J. Biosci., Vol. 6, Number 4, October 1984, pp. 491–502.

Liposomes in immunology

NAHID LATIF and BIMAL KUMAR BACHHAWAT
Department of Enzyme Engineering, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Calcutta 700032, India

MS received 18 July 1984

Abstract. Liposomes, the artificial phospholipid vesicles, have the capacity of entrapping water soluble substances in their aqueous compartments. Of the many possible potentials of liposomes their application in immunology is most significant. Recent studies have shown an adjuvant and a carrier effect of liposomes to a number of antigens. Liposomes used in these studies are generally multilamellar vesicles with the antigen encapsulated in the aqueous phase. Some antigens may also be associated with the lipid lamellae covalently or noncovalently. The adjuvant property of liposomes is greatly affected by the surface charge of the vesicle as well as the site of association of the antigen. The other factors which may have a role in immunopotentiation by liposomes are the size and structure of the vesicles, the lipid composition, route of administration and their surface sugars. In addition, liposomes may function as carriers to haptens and other antigens. In association with liposomes the nature of the immune response may be modulated. For a further enhancement of the adjuvant activity of liposomes use has been made of immunomodulators.

Keywords. Liposomes; adjuvant; protein antigens.

Introduction

Liposomes are artificial vesicles comprised of lipid and aqueous compartments where the lipid exists in the bilayer form. Such vesicles can be composed solely of phospholipids or in combination with other amphipathic molecules such as sterols, long chain organic bases or acids. When phospholipids are suspended in an excess of aqueous solution they spontaneously form multilamellar concentric bilayers with lipid layers separated by layers of aqueous medium. Water soluble substances such as drugs, proteins, nucleic acids and dyes, present in the aqueous phase during the formation of liposomes, can be encapsulated into the aqueous compartments of the vesicles. This unique property of liposomes has made them a versatile tool for an increasing number of studies in biology and medicine.

The earliest suggestion of a therapeutic potential for liposomes was for its possible application as carriers of enzymes and drugs *in vivo* in the therapy of various metabolic and physiological disorders (Gregoriadis *et al.,* 1971). The concept behind the use of

Abbreviations used: CFA, Complete Freund's adjuvant; IFA, incomplete Freund's adjuvant; MLV, multilamellar vesicle; T_c, temperature; SPDP, succinimydl β-(2-pyridyldithiopropionate); BSA, bovine serum albumin; GCSA, gross cell surface antigen; ULV, unilamellar vesicles; DPPC, dipalmitoyl phosphatidyl-choline; DSPC, distearyl phosphatidylcholine; MDP, muramyl dipeptide; MAF, macrophage-activating factor.

liposomes as carriers of drugs and macromolecules was mainly related to an expected protection of the encapsulated molecules in the blood stream. On the contrary, it was found that liposome encapsulation of antigens resulted in elevated antibody titres in comparison to the free antigen (Allison and Gregoriadis, 1974). This interesting observation opened the field for the study of the immunopotentiating properties of liposomes. The common adjuvants used for experimental immunization in laboratory animals are Freund's complete and incomplete adjuvants (CFA and IFA). They are composed of a water-in-oil emulsion with or without heat killed mycobacteria. Though these adjuvants evoke high level and long lasting immunity they are not suitable for use in humans since they cause the formation of granulomas at the site of injection. Other adjuvants such as alum, aluminium hydroxide and calcium chloride used in humans are far from ideal. Hence, a need is felt for a safe and effective adjuvant for use in human immunization. Liposomes have a decided advantage over the above mentioned adjuvants since they are prepared from biodegradable phospholipids which do not produce granulomas (Allison and Gregoriadis, 1976). Further, liposomes protect the entrapped antigens from the hypersensitivity reactions (Gregoriadis and Allison, 1974). The adjuvant and carrier property of liposomes to a number of antigens has been established in recent years (van Rooijen and van Nieuwmegen, 1981; Alving and Richards, 1983). Considering the potential of liposomes in immunopotentiation it is likely that their application in this field may be realized first.

In addition to the development of liposomes as immunopotentiators, the vesicles have been used earlier in investigating a wide range of immunological events. These include lysis of foreign cells as a consequence of membrane damage and the ability of membrane sensitized haptenic antigens to induce formation of antibodies and/or cytotoxic effector cells. In the present review attention will be directed to the adjuvant properties of liposomes with respect to protein antigens and as carriers of haptens.

