the terminal phase of tumour growth could be correlated with the activity of a serum opsonin precipitable by 35–50% ammonium sulphate (Table 1 and Fig. 4). Earlier, we demonstrated that the activity of this liver-specific opsonin is regulated by calcium [15]. An increase in physiological concentration of calcium will suppress and a decrease will enhance its activity with regard to the recognition of multilamellar liposomes (composed of egg PC: Chol: DCP, in a mole ratio of 7:2:1) by freshly isolated rat liver macrophages in suspension (see also Fig. 3). The findings reported here (Figs. 1–3) suggest that the elevated levels of calcium in the blood at terminal stages of tumour growth can suppress opsono-recognition of liposomes by the liver phagocytes. Although there was no sign of metastases in the host, differences were observed between the liver phagocytes of normal and tumour-transplanted rats with regard to liposome recognition in the presence of dialyzed or EGTA-treated serum of tumour-bearing animals. Therefore, we cannot exclude the possibility of certain changes associated with liver macrophages that can affect opsono-recognition of liposomes in cancer. Nevertheless, the results of liposome recognition by Kupffer cells are in close agreement with the earlier observations of Ryder et al. [17] and Saba and Antikatzides [23], although the nature of the opsonic molecule and the test particles are different between the present and these reported studies. These investigators [17,23] used gelatin-coated lipid emulsions as the phagocytic substrate and the responsible opsonin has been identified as fibronectin. Our previous ob-

servations disregarded fibronectin as an opsonin responsible for the recognition of multilamellar liposomes by isolated rat Kupffer cells [6].

The enhanced liposome clearance by the spleen in tumour-bearing rats can be closely correlated to an increase in the opsonic activity of serum, since splenic phagocytes from both normal and tumour-bearing rats manifest liposome hyperphagocytosis in the presence of serum derived from tumour-bearing animals when compared to control serum. The increased opsonic activity in the serum from tumour-transplanted rats is unlikely to be due to elevated levels of calcium, since the addition of calcium to normal serum to the level that is encountered in tumour-bearing animals did not have a significant effect on liposome recognition by both cell types (data not shown). Furthermore, unlike liver phagocytes, spleen cells manifest hypophagocytosis in the presence of EGTA-chelated and dialysed sera and the addition of calcium to such sera fails to fully restore the lost opsonic activity [15]. These observations therefore support our earlier suggestion that blood may contains opsonins which exhibit tissue-specificity; at least with respect to recognition and the clearance of DCP-containing egg PC vesicles of different cholesterol content by hepatic and splenic phagocytic cells, respectively [5,6,15].

Previous studies have shown elevated lung uptake and poor hepatic localization of technetium tin colloid (TTC) in numerous malignant and benign diseases, particularly in those of the chest region [27]. In contrast, enhanced bone marrow uptake of TTC was noted in patients with gastrointestinal malignancies, whereas patients with lymphoreticular malignancies seemed to cause abnormally increased spleen uptake of TTC [27]. The cause for these discrepancies has been hypothesized to be related to regional stimulation of the macrophages by the tumour [27]. However, in the light of the present study we additionally propose that altered tissue distribution of colloidal particles in various malignancies may be due to changes in the activity of tissue-specific opsonins induced by factors such as the elevated blood calcium levels. For instance, it is likely that an increase in levels of calcium concentration in the blood may have suppressed TTC clearance by Kupffer cells in cancer patients, since hypercalcaemia is known to be associated with both primary and metastatic lung carcinoma as well as in breast carcinoma [22]. The enhanced clearance of TTC by other organs of the reticuloendothelial system could also be the result of elevated activity of those opsonins which exhibit phagocyte specificity. The relationship of the present observations to disturbances of the macrophage system during malignancy, which is known for its ability to alter the serum calcium concentration, warrants further investigation.

References

[1] Patel, H.M., Tuzel, N.S. and Ryman, B.E. (1983) Biochim. Biophys. Acta 761, 142–151.
[2] Senior, J., Crawley, J.C.W. and Gregoriadis, G. (1985) Biochim. Biophys. Acta 839, 1–8.
[3] Roerdink, F.H., Regts, J., Handel, T., Sullivan, S.M., Baldeschwieler, J.D. and Scherphof, G.L. (1989) Biochim. Biophys. Acta 980, 234–240.
[4] Derksen, J.T.P., Baldeschwieler, J.D. and Scherphof, G.L. (1988) Proc. Natl. Acad. Sci. USA 85, 9768–9772.
[5] Moghimi, S.M. and Patel, H.M. (1988) FEBS Lett. 233, 143–147.
[6] Moghimi, S.M. and Patel, H.M. (1989) Biochim. Biophys. Acta 984, 379–383.
[7] Funato, K., Yoda, R. and Kiwada, H. (1992) Biochim. Biophys. Acta 1103, 198–204.
[8] Devine, D.V., Wong, K., Serrano, K., Chonn, A. and Cullis, P.R. (1994) Biochim. Biophys. Acta 1191, 43–51.
[9] Liu, D., Liu, F. and Song, Y. (1995) Biochim. Biophys. Acta 1235, 140–146.
[10] Nagayasu, A., Uchiyama, K., Nishida, T., Yamagiwa, Y., Kawai, Y. and Kiwada, H. (1996) Biochim. Biophys. Acta 1278, 29–34.
[11] Ellens, H., Morselt, H.W.M., Dontje, B.H.J., Kalicharan, D., Hulstaert, C.E. and Scherphof, G.L. (1983) Cancer Res. 43, 2927–2934.
[12] Moghimi, S.M., Hedeman, H., Christy, N.M., Illum, L. and Davis, S.S. (1993) J. Leukoc. Biol. 54, 513–517.
[13] Moghimi, S.M. and Murray, J.C. (1996) J. Natl. Cancer Inst. 88, 766–768.
[14] Moghimi, S.M. and Patel, H.M. (1993) Biochem. Soc. Trans. 21, 128S.
[15] Moghimi, S.M. and Patel, H.M. (1990) Biochim. Biophys. Acta 1028, 304–308.
[16] Stossel, T.P. (1973) J. Cell Biol. 48, 346–356.
[17] Ryder, Jr., K.W., Kaplan, J.E. and Saba, T.M. (1975) Proc. Soc. Exp. Biol. Med. 149, 163–167.
[18] Gardner, B. (1969) Surg. Gynecol. Obstet. 128, 369–374.
[19] Buckle, R.M., McMillan, M. and Mallinson, C. (1970) Br. Med. J. 4, 724–726.
[20] Haskell, C.M., De Vita, V.T. and Canellos, G.P. (1971) Cancer 27, 872–880.

[21] Tashjian, A.H., Voelkel, E.F., Levine, L. and Goldhaber, P. (1972) J. Exp. Med. 136, 1329–1343.
[22] Gislason, T., Palmer, M. and Nou, E. (1987) Eur. J. Respir. Dis. 70, 8–13.
[23] Seba, T.M. and Antikatzides, T.G. (1975) Br. J. Cancer 32, 471–482.
[24] Breilkreutz, D., Diaz de Leon, L., Paglia, L., Gay, S., Sarm, R.L. and Stern, R. (1979) Cancer Res. 39, 5093–5100.
[25] Souhami, R.L., Patel, H.M. and Ryman, B.E. (1981) Biochim. Biophys. Acta 674, 354–371.
[26] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[27] Lantto, T., Luostarinen, M. and Vorne, M. (1986) Nucl. Med. 25, 15–18.