Volume 233, number 1, 143–147 FEB 05925 June 1988

Tissue specific opsonins for phagocytic cells and their different affinity for cholesterol-rich liposomes

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Received 14 April 1988

In accordance with the finding of our in vivo experiments reported earlier [(1983) Biochim. Biophys. Acta 761, 142–157; (1986) Biochim. Biophys. Acta 888, 184–190], the results of in vitro experiments show that Kupffer cells avidly take up cholesterol-poor but not cholesterol-rich liposomes, whereas splenic phagocytic cells take up preferentially cholesterol-rich rather than cholesterol-poor liposomes in the presence of serum. Evidence presented here suggests that serum contains opsonins specific for hepatic and splenic phagocytic cells and these opsonins have different affinities for cholesterol-rich and cholesterol-poor liposomes.

Opsonin; Macrophage; Cholesterol; Liposome; (Liver, Spleen)

1. INTRODUCTION

Intravenously injected liposomes are rapidly sequestered by mononuclear phagocytes of the reticuloendothelial system and liver and spleen are the major organs involved in the clearance of liposomes from the circulation [1]. Inclusion of cholesterol in liposomes is known to increase their half-life in the bloodstream [2,3]. This is achieved by cholesterol playing a dual role [4], firstly, it stabilizes the liposomal membrane and makes it less susceptible to destruction by various serum components [5,6]. Secondly it makes liposomes unfavourable for phagocytosis by hepatic Kupffer cells [4,7,8]. The reason why Kupffer cells have a poor affinity for cholesterol-rich liposomes is not yet known. Cholesterol influences the fluidity of liposomal membranes and binding of serum opsonin(s) on liposomes [9–11] and may perhaps affect the uptake of liposomes by these cells.

Furthermore, we reported earlier [7] that liver and spleen respond differently to cholesterol-free and cholesterol-rich liposomes when injected to produce a reticuloendothelial blockade but the mechanism by which these organs handle the two types of liposomes differently is not known. Hence to investigate the possible mechanism responsible for the suppression of cholesterol-rich liposome uptake in liver and factors which stimulate a differential response in hepatic and splenic phagocytic activity, we have studied the uptake of liposomes in the absence and presence of rat serum by using the isolated hepatic and splenic cells in suspension.

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2. MATERIALS AND METHODS

Negatively charged multilamellar liposomes containing ^{125}I-labelled poly(vinylpyrrolidone) were prepared as described earlier [4,7] from a mixture of phosphatidylcholine/cholesterol/dicetylphosphate in a molar ratio 7:7:1 for cholesterol-rich, 7:2:1 for cholesterol-poor and 7:0:1 for cholesterol-free liposomes, respectively. Thus the cholesterol content of cholesterol-rich liposomes was 46.6 mol%, for cholesterol-poor 20 mol% and for cholesterol-free 0 mol%.

Cholesterol and dicetylphosphate were purchased from Sigma (England), phosphatidylcholine from Lipid Products (England) and ^{125}I-labelled poly(vinylpyrrolidone) from Amersham. Liposomes (15 mg) were opsonised by incubation in 50% fresh rat serum (diluted with saline) for 15 min at 37°C, follow-

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00145793/88/$3.50

ed by centrifugation at 200000 × g for 30 min to pellet the opsonised liposomes. In some experiments the pellet was washed once with saline and centrifuged to obtain a liposome pellet as described above.

Hepatic non-parenchymal cells, containing predominantly endothelial and Kupffer cells, and splenic white cells were prepared from CFY rats of body weight 250 ± 25 g by methods described by Doolittle and Richter [12] and Kleiman et al. [13], respectively.

