Biochimica et Biophysica Acta, 984 (1989) 384–387
Elsevier

BBAMEM 70622

BBA Report

Serum opsonins and phagocytosis of saturated and unsaturated phospholipid liposomes

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(Received 8 February 1989)
(Revised manuscript received 17 May 1989)

Key words: Organ specific opsonin; Opsonin; Liposome; Macrophage; Phagocytosis; (Rat liver); (Rat spleen)

Recently we reported that serum contains opsonins specific for hepatic and splenic phagocytic cells and that these opsonins have different properties and affinities for cholesterol-rich and cholesterol-free egg phosphatidylcholine liposomes (Moghimi, S.M. and Patel, H.M. (1988) FEBS Lett. 233, 143–147). In the present report we investigate the affinity of these opsonins for the liposomes prepared from sphingomyelin and saturated phospholipids, as measured by their effect on the uptake of these liposomes by hepatic and splenic phagocytic cells. Results presented here suggest that neither liver- nor spleen-specific opsonins have affinity for sphingomyelin or saturated phospholipid liposomes since serum fails to enhance their uptake in liver or splenic cells. On the contrary, these liposomes attract serum dysopsonins which inhibit their uptake by liver cells. Inclusion of cholesterol in these liposome preparations enhances their uptake in splenic cells but not in liver cells. It is suggested that fluidity and hydrophobicity of liposomal membranes play an important role in attracting the right opsonins which determine their phagocytic fate.

Liposomes containing cholesterol prepared from sphingomyelin and saturated phospholipids such as dimyristoyl/phosphatidylcholine, dipalmitoyl/phosphatidylcholine and distearoyl/phosphatidylcholine, when injected intravenously, have an extremely long half-life in the circulation [1–3]. These liposomes are less susceptible to destruction by serum components [3,4] and hence it is suggested that their stability in the circulation contributes to their long half-life. However, the clearance of liposomes from the blood by phagocytic cells of the organs of the reticuloendothelial system (RES) may be the major factor in determining their half-life [5,6] and hence it is important to investigate the reasons for the slow uptake of these liposomes by phagocytic cells.

The clearance of liposomes from the circulation by the RES depends on several factors such as the physical and chemical properties of liposomes and serum components associated onto the surface of liposomes in the circulation. Various investigators [7–10] have examined the possible involvement of serum opsonins in phagocytosis of liposomes by macrophages from the same animal species, both in vitro and in vivo. Some of these studies [8–10] failed to demonstrate the opsonic effect of serum on uptake of liposomes by macrophages and yet these authors [8,10] speculated the possible role of opsonins in enhancing the uptake of liposomes by macrophages. Recently we have for the first time demonstrated [11,12] the presence of tissue-specific opsonins in serum which exhibit a different affinity for cholesterol-poor and cholesterol-rich liposomes. This suggests that the failure of the earlier attempts [8–10] to demonstrate the opsonic effect of serum on phagocytosis of liposomes by macrophages may possibly be attributed to the different affinity of these opsonins for liposomes prepared from various phospholipids used in these studies. Hence in this report we examine the role of serum opsonins on the uptake of liposomes prepared from saturated and unsaturated phospholipids by rat liver and spleen phagocytic cells.

Negatively charged multilamellar vesicles (containing ^{125}I-labelled iodinated polyvinylpyrrolidone (^{125}I-PVP) or inulin-[^{14}C]carboxylic acid) were prepared as previously described [5,11] from a mixture of various phospholipids, cholesterol and dicetyl phosphate (DCP) in a molar ratio of 7:0:1 for cholesterol-free, 7:2:1 for cholesterol-poor or 7:7:1 for cholesterol-rich liposomes. Hepatic non-parenchymal cells containing predominantly endothelial and Kupffer cells and splenic

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TABLE I

Interaction of negatively charged saturated and unsaturated phospholipid liposomes with liver non-parenchymal cells in the absence and presence of serum

For experimental details see Ref. 11. A and B are separate experiments.

