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Dual role of liposomes as adjuvant and carrier for the production of sugar-specific antibodies

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Abstract. Aminophenyl derivatives of monosaccharides were coupled to the surface of phosphatidylethanolamine containing liposomes. The glycosylated liposomes elicited in rabbits a hapten-specific immune response. The magnitude of antibody formation was nearly equivalent in the presence and the absence of Freund's adjuvant. The antibody response mediated through liposomes was better than that obtained through the protein carrier.

Keywords. Liposomes; immunological carrier; adjuvant; carbohydrates; antibodies.

1. Introduction

The use of liposomes (phospholipid vesicles) in biology and medicine is a promising area of research. An interesting application of liposomes is their use as immunological adjuvant that has been noted in the case of various proteins and viral antigens (Allison and Gregoriadis 1974; Van Rooijen and Van Nieuwmegen 1979; Gerlier *et al* 1980). Liposomes themselves being bio-degradable and poorly immunogenic offer an excellent alternative for mycobacteria in mineral oil suspensions of Freund's adjuvant. The role of liposomes in the anti-carbohydrate immune response has not been studied, and so this project was initiated. Our work has shown that liposomes can act as immunological carriers for the production of antigalactosyl, antimannosyl and anti-N-acetylglucosaminyl antisera (Das *et al* 1982a, b). We have also demonstrated that apart from acting as carriers liposomes also exhibit the adjuvant effect (Sarkar *et al* 1982).

2. Materials and methods

Egg lecithin, cholesterol and phosphatidylethanolamine were purchased from the CSIR Center for Biochemicals, New Delhi, bovine serum albumin (BSA), ovalbumin (OA), various *p*-aminophenylated sugars were obtained from the Sigma Chemical Co., USA. *p*-aminophenylated derivatives of sugars were coupled to the surface of negatively charged, multilamellar liposomes and also to BSA and OA according to previous procedures (Das *et al* 1982; Sarkar *et al* 1982).

2.1 *Preparation of antisera*

This was done as follows (Sarkar *et al* 1982):

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2.1a *Immunization with galactosylated liposomes in* CFA: Galactosylated liposome suspensions (30 mg lipid/ml and 6 mg sugar/ml) were emulsified with an equal volume of complete Freund's adjuvant (CFA) and an aliquot containing 2 ml of the emulsion was injected intradermally into rabbits. Three injections were given to each at 10-day intervals of which the last two were given in incomplete Freund's adjuvant.

2.1b *Immunization with galactosylated liposomes*: One ml of a galactosylated liposome suspension (30 mg lipid/ml and 6 mg sugar/ml) was used for immunization. The schedule was maintained as in §2.1a, in this case, however, the animals did not receive Freund's adjuvant at any stage of immunization.

2.1c *Immunization with protein carrier in* CFA: *p*-aminophenyl-β-D-galactoside-BSA (50 mol of sugar/mol of protein) conjugate was emulsified with CFA. A dose of 1 mg conjugate per rabbit was used for each injection.

In each case, seven days after the third injection, the animals were bled by cardiac puncture and the antisera were decomplemented at 56°C for 30 min and stored at −20°C.

2.2 *Antibody titers*

The radioactive antigen binding assay (Sparks *et al* 1980), complement mediated lysis of antigen associated liposomes (Six *et al* 1974) and agglutination of galactosylated liposomes (Hamers *et al* 1978) were carried out to estimate the anti-sugar antibody response in the sera.

3. Results and discussion

Glycosylated liposomes when injected into rabbits with CFA produced anti-sugar antibodies as checked against the glycosylated BSA conjugates by immunodiffusion analysis. The adjuvant property of liposomes was tested by immunizing the liposome preparation *e.g.* galactosylated liposomes without Freund's adjuvant. It also produced anti-galactosyl antiserum. The antisera raised in the presence and the absence of CFA were tested for their ability to precipitate Gal_{50}-BSA and Gal_{14}-BSA conjugates (figure 1). The antiserum raised without CFA precipitated these synthetic conjugates to a slightly lesser degree than that of the serum raised with CFA.

Various ligands were tried to inhibit the precipitation of Gal_{50}-BSA conjugate with anti-galactosyl antiserum raised against the galactosylated liposomes without CFA (table 1). The results have been compared with the antiserum raised in presence of CFA. Maximum inhibition was obtained with *p*-aminophenyl-β-D-galactopyranoside in both the sera. Methyl-β-D-galactopyranoside, D-galactose and lactose are all shown to be good inhibitors. The specificity of the serum for D-galactose is shown by the fact that D-glucose and methyl-α-D-mannopyranoside do not inhibit the precipitation at all. The inability of melibiose in contrast to lactose to inhibit the precipitation suggests the preference of a β-anomeric linkage for the antiserum. The contribution of the aromatic aglycone moiety to the specificity is shown with *p*-aminophenyl-α-D-mannopyranoside. It should be noted here that the antisera raised against saccharide haptens by conjugation as the aromatic derivative to BSA or keyhole limpet hemocyanin are also known to have antibody population specific for the aglycone part (Ebisu *et al* 1978).