Splenic cells (≡ 8 × 10^7 cells) were incubated in polythene vials at 37°C for 1 h with liposomes (≡ 0.6 mg lipid) in the absence and presence of 25% fresh rat serum in a total volume adjusted to 2.0 ml with 10 mM oxygenated phosphate-saline buffer, pH 7.4 containing 5.0 mM glucose and 1% bovine serum albumin. The incubation media for hepatic cells (≡ × 10^7) was the same as that described for the splenic cells except that phosphate-saline buffer was replaced with Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution (Gibco). The uptake of ^{125}I-labelled poly(vinylpyrrolidone) radioactivity was measured in the cells at the end of the incubation [14]. Each incubation was performed in duplicate and each experiment was repeated more than twice and since opsonic activity varies from day to day and animal to animal the results of a typical experiment expressed as mean (of duplicate incubations) percentage of the total ^{125}I-radioactivity initially added to each incubation are presented. Since free ^{125}I-labelled poly(vinylpyrrolidone) is not degradable and not adsorbed onto or taken up by cells, the radioactivity associated with cells indicates the presence of intact liposomes [7,15]. In our experiments, the uptake of liposomes in the absence of serum was suppressed by 40–50% by substances known to inhibit endocytosis [16].

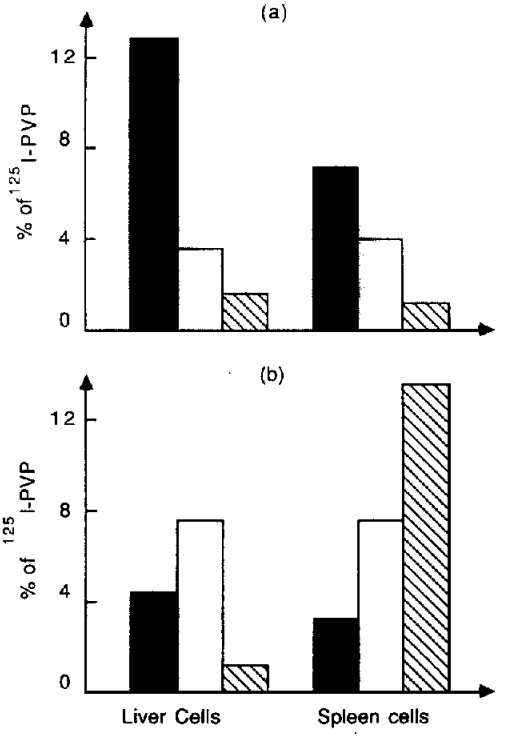

Fig.1. Uptake of liposomes by liver and spleen cells in the absence (a) and presence (b) of rat serum. Cholesterol-free (▬), cholesterol-poor (▭) and cholesterol-rich (▨). For experimental details see section 2.

3. RESULTS

The results in fig.1a show that hepatic and splenic cells take up cholesterol-free liposomes much more than cholesterol-containing liposomes in the absence of serum. Serum suppressed the uptake of cholesterol-free liposomes by both hepatic and splenic cells (fig.1b). This may be attributed to the fact that cholesterol-free liposomes are degraded in serum [2–6], as a consequence a smaller number of intact liposomes may be available to cells for phagocytosis which may apparently result in the poor uptake [4]. Among cholesterol-containing liposomes, cholesterol-poor are taken up much more by hepatic and splenic cells in the absence of serum than cholesterol-rich liposomes. Serum enhances the uptake of cholesterol-poor by hepatic cells but suppresses that of cholesterol-rich liposomes, in comparison to the results obtained in the absence of serum. In contrast, serum stimulates uptake of cholesterol-containing liposomes by splenic cells (fig.1a,b) but its opsonic effect is greater on the uptake of cholesterol-rich than cholesterol-poor liposomes, since cholesterol-rich liposomes are taken up twice as much as cholesterol-poor by splenic cells.

The negatively charged liposomes used here have been shown to be endocytosed rapidly by Kupffer cells [17], and endothelial cells present in our hepatic cell preparation do not take up liposomes [15]. Since the main mechanism of interaction of liposomes with Kupffer cells in vitro is adsorptive endocytosis [18], we examined the effect of cholesterol and serum on adsorption of liposomes at 4°C. At this temperature liposomes are predominantly adsorbed onto the cell surface and not phagocytosed [18]. It was found (see table 1) that the presence of cholesterol in liposomes reduces adsorption and serum further hinders this process for all liposomes in hepatic and splenic cells except the adsorption of cholesterol-rich liposomes on splenic cells which, in contrast, is enhanced. The total amount of all liposomes adsorbed on the cells did not constitute more than a