Preparation of liposomes

Liposomes generally used in the immunological studies are multilamellar in structure. The ease of encapsulation of proteins and haptenic groups and the variety of lipid compositions used in the preparation of multilamellar vesicles (MLVs) makes them the choice for these studies. The method described by Bangham *et al.* (1974) for the preparation of MLVs is exceptionally simple. The lipid mixture taken in organic solvents is allowed to deposit upon the walls of a round bottomed flask by rotary evaporation under reduced pressure. The thin film of lipids is dispersed in aqueous buffer at a temperature above the transition temperature (T_c) of the lipid or above the T_c of the highest melting component in the mixture.

A higher percentage of encapsulated aqueous phase per mol of lipid may be achieved by a long hydration and gentle shaking than a faster and vigorous preparation. The entrapped volume of the MLVs can be further increased by including charged lipids in the bilayer. The main disadvantages of MLVs are the heterogenous population of vesicles and relatively low ratio of internal aqueous space per total lipid. A more uniform preparation can be obtained by passing the vesicles through polycarbonate membrane (Olson *et al.,* 1979) or by extrusion through a French Press (Barenholz *et al.,* 1979).

Association of materials with liposomes

The original and still most commonly used mode of association of materials with liposomes is the aqueous phase entrapment of water soluble molecules. However, macromolecules such as proteins during entrapment in the aqueous phase may bind to the surface of liposomes (Tyrrell *et al.,* 1976). This is similar to membrane-protein interaction which may be electrostatic or hydrophobic in nature. Such a preparation results in antigens exposed on the surface of liposomes. For a specific surface expression of antigens, preformed liposomes are incubated with the protein (Raphael and Tom, 1984). For an incorporation of the materials in the lipid bilayers the nonpolar molecules are added to and dried down with the lipid phase in organic solvent (Bangham *et al.,* 1974). The higher the solubility of the molecules in the non-polar solvent the greater the amount which can be sequestered in the hydrophobic regions of the liposomes. A more stable surface expression of antigens is obtained by covalent coupling of these molecules to preformed liposomes. Four general methods have been described in literature for the coupling of proteins to the surface of liposomes.

Periodate oxidation of visinal cis-hydroxyl groups of oligosaccharide chains of glycoproteins followed by reduction of Schiff's bases formed between the amino groups of the phospholipids of the liposomes

For example, horse raddish peroxidase has been covalently coupled to preformed liposomes containing phosphatidyl ethanolamine, stearylamine or phosphatidyl serine (Heath *et al.,* 1980). The major disadvantage of this method is that it can be applied for coupling of only those proteins having available oligosaccharide residues for the generation of aldehydes. However, in recent years a number of novel methods have been developed for glycosylation of proteins. This method may then be applied for the glycosylated proteins.

Periodate oxidation of the oligosaccharide chains or glycosphingolipids of liposomes followed by reduction of the Schiff's bases formed between the amino groups of the proteins and aldehyde groups of the lipid

In this way proteins such as immunoglobulins and $F(ab')_2$ fragments have been covalently coupled to vesicles (Heath *et al.,* 1981). This method, too, has a disadvantage as it necessitates the presence of glycosphingolipids in the liposome membrane.

Homobifunctional cross linking between the amino groups of phospholipids and proteins mediated by glutaraldehyde, dimethyl suberimidate or carbodiimide

Torchilin *et al.* (1978) used these coupling agents and a stable immobilization of α-chymotrypsin on the surface of liposomes was achieved. However, utilization of homobifunctional coupling agents might lead to the formation of homopolymers of vesicles, protein or both.

Chemical cross linking using heterobifunctional agents

In order to minimize homopolymerization and intramolecular cross linking the use of heterobifunctional agents has been suggested. An example of such a reagent is N-

hydroxyl-succinimydl *β*-(2-pyridyldithiopropionate) (SPDP) for covalent coupling of antibody and *S. aureas* protein A to liposomes (Laserman *et al.,* 1980). For coupling of protein to liposomes SPDP is first reacted with phosphatidylethanolamine and the stable derivative PE-DTP is used for the formation of the liposomes. Subsequently, proteins containing activated thiol, groups are coupled to the liposomes. A similar method, introduced by Martin and Paphadjopoulos (1982) involves the formation of a phospholipid derivative N-[4-(*p*-maleimidophenyl) butyryl] phosphatidyl ethanolamine. The maleimide moiety incorporated in liposomes is reacted with the sulphydryl groups of *F*(*ab'*) fragments leading to an efficient and stable protein-vesicle linkage. Recently a modified method involving thiolation of antigen and coupling to maleimide moiety of preformed liposomes has been described for proteins lacking sulphydryl groups (Shek and Heath, 1983).

Haptens such as lipids and peptides are incorporated in liposomes by covalently coupling them to phsophatidylethanolamine prior to liposome preparation. This method of liposome sensitization has been found to enhance the immuno-genicity of a wide variety of agents (Kinsky, 1980; van Houte *et al.,* 1981).