Liposome composition	% of initial ^{125}I-PVP	
	control	serum
(A) Chol-free liposomes (i.e. O mol% Chol)		
PC:DCP	13.4±0.7	10.3±0.1
SM:DCP	0.6±0.1	0.3±0.1
DMPC:DCP	1.2±0.2	0.2±0.1
DPPC:DCP	2.7±0.3	0.1±0.1
DSPC:DCP	13.0±0.2	0.2±0.1
(B) Chol-poor liposomes (i.e. 20 mol% Chol)		
PC:Chol:DCP	1.4±0.3	4.1±1.0
SM:Chol:DCP	0.6±0.1	< 0.1
DMPC:Chol:DCP	1.1±0.3	< 0.1

TABLE II

Interaction of negatively charged saturated and unsaturated phospholipid liposomes with splenic phagocytes in the absence and presence of serum

Liposome composition	% of initial ^{125}I-PVP	
	control	serum
(A) Chol-free liposomes (7:1, mole ratio)		
PC:DCP	6.5±0.2	3.4±0.1
SM:DCP	1.7±0.6	1.4±0.3
DPPC:DCP	0.9±0.1	1.0±0.1
(B) Chol-poor liposomes (7:2:1, mole ratio)		
PC:Chol:DCP	2.0±0.2	4.4±0.8
SM:Chol:DCP	0.2±0.1	0.6±0.1
DPPC:Chol:DCP	0.4±0.1	0.9±0.1
(C) Chol-rich liposomes (7:7:1, mole ratio)		
PC:Chol:DCP	1.5±0.2	13.1±0.1
SM:Chol:DCP	0.3±0.1	2.2±0.2
DPPC:Chol:DCP	0.6±0.1	3.9±0.1

white cells were prepared from male CFY rats and incubated with liposomes at 37°C in the presence and absence of serum, as described earlier [11]. For the reasons described elsewhere [12], each incubation was performed in triplicate and each experiment was repeated three times and the results of a typical experiment expressing the uptake of ^{125}I-PVP or [^{14}C]inulin radioactivity are presented as means ± S.E. The amount of liposomes adsorbed on the surface of the cells was measured at 4°C and by trypsinisation of the cells incubated at 37°C in the presence of liposomes and serum, as described earlier [11].

The results in Table I show that in the absence of serum liposomes prepared from sphingomyelin (SM) interact poorly with liver cells, while substitution of SM with egg phosphatidylcholine (PC) considerably increases their interaction with these cells, at 37°C. In the absence of serum the interaction of saturated phospholipid liposomes with liver cells increases with an increase in the chain length of phospholipid used. The inclusion of 25% serum in the incubation reduces the uptake of all preparations of liposomes by liver cells and this effect of serum is much more pronounced with the saturated than unsaturated phospholipid (Table IA). Incorporation of 20 mol% cholesterol in SM, DMPC and PC liposomes does not make a significant difference to the interaction of SM and DMPC liposomes with liver cells in the absence of serum (Table IB), but serum reduces the uptake of SM and DMPC to less than 0.1%, whereas it enhances the uptake of PC liposomes by 3-fold as compared to that in the presence of serum.

Splenic phagocytic cells respond differently to liver cells in handling SM and DPPC liposomes in the presence of serum (see Table II). For example, the uptake of the entrapped ^{125}I-PVP from SM and DPPC liposomes is very similar in the presence and absence of serum. Incorporation of cholesterol into these liposomes reduces their uptake in the absence of serum. Serum enhances the uptake of cholesterol-containing liposomes, particularly that of cholesterol-rich liposomes. For example, inclusion of 20 mol% cholesterol into SM, and DPPC liposomes enhances their uptake by 2- to

TABLE III

Interaction of double-labelled saturated and unsaturated phospholipid liposomes with liver and spleen phagocytes in the absence and and presence of

Liposome composition	% of initial radioactivity			
	control		serum	
	[^{3}H]cholesterol	[^{14}C]inulin	[^{3}H]cholesterol	[^{14}C]inulin
(A) Liver cells (7:2:1, mole ratio)				
PC:Chol:DCP	14.2±0.7	4.2±0.2	8.6±0.8	7.3±1.0
SM:Chol:DCP	0.7±0.4	0.2±0.02	0.3±0.1	< 0.1
DMPC:Chol:DCP	2.7±0.1	1.1±0.2	1.3±0.1	1.3±0.1
(B) Spleen cells (7:7:1, mole ratio)				
PC:Chol:DCP	8.5±0.5	2.0±0.2	13.6±0.41	11.7±0.3
DMPC:Chol:DCP	5.5±0.1	0.5±0.1	6.9±0.1	7.2±0.2

3-fold but 46.6 mol% cholesterol enhances their uptake by 6- to 7-fold as compared to their uptake in the absence of serum (see Table II).