Table 1

Adsorption of liposomes onto liver and spleen cells at 4°C

	Adsorption (% of initial [^{125}I]-PVP radioactivity)[a]					
Liposomes:	Chol-free		Chol-poor		Chol-rich	
Serum:	−	+	−	+	−	+
Liver cells	4.50	3.80	0.90	0.27	0.16	0.19
Spleen cells	1.60	0.67	0.17	0.13	0.23	0.99

[a] Mean of duplicate incubations

For experimental details see section 2. These experiments were done twice and the results of a typical experiment are presented

few per cent of total liposomes taken up by the cells at 37°C, except in the case of cholesterol-free liposomes in hepatic cells where the results of absorption at 4°C and uptake at 37°C (fig.1b) in the presence of serum are similar. This makes it difficult to assess the role of serum on the uptake of cholesterol-free liposomes by these cells and hence in our subsequent studies cholesterol-free liposomes were omitted.

The effect of serum on uptake of liposomes is further confirmed when opsonised cholesterol-poor and cholesterol-rich liposomes were incubated with hepatic and splenic cells at 37°C. The results in table 2 show that opsonisation enhances uptake of cholesterol-poor but suppresses that of cholesterol-rich liposomes by liver cells. When opsonised cholesterol-poor vesicles were washed once with saline prior to the incubation with liver cells, the opsonic effect was lost.

On the other hand, although serum enhances uptake of both cholesterol-poor and cholesterol-rich liposomes in splenic cells, opsonisation of cholesterol-poor liposomes has no opsonic effect on their uptake by these cells. However, opsonised cholesterol-rich liposomes are taken up 2-fold more than unopsonised control liposomes, but this represents only 12% of the total liposomes taken up by splenic cells in the presence of serum. This small opsonic activity of the opsonised cholesterol-rich liposomes is not lost when liposomes were washed prior to the incubation with cells.

Electrophoretic studies of the opsonised cholesterol-poor and cholesterol-rich liposomes

Table 2

Uptake of opsonized liposomes by liver and spleen cells

Treatment	Uptake (% of initial [^{125}I]-PVP radioactivity)[a]	
	Liver cells	Spleen cells
Chol-poor liposomes		
Liposomes (control)	7.6	5.3
Liposomes + serum	22.7	9.1
Opsonized liposomes	15.4	5.3
Washed opsonized liposomes	7.2	ND
Chol-rich liposomes		
Liposomes (control)	5.0	1.6
Liposomes + serum	2.1	31.7
Opsonized liposomes	2.8	3.8
Washed opsonized liposomes	ND	3.1

[a] Mean of duplicate incubations

ND, not done. For experimental details see section 2. The whole experiment was repeated three times and only the results of a typical experiment are presented here

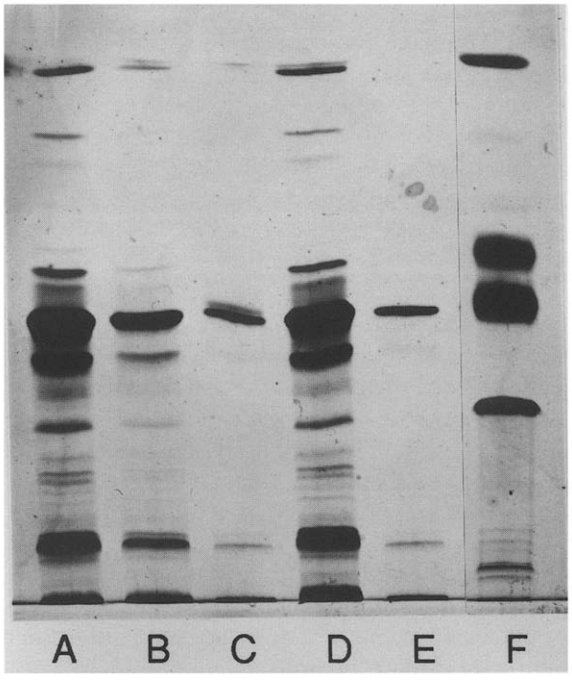

Fig.2. SDS-PAGE electrophoresis of opsonized cholesterol containing liposomes by Pharmacia Phast-Electrophoresis system on a 10–15% gradient gel. Direction of migration is from bottom to the top. A, Serum; B, opsonized cholesterol-rich liposomes; C, washed opsonized cholesterol-rich liposomes; D, opsonized cholesterol-poor liposomes; E, washed opsonized cholesterol-poor liposomes; F, standards (from bottom to top: phosphorylase *a*, bovine albumin, ovalbumin and cytochrome *c*).