Adjuvant Properties of liposomes

The adjuvant effect of liposomes was first reported by Allison and Gregoriadis (1974) for the antigen, diptheria toxoid. Since then the immunopotentiating effect of liposomes has been the subject of an increasing number of studies. The immunogenicity of a wider variety of antigens for example proteins (van Rooijen and van Nieuwmegen, 1980b; Heath *et al.,* 1976), peptides (Liftshitz *et al.,* 1981), sugars (Das *et al.,* 1982a,b) and lipids (Alving, 1977) have been found to be significantly enhanced in association with liposomes.

The properties of the liposomes may be varied at will. For instance the vesicle size phospholipid composition, surface charge and lamellar structure may be altered as desired. As a consequence, the immunopotentiating capacity of the vesicle to the associated antigen also changes. A number of parameters affecting the adjuvant property of liposomes have been studied in the past decade which is discussed below.

The effect of surface charges of the vesicles

Liposomes may be neutral, negative or positvely charged depending on the lipid used in the liposome preparation. The effect of surface charge on the immunopotentiating activity of liposomes was first observed by Allison and Gregoriadis (1974). It was found that inoculation of diptheria toxoid in negatively charged liposomes elicited significantly higher antibody levels than when entrapped in neutral or positively charged liposomes. A later report showed that though negatively charged liposomes could act as adjuvants, positively charged liposomes too could do the same (Heath *et al.,* 1976). Similar results were obtained by van Rooijen and van Nieuwmegen (1980b) showing that positively charged and neutral liposomes have the same adjuvant activity as negatively charged liposomes. Still most of the workers studying the adjuvant properties of liposomes to antigens made use of negative liposomes. Recently the

effectiveness of positively charged liposomes in producing significant levels of antibodies has been demonstrated (Latif and Bachhawat, 1984a). The antibody titres obtained with lysozyme entrapped in positively charged liposomes has been found to be higher than neutral, negatively charged liposomes and even CFA. The high level of immunopotentiation by positive liposomes cannot be attributed to a higher affinity of the antigen for these liposomes since both carry the same charges. In contrast to previous reports that liposomes do not cause granuloma formation (Allison and Gregoriadis, 1976) it was found that positively charged liposomes, with or without antigen, lead to the formation of mild granulomas at the sites of injection. Though the exact mechanism of action of the positively charged liposomes is not understood, it is clear that these liposomes interact differently with cells *in vivo* in comparison to the neutral and negatively charged liposomes possibly eliciting a cell-mediated immunity, in addition. There is a possibility that the positive charge may hamper the fusion of the vesicles with the lysosomes thus allowing protection and prolonged exposure of the entrapped antigen to the immune system. Further studies on the interaction of liposomes with cells in culture may provide an insight into the mode of action of these vesicles. For the present, the profound immunopotentiating activity of positively charged liposomes is of considerable interest.

Table 1. Antibody response of rabbits to lysozyme

	Antibody titres in weeks**				
	1	2	3	4	5
Immunogen*	Primary response		Secondary response		
Lysozyme in saline†	ND	ND	4·67	ND	5·3
Lysozyme plus CFA†	ND	ND	213·3	ND	170·7
Lysozyme entrapped in neutral liposomes†	1	1·3	26·7	16	37·3
Lysozyme entrapped in negatively charged liposomes†	0·7	2	22·7	22·7	53·3
Lysozyme entrapped in positively charged liposomes†.	2·3	85·3	384	426·7	768
Lysozyme coupled to neutral liposomes‖.	4·3	17·3	37·3	26·7	64
Lysozyme coupled to neutral liposomes plus CFA‖.	2	192	512	853·3	1706·7
Lysozyme entrapped in β-Gal liposomes§	1.3	2	37.3	42.7	42.7
Lysozyme entrapped in α-Man liposomes§	1	1	3	4·7	12

* Subcutaneous injections of lysozyme administered at 0, 2 and 4 weeks.
** Sera assayed by passive haemagglutination and expressed as the reciprocal dilution of the end point. Each datum point represents the mean response of three animals.
† Latif and Bachhawat, 1984a.
§ Latif and Bachhawat, 1984b.
‖ Latif, 1984.
ND Not done.

The effect of the sites of association of the antigen with the liposome vesicles

As discussed earlier there are a number of ways by which proteins can be associated with liposomes and this depends upon the physicochemical properties of the proteins.