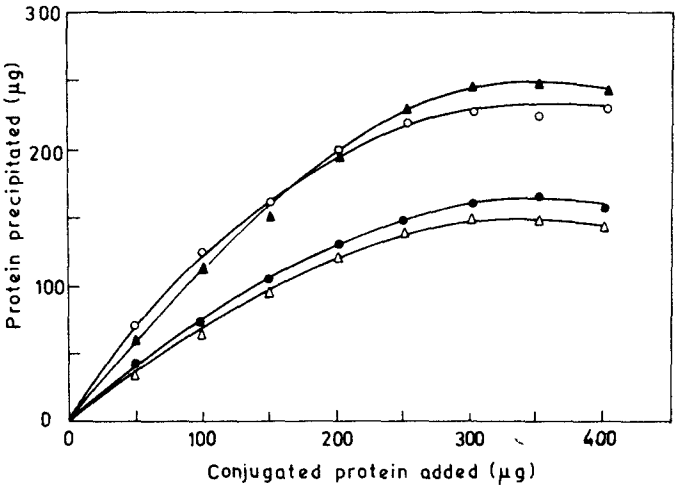

Figure 1. Quantitative precipitation curves of the anti-galactosyl antisera raised through galactosylated liposomes in the presence and the absence of CFA with synthetic conjugates (BSA-galactosides). Gal_{50}-BSA with the antisera obtained in presence (—○—) and in absence (—▲—) of CFA. Gal_{14}-BSA antiserum obtained in presence (—●—) and absence(—△—) of CFA.

Table 1. Percent inhibition of antigalactosyl antisera precipitation with Gal_{50}-BSA.

Ligands (50 mm)	Antisera (no. 1) raised through galactosylated liposomes + CFA	Antisera (no. 1) raised only through galactosylated liposomes
p-Aminophenyl-β-D-galactopyranoside	32	43
p-Nitrophenyl-β-D-galactopyranoside	30	33
p-Aminophenyl-α-D-mannopyranoside	20	27
Methyl-α-D-mannopyranoside	0	0
Methyl-β-D-galactopyranoside	20	31
D-galactose	16	20
Lactose	12	23
Melibiose	0	0
D-glucose	0	0

To compare the adjuvant role of liposomes, anti-galactosyl antiserum was also raised by immunizing rabbits with galactoside-BSA emulsified in CFA. From the complement-mediated lysis (table 2) it can be seen that the antiserum raised using galactosylated liposome without CFA was able to lyse nearly to the same level as that of the serum raised with CFA. But the antiserum raised conventionally *i.e.* galactoside-BSA in CFA does not lyse very well. From the modified Farr assay (table 3), galactosylated liposomes in the presence of CFA are found to give the best immune response followed by galactosylated liposomes in the absence of CFA, and galactoside-BSA in CFA. Similar results are obtained from the agglutination data (figure 2). Rabbit antiserum to galactosylated liposomes

Table 2. Complement mediated lysis of antigen associated liposomes by antigalactosyl antisera.

Immunizing material	Liposomes	Guinea pig complement	Antiserum	Antiserum no.	Percentage lysis
	(μl)	(μl)	(μl)		
Galactosylated liposomes + CFA	20	25	25	I	80
				II	75
				III	76
				IV	70
Galactosylated lipsomes	20	25	25	I	78
				II	75
				III	76
				IV	70
				V	73
BSA-galactoside + CFA	20	25	25	I	55
				II	60
				III	58
				IV	50
				V	42

Table 3. Radioactive antigen binding assay.

Immunizing material	Antisera No.	Percentage ^{125}I Gal_{14}-BSA bound	Percentage ^{125}I Gal_{20}-OA bound
Galactosylated liposome + CFA	I	81	60
	II	75	58
	III	78	55
	IV	70	50
Galactosylated liposome	I	78	45
	II	70	43
	III	71	46
	IV	65	40
	V	73	44
BSA-galactoside + CFA	I	—	40
	II	—	45
	III	—	42
	IV	—	38
	V	—	41

raised in the absence of CFA agglutinates slightly less than that of the antiserum raised in the presence of CFA, but better than that of the serum raised using BSA as the carrier.

Our results demonstrate the adjuvant property of liposomes in eliciting an antigalactosyl immune response. This is the first report showing the production of anti-saccharide antiboides using liposomes as both adjuvant and carrier. Glycosylated liposomes themselves could function as adjuvant, substituting the need for CFA. Previous methods require proteins as the immunological carrier (Landsteiner 1945).

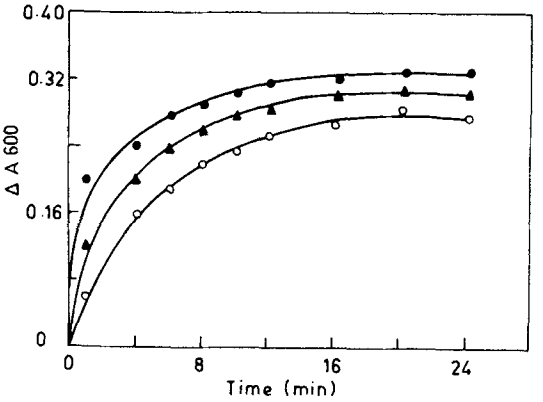

Figure 2. Agglutination of 10 μl galactosylated liposomes with 10 μl antisera at 600 nm. Antigalactosyl antisera raised through liposomes with CFA (—●—) and without CFA (—▲—), and the serum raised against Gal_{50}-BSA with CFA (—○—).

The most obvious advantages of our method are the easy bio-degradability, and the low immunogenicity of liposomes compared to that of proteins thereby eliminating the need to remove carrier specific antibodies. Another factor to be noted is that the glycosylated liposome preparation should be a more homogeneous immunogen with respect to the glycosylated protein considering the differences in the microenvironments of overall protein structure. The effect of a homogeneous immunogen on the pattern of antibodies produced is to be studied.

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