show that more serum proteins are associated with cholesterol-poor liposomes than cholesterol-rich liposomes (fig.2). This may be attributed to the increased rigidity of the membrane due to a high concentration of cholesterol (≡46.6 mol%) in cholesterol-rich liposomes [9]. Washing opsonised liposomes with saline removes most of the serum proteins associated on both cholesterol-poor and cholesterol-rich opsonised liposomes and the proteins retained give very similar bands on gels in both preparations.

4. DISCUSSION

The results presented here confirm our earlier findings of in vivo studies [4,7] and show that Kupffer cells take up avidly cholesterol-poor but not cholesterol-rich liposomes, whereas splenic cells take up preferentially cholesterol-rich more than cholesterol-poor liposomes in the presence of serum. The experiments with opsonised liposomes (table 2) suggest that there are opsonins present in serum which are specific for liver and spleen phagocytes. Opsonin(s) specific for liver cells have no affinity for cholesterol-rich liposomes since no enhancement in uptake of these liposomes is observed either in the presence of serum (fig.1) or when liposomes are pre-opsonised with serum. This opsonin exerts its action probably on liver phagocytic cells by being adsorbed loosely on the surface of cholesterol-poor liposomes, since when the opsonised liposomes are washed prior to incubation with these cells, its opsonic effect is lost. Cholesterol (≡46.6 mol%) incorporated in cholesterol-rich liposomes decreases the fluidity of their membrane [19] and this could interfere with the interaction of liposomes with Kupffer cells, since it is known that the fluidity of membrane determines the ability of liposomes to interact with cells [11,20]. Our results also indicate that certain serum components hinder the absorption and eventually uptake of liposomes by Kupffer cells. However, the most likely explanation for the poor uptake of cholesterol-rich liposomes by Kupffer cells is that cholesterol has been shown to reduce the interaction of liposomes with serum proteins (see [10] and fig.2) and serum components [5,6], it is therefore not surprising that incorporation of 46.6 mol% cholesterol in these liposomes renders them less attractive for the interaction with liver specific opsonin and hence no stimulant for Kupffer cells.

On the other hand, the opsonin(s) specific for splenic cells stimulate phagocytosis of both preparations of cholesterol-containing liposomes but its effect is greater on the uptake of the rigid cholesterol-rich liposomes. However, unlike the opsonin specific for liver cells, this factor(s) does not exert its effect on splenic phagocytes by being adsorbed on the surface of liposomes, since no enhancement in uptake of the opsonised liposomes is observed (table 2). This probably suggests that, unlike in the case of liver, this opsonin may exert its effect by binding to the site other than the liposome-binding site on the spleen cell surface. However, since a small increase in uptake of the opsonised cholesterol-rich liposomes is found (table 2), it may indicate that more than one opsonin specific for spleen cells may be present in serum. Among these opsonins, one probably binds tightly to cholesterol-rich liposomes and enhances their uptake to a smaller extent than that observed in the presence of serum. It is also possible that this opsonin may also act on cells like lymphocytes in spleen cell preparations, which may be stimulated to take up liposomes [7].

The results discussed here probably explain why early attempts to show the opsonic effect of serum on phagocytosis of liposomes by Kupffer cells in vivo [21,22] and in vitro [18] were not successful. These investigators used cholesterol-rich liposomes (≡50 mol% cholesterol content) and sometimes they used phospholipids other than phosphatidylcholine [19]. Our experimental data (manuscript in preparation) suggest that like cholesterol, certain phospholipids have a poor affinity for opsonin specific for liver and hence their uptake in liver and consequently distribution in the whole body is altered. The chemical nature of the opsonins specific for both liver and spleen cells is not known, but experiments are in progress to characterise some of their properties in order to evaluate their role in various diseases and their potential in targetting drug carriers to phagocytic cells of specific organs of the reticuloendothelial system.

Acknowledgements: We are grateful to Linda Readings for the preparation of this manuscript. Sadly this work was not supported by any grants.

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