Water-soluble molecules may be entrapped within the aqueous compartments between the lipid lamellae whereas hydrophobic proteins may interact with the lipid bilayers (Tyrrell *et al.,* 1976). Further, amphipathic substances may be associated with the lipid bilayers; at the same time their hydrophilic tails may project on the surface of the liposomes (Fendler, 1980). Recent interest has been centred on the effect of the modes of association of antigen with liposomes on antibody production. Entrapment of a variety of antigens in liposomes has been found to induce enhanced humoral responses compared to similar proteins injected free in saline (Allison and Gregoriadis, 1974; van Rooijen and van Nieuwmegen, 1977; Hudson *et al.,* 1979). That a liposome protein association was a prerequisite for the adjuvant effect was evident when mixtures of free antigen and liposomes did not lead to any immune stimulation (Shek and Sabiston, 1982b). During encapsulation in liposomes some antigens for example serum proteins, may associate with the liposome surface membrane (Hoekstra and Scherphof, 1979). Conflicting reports have appeared regarding the significance of surface exposure (van Rooijen and van Nieuwmegen, 1980a) versus internal entrapment (Six *et al.,* 1980) of the antigen in enhancing the immunogenicity of liposome-associated antigens.

Van Rooijen and van Nieuwmegen (1977, 1978) reported comparable anti-body titres elicited by human serum albumin and bovine γ-globulin adsorbed on and entrapped within the liposomal vesicles. Subsequently it was postulated that only those ligands that are exposed on the liposome surface are immunogenic. However, substantial data to prove or disprove this hypothesis was lacking. On the contrary internalizing of a number of antigens in liposomes was reported to induce antibody production (Allison and Gregoriadis, 1974; Hudson *et al.,* 1979). The significance of surface adsorbed and entrapped antigens in mediating immune responses has been further investigated using bovine serum albumin (BSA) and another serum protein (Shek and Sabiston, 1982b). It has been found that antigens exposed on the surface of liposomes are immunogenic. At the same time trypsinization of liposome encapsulated antigens does not reduce or abolish the immunological response. Additional information regarding the immunogenicity of entrapped antigen has been obtained using lysozyme as an antigen (Latif, 1984). Lysozyme does not associate with the liposome surface and as such it leads to almost negligible antibody production. However, when entrapped in liposomes the antibody titre is highly significant.

Viral antigens are mostly membrane proteins and when incorporated in liposomes may associate with the aqueous compartments as well as the lipid membranes. Such a polypeptide derived from Hepatitis B surface antigen showed a high percentage of incorporation in liposomes (Sanchez *et al.,* 1980). When administered in guinea pigs higher antibody titres were obtained with liposome-associated antigen than the antigen administered in aluminium gel. Similarly, gross cell surface antigen (GCSA), extracted from syngenic (C58NT)D lymphoma cells and incorporated in liposomes showed a strong association with the lipid membranes and only 25 % of the total protein was trapped in the aqueous compartment. Immunization with liposome-associated GCSAa showed that the antigen present on the liposome surface could induce antibodies to GCSAa reaching in some instances the level obtained after immunization with viable syngenic tumour cells (Gerlier *et al.,* 1980). The immunogenicity of surface expressed antigens has been further demonstrated using human LS174T colon tumour cell membranes incorporated in vesicles (Raphael and Tom, 1984).

Proteins, as described earlier could be associated with the liposomes by covalent linkage with the vesicles. However, the effect of such an association on immunopotentiation by liposomes has not been extensively investigated. Recently this phenomenon for enhancing the immunogenicity of BSA has been employed by Shek and Heath (1983). BSA covalently linked to the surface of preformed unilamellar vesicle composed of phosphatidyl choline, cholesterol and N-(4-maleimidophenyl butyryl) phosphatidyl ethanolamine has been found to be immunogenic. A simpler method for coupling lysozyme to liposomes through phosphatidyl ethanolamine has been carried out earlier (Latif and Bachhawat, 1981). The surface-coupled antigen has been found to be significantly immunogenic. However, in association with another adjuvant, CFA coupled ligands are several fold more immunogenic than the native antigen in CFA (Latif, 1984). These studies demonstrate the potential of liposomes as carriers and adjuvants to protein antigens and may find application in vaccine preparation.

Effect of size and structure of liposomes

The size and structure of liposomes may be modulated as required. It is anticipated that these factors may affect the immunogenicity of liposome associated antigens. However, comparative studies of the immunogenicity of antigens associated with different preparations of liposomes are not available. Most of the immunological studies on liposomes have been carried out using MLVs. Recently the differences in multilamellar and unilamellar liposomes of comparable size in promoting antibody response to a protein antigen has been analyzed (Shek *et al.,* 1983). Results obtained with the negatively charged liposomes prepared from dimyristoyl-lecithin indicate that unilamellar vesicles (ULVs) are more effective than MLVs in promoting an immune response to the entrapped BSA. Though the exact mechanism responsible for the difference in the immunopotentiating capacity of the two liposome preparations remains to be established, it is postulated that the extent of BSA molecules embedded in the phospholipid bilayers of the two kinds of vesicles might have a role to play.