The possible mechanism of interaction of liposomes with liver and spleen cells was examined by using double-labelled liposomes with [^{3}H]cholesterol as a membrane marker and [^{14}C]inulin as an aqueous marker. The amount of the lipid marker [^{3}H]cholesterol associated with the cells in the absence of serum is greater than that of an aqueous marker [^{14}C]inulin entrapped in DMPC and PC liposomes (Table III). This suggests that a few intact liposomes are absorbed on the surface of liposomes but a large amount of [^{3}H]cholesterol is exchanged with plasma membrane [15], whereas in the presence of serum, the mechanism of interaction is different since the ratio of the two markers is close to 1, indicating that in the presence of serum, [^{3}H]cholesterol exchanged is minimal and most of the intact liposomes are associated with the cells, with serum components making a bridge between liposomes and the cell.

In vitro, in the absence of serum, liposomes may be taken up by hepatic and splenic phagocytic cells either by fusion or by endocytosis [13,15]. Both of these mechanisms involve direct interaction of the liposomal membrane with the plasma membrane of the cells. However, in the presence of serum the mode of interaction of liposomes with the cells is different since their interaction will be mediated via serum components associated on the surface of liposomes (see Table III). The serum components may be opsonins which may stimulate or dysopsonins which will suppress phagocytosis of liposomes by liver and spleen macrophages. Binding of the opsonins onto the liposome surface may depend on the chemical and physical properties of liposomes and these properties can be manipulated by selecting the lipid composition and phospholipids of the vesicles [16]. For example the opsonin(s) specific for liver phagocytic cells have a poor affinity for cholesterol-rich liposomes, probably due to the rigidity of these liposomal membrane [11]. Sphingomyelin (SM) liposomes are less fluid than PC liposomes at the incubation temperature of 37°C [16], and this may explain the fact that no enhancement in the uptake of SM liposomes is observed in the presence of serum, since the liver-specific opsonins may not associated with the less fluid SM liposomes. Perhaps other serum components which may act as dysopsonins may absorb on the surface of such liposomes and inhibit their uptake. Similarly, the liver-specific opsonins have no affinity for 'solid' saturated phospholipid liposomes, DMPC, DPPC and DSPC, since no enhancement in their uptake by liver cells is observed in the presence of serum. However, the rigidity of DPPC and DSPC liposomes can be reduced by incorporation of cholesterol into the preparation [17,18], but there is no evidence of serum stimulating the uptake of DPPC vesicles containing 46.6 mol% cholesterol by liver cells. This probably suggests that the fluidity of the membrane may not be the only factor that regulates the binding of the liver-specific opsonin onto the liposomal surface. Properties such as hydrophobicity of the phospholipid may also play an important role in attracting the right serum protein or other components.

The opsonin(s) for splenic phagocytic cells is different from that for Kupffer cells [11,12]. Our results show that spleen-specific opsonins like those of liver have no affinity to bind on the surface of SM or saturated phospholipid liposomes, perhaps due to the different properties of these phospholipids as compared to those of unsaturated PC liposomes, but unlike its effect on liver cells, serum does not inhibit the uptake of these vesicles by the spleen cells. This suggest that serum components which may act as dysopsonins and inhibit the uptake of SM and saturated phospholipid vesicles by liver cells have no effect on the uptake of these liposomes by splenic cells. However, serum enhances the uptake of cholesterol-containing liposomes, particularly cholesterol-rich (i.e., 46.6 mol% cholesterol content) liposomes, despite their phospholipid composition. This suggests that by increasing the hydrophobicity of the liposomal membrane by inclusion of cholesterol perhaps spleen-specific opsonin(s) can be attracted to these liposomes, resulting in enhancement in their uptake by spleen cells.

In conclusion, our results explain why the early attempts to show the opsonic effect by serum on SM and saturated phospholipid liposomes were not successful [8–10]. It appears that liver- and spleen-specific opsonins do not have an affinity for these liposomes and hence they are not readily phagocytosed by liver and spleen cells in the presence of serum. On the contrary, these liposomes attract some serum components which act as dysopsonins and inhibit their phagocytosis by liver cells. However, these dysopsonins [5] have no effect on the uptake of liposomes by spleen cells. We have characterised some of the properties of liver and spleen specific opsonins [12] and experiments are in progress to purify these opsonins.

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