Effect of lipid composition

Most of the liposomal vesicles for immunological studies have been prepared from egg lecithin, cholesterol and a negatively charged phospholipid, diacetyl phosphate or phosphatidic acid. In order to introduce a positive charge stearylamine is used. The molar ratio of the lipids used in the preparation of liposomes which promotes effective antibody production (Allison and Gregoriadis, 1974) is usually 7:2:1 of egg leicthin, cholesterol and charged lipid, respectively. However, whether this ratio is indeed optimum for immune enhancement remains to be established.

Egg lecithin or phosphatidylcholine is the most important ingredient of liposomes used for adjuvant activity. It is bio-degradable and a harmless compound when administered as liposomes (van Rooijen and van Nieuwmegen, 1980b) although some exchange may occur with the phopholipid of cells (Gregoriadis *et al.,* 1977). The most important advantage of phosphatidylcholine liposomes as adjuvants is that, in contrast to phosphatidyl inositol, phosphatidyl glycerol and phosphatidic acid, phosphatidyl-

choline is a very poor antigen (Alving, 1977). Liposomes prepared with phosphatidylcholine by themselves, do not evoke an immune response in rabbits even when incorporated in IFA. However an immune response against phosphatidylcholine is induced when lipid A is incorporated in the liposomes (Shuster *et al.,* 1979).

Liposomes prepared from sphingomyelin have been reported to be more effective in eliciting an immune response to incorporated antigen than liposomes from phsophatidylcholine (Uemura *et al.,* 1974; Yasuda *et al.,* 1977). There is a striking difference in the T_c of these lipids (Phosphatidylcholine –8° to 15°, sphingomyelin 42°) and it is suggested that high immunological response by sphingomyelin liposomes may be due to their greater stability. In contrast to these observations van Rooijen and van Nieuwmegen (1980b) reported lower adjuvant activity of sphingomyelin liposomes than phosphatidylcholine liposomes to the associated protein antigen.

Liposomes composed of dipalmitoyl phosphatidyl-choline (DPPC) and distearyl phosphatidylcholine (DSPC) (T_c 41·4° and 54·9°, respectively) have also been reported to be more effective immunogens than those prepared from egg lecithin (Hudson, 1977). It is postulated that these liposomes may have greater bilayer stability at physiological temperature and may thus persist longer *in vivo* than egg lecithin. In contrast, it has been found that though DPPC and DSPC liposomes are strong immunopotentiators to the entrapped antigens liposomes prepared from egg lecithin are better adjuvants (Latif and Bachhawat, 1984a).

Effect of the route of administration

For potentiating an immunological response to liposome-associated antigens, animals may be immunized intravenously, intraperitoneally, intramuscularly or subcutaneously. A strong primary immune response is induced by intravenous administration of liposome-associated antigens (Allison and Gregoriadis, 1974; Heath *et al*.,1981). However, the response does not persist and may not show a secondary induction. In contrast, intramuscular and subcutaneous route elicit higher concentration of antibodies (Allison and Gregoriadis, 1974; Heath *et al.,* 1976) on secondary immunization. Intraperitoneal injections of liposome-associated antigens too, induce a substantial antibody production (Shek and Sabiston, 1982a,b).

After intravenous injection liposomes are removed from the circulation by reticuloendothelial cells and are rapidly degraded (Tyrrell *et al.,* 1976). Thus accumulation of liposomes in order to trigger a high antibody response does not take place. Still, significantly higher titres of anitbody were obtained after intravenous administration of liposome entrapped antigen that the free foreign protein (Allison and Gregoriadis, 1974; Hudson *et al.,* 1979). The adjuvant property of intravenously administered liposomes is evident from the observation that liposome carrier itself could activate phagocytic cells in the reticuloendothelial system (Hudson *et al.,* 1979). Significant stimulation of the immune system is observed when liposomes are injected subcutaneously intramuscularly or intraperitoneally. It is possible that liposomes injected by these routes will remain at the site of injection for a long period. Thus liposomes exert their adjuvant effect by retaining a ‘depot’ of an antigen at the site of injection (Tyrrell *et al.,* 1976). However, the adjuvant action is not entirely physical.

Some interaction between liposome and cells of the immune system has been reported (Hudson, 1977). The appearance of activated mononuclear phagocytes and increased specific activities of several lysosomal hydrolases in these cells by a single liposomal sensitization in mice (Hudson *et al.,* 1979) indicates that liposomes may affect the reticuloendothelial function directly by activating phagocytic cells.

The effect of sugars on the surface of liposomes

Most adjuvants exert their major effects in macrophages (Allison, 1979). However, the mechanism of cellular action of liposomes in stimulating antibody production remains to be established. It is clear that liposomes administered either intravenously, intraperitoneally or subcutaneously are sequestered by the organs of high reticulo-endothelial activity such as liver, spleen, lymph nodes (Tyrrell *et al.,* 1976). Thus they finally gain access to the phagocytic cells of the reticuloendothelial system which are responsible for the clearance of the liposomes. However, the participation of these cells *i.e.,* the macrophages, in potentiating the immune response to liposome-associated antigens remains unresolved. Recently it has been shown that macrophages are necessary for the induction of a humoral response to lipsome-associated protein antigens (Shek and Lukovich, 1982). Liposomes designed specially to interact with macrophages would facilitate a better immunopotentiation. It is well established that macrophages possess recognition sites for sugars such as mannose, galactose and fucose (Weir, 1980). Liposomes carrying terminal galactose and mannose residues are specifically recognized by lecithin-like molecules present on the plasma membranes of macrophages of parenchymal and nonparenchymal tissues (Ghosh and Bachhawat, 1980). A recent study of the immunological response of sugar grafted liposomes shows that antigen entrapped liposomes bearing galactose on the surface induce an immune response comparable to sugar-free neutral liposomes. However, the immune response by mannose-coupled liposomes is alsmost equal to that of the free antigen (Latif and Bachhawat, 1984b). Based on these results, it is postulated that subcutaneous administration of liposomes facilitates a receptor mediated uptake by peritoneal macrophages and macrophages derived from bone marrow by a recognition system specific for mannose. A greater accessibility of these liposomes to the phagocytic cells leads to a rapid degradation of the antigen and subsequently low immune response results.

Liposomes as carriers of antigens

In addition to their adjuvant effect, liposomes have been recognised as efficient carriers of antigens in recent years. Haptenic groups are generally inserted in liposomes by coupling them to one of the phospholipids used in the liposome preparation. With such liposomes, Kinsky (1978) investigated the immune response of haptens such as dinitrophenyl and phosphorylcholine. The most obvious advantages of the use of liposomes as carriers are the easy biodegradability and the low immunogenicity of liposomes compared to protein carriers thus eliminating the need to remove carrier

specific antibodies. These potentials of liposomes attracted extensive research in the production of antibodies to a number of haptens.

This approach for obtaining specific antibodies has been extended to molecules of clinical interest such as hormones, drugs, vitamins etc. For example, anti-L-thyroxine antiserum has been produced with liposomes sensitized with L-thyroxine derivative of phosphatidylethanolamine (Tan *et al.,* 1981). In addition, extensive studies on the immunogenic properties of haptenated liposomes have been carried out using tripeptide enlarged haptens such as 3-(*p*-azobenzene arsonate)-N-acetyI-L-tyrosylglycylglycine and N-(2,4-dinitrophenyl)-*β*-alanylglycylglycine (van Houte *et al.,* 1981). Wood and Kabat (1981) showed the production of antiglycolipid antibodies and Das *et al.* (1982a,b, 1984) report the production of antibodies against sugars such as mannose, galactose and N-acetyl-D-glucosamine using liposomes as carriers. The carrier effect of liposomes to protein antigens has also been reported (Latif, 1984).

Nature of the immune response mediated *by* liposomal antigens

For a better understanding of the mechanism of adjuvant action of liposomes to associated antigens and for their applicability in vaccines, information regarding the kind of immunoglobulin produced by liposomal antigen is required. Protein antigens are generally *T*-dependent with respect to eliciting antibody production and in association with liposomes their humoral response is not altered (Shek and Sabiston, 1982a; Latif, 1984). However, these reports are at variance with the study of van Rooijen and van Nieuwmegen (1983) who found IgM antibodies produced during primary immunization of BSA associated with liposomes. BSA molecules are associated with the liposome surface and it is possible that the surface-association is responsible for the observed IgM response. Haptens such as tripeptides (van Houte *et al*., 1981) and sugars such as N-acetyl-D-glucosamine (Das *et al.,* 1984) and galactose (Sarkar and Das, 1984) result in the formation of 1 gM type of antibodies. Though this difference in the kind of antibody produced cannot be resolved at present it may be possible that the type of antigen has a significant role to play.

Liposomes as carriers of additional immunomodulators

The immunoadjuvant activity of liposomes to associated antigens may be enhanced further by the incorporation of immunomodulators. Studies carried out with endotoxin and lipid A show an enhanced adjuvant effect to the antigen in association with liposomes (van Rooijen and van Nieuwmegen, 1980c; Dancey *et al.,* 1977). However, further increase in the adjuvant activity is observed when the antigen and the immunomodulator are incorporated in the same liposomes.

Another compound possessing immune potentiating activity is N-acetylmuramyl-L-alanyl-D-isoglutamine [muramyl dipeptide (MDP)] the minimal structural unit that can replace the immunoadjuvant activity of mycobacteria in CFA. Encapsulation of MDP in liposomes enhances its effectiveness in producing an immune response against antigen compared to the unencapsulated MDP (Chedid *et al.,* 1979). In addition, encapsulation of M D P in liposomes reduces the amount of drug needed for protection

against infections (Fraser-Smith *et al.,* 1983). Liposome-encapsulated MDP has been found to activate macrophages more efficiently *in vitro* than free MDP (Sone and Fidler, 1981). This property to activate macrophages has been further extended *in vivo* for the destruction of spontaneous lymph mode and visceral metastasis (Fidler *et al.,* 1981). A more specific mediator involved in the activation of normal noncytotoxic cells of the macrophage-histocyte series to become cytotoxic against tumour cells is the soluble lymphokine referred to as macrophage-activating factor (MAF) (Churchill *et al.,* 1975). However, the *in vivo* administration of MAF to bring about systemic activation of macrophages has not been accomplished. Lymphokines have a short life and after injection into circulation they rapidly bind to serum proteins. Moreover, preparations of MAF are antigenic and therefore their repeated injections may not be possible. Encapsulation of MAF in liposomes prevents these undesirable side effects at the same time induces an efficient macrophage activation (Kleinerman *et al.,* 1983).

Concluding remarks

The recent development in liposome research demonstrate the promising role of liposomes in immunology, in particular, as immunopotentiators and carriers of antigens. The major factors affecting the immunoadjuvant action of liposomes appear to be the charge of the vesicles and the site of association of the antigen with the lipid bilayer. The presence of sugar ligands on the surface of liposomes, further seem to regulate the production of antibodies to the associated antigens. However, for a clear understanding of the mechanism of action of liposomes further investigations are required. Still, the potential value of liposomes as immunological adjuvants has emerged as one of the brightest prospects among the many projected clinical applications of liposomes.

References

Allison, A. C. (1979) *J. Reticuloendoi Soc.,* **26,** 610.

Allison, A. C. and Gregoriadis, G. (1974) *Nature* (*London*)*,* **252,** 252.

Allison, A. C. and Gregoriadis, G. (1976) in *Recent results in cancer research,* (eds. G. Mathe, I. Florintin and M. C. Simmler) (Heideberg: Springer Verlag), p. 58.

Alving, C. R. (1977) in *The antigens,* (ed. M. Sela) (New York: Academic Press), Vol. 4, p. 1.

Alving, C. R. and Richards, R. L. (1983) in *Liposomes,* (ed. M. Ostro) (New York: Marcel Dekker Inc.). p. 209.

Bangham, A. D., Hill, M. W. and Miller, N. G. (1974) *Meth. Memb. Biol.,* **1,** 1.

Barenholz, Y., Amselen, S. and Lichtenberg, D. (1979) *FEBS Lett.,* **99,** 210.

Chedid, L., Carelli, C. and Audibert, F., (1979) *J. Reticuloendo. Soc.,* **26,** 631.

Churchill, W. H. Jr., Piessens, W. F., Sulis, C. A. and David, J. R. (1975) *J. Immunol.,* **115,** 781.

Dancey, G. F., Yasuda, T. and Kinsky, S. C. (1977) *J. Immunol.,* **119,** 1868.

Das, M. K., Roy, S. K. and Sarkar, D. P. (1984) *Carbohyd. Res.* **128,** 335.

Das, P. K., Ghosh, P., Bachhawat, B. K. and Das, M. K. (1982a) *Experentia,* **38,** 629.

Das, P. K., Ghosh, P., Bachhawat, B. K. and Das, M. K. (1982b) *Immunol. Commun.,* **11,** 17.

Fendler, J. H. (1980) in *Liposomes in Biological Systems,* (eds. G. Gregoriadis and A. C. allison) (New York: Wiley), p. 87.

Fidler, I. J., Sone, S., Fogler, W. E. and Barnes, Z. L. (1981) *Proc. Natl. Acad. Sci. USA,* **78,** 1680.

Fraser-Smith, F. B., Eppstein, D. A., Larsen, M. A. and Matthews, T. R. (1983) *Infect. Immun.,* **39,** 172.

Gerlier, D., Sakai, F. and Dore, J. F. (1980) *Br. J. Cancer,* **41,** 236.
Ghosh, P. and Bachhawat, B. K. (1980) *Biochim. Biophys. Acta,* **632,** 562.
Gregoriadis, G. and allison, A. C. (1974) *FEBS Lett.,* **45,** 71.
Gregoriadis, G. Leathwood, P. D. and Ryman, B. E. (1971) *FEBS Lett.,* **14,** 95.
Gregoriadis, G., Siliprandi, N. and Turchetto, E. (1977) *Life Sci.,* **20,** 1773.
Heath. T. D., Edwards, D. C. and Ryman, B. E. (1976) *Biochem. Soc. Trans.,* **4,** 129.
Heath, T. D., Macher, B. A. and Paphadjopoulos, J. (1981) *Biochim. Biophys. Acta,* **640,** 66.
Heath, T. D., Robertson, D., Birbeck, M. S. C. and Davies, A. J. S. (1980) *Biochim. Biophys. Acta,* **599,** 42.
Hoekstra, D. and Scherphof, G. (1979) *Biochim. Biophys. Acta,* **551,** 109.
Hudson, L. D. S. (1977) *Studies in enzyme therapy: Evaluation of enzyme delivery strategies in a mammalian system,* Ph.D. Thesis, University of Minnesota, Minnesota.
Hudson, L. D. S., Fidler, M. B. and Desnick, R. J. (1979) *J. Pharm. Exp. Ther.,* **208,** 507.
Kinsky, S. C. (1978) *Ann. N. Y. Acad. Sci. USA,* **308,** 111.
Kinsky, S. C. (1980) in *Liposomes and immunobiology,* (eds. B. H. Tom and H. R. Six) (New York: Elsevier, North Holland, Inc.), p. 79.
Kleinerman, E. S., Schroit, A. J., Fogler, W. E. (1983) *J. Clin. Invest.,* **72,** 304.
Laserman, L. D., Barbet, J. and Kourilsky, F. (1980) *Nature* (*London*)*,* **288,** 602.
Latif, N. (1984) *Liposomes as tools in biomedicine,* Ph.D. Thesis, University of Calcutta, Calcutta.
Latif, N. and Bachhawat, B. K. (1981) *Proceedings of the* 50*th Annual General Meeting of the Society of Biological Chemists,* Baroda, India.
Latif, N. and Bachhawat, B. K. (1984a) *Biosci. Rep.,* **4,** 99.
Latif, N. and Bachhawat, B. K. (1984b) *Immunol. Lett,* (in press).
Liftshitz, R., Gitler, C. and Mozes, E. (1981) *Eur. J. Immunol.,* **11,** 398.
Martin, F. J. and Papahadjopoulos, D. (1982) *J. Biol. Chem.,* **257,** 286.
Olson, F., Hunt, L. A., Szoka, E. C, Vail, W. J. and Papahadjopoulos, D. (1979) *Biochim. Biophys. Acta,* **537,** 9.
Raphael, L. and Tom, B. H. (1984) *Clin. Exp. Immunol.,* **55,** 1.
Sanchez, Y., Iones Cu-Matin, I., Dressman, G. R., Kramp, W., Six, H. R., Hollinger, F. B. and Melnick, J. L. (1980) *Infect. Immun.* **30,** 728.
Sarkar, D. P. and Das, M. K. (1984) *Immunol. Commun.,* **13,** 5
Shek, P. N. and Heath, T. D. (1983) *Immunology,* **50,** 101.
Shek, P. N. and Lukovich, S. (1982) *Immunol. Lett.,* **5,** 305.
Shek, P. N. and Sabiston, B. H. (1982a) *Immunology,* **45,** 347.
Shek, P. N. and Sabiston, B. H. (1982b) *Immunology,* **47,** 627.
Shek, P. N., Yung, B. Y. K. and Stanacev, N. Z. (1983) *Immunology,* **49,** 37.
Shuster, B. G., Naidig, M., Alving, B. M. and Alving, C. R. (1979) *J. Immunol.,* **122,** 900.
Six, H. R., Kramp, W. J. and Kosel, J. A. (1980) in *Liposomes and Immunobiology,* (eds. B. H. Tom and H. R. Six) (New York: Elsevier, North Holland Inc.), p. 119.
Sone, S. and Fidler, I. J. (1980) *Cell. Immunol.,* **57,** 42.
Tan, C. T., Chan, S. W. and Hsia, J. C. (1981) *Immunol. Commun.,* **10,** 27.
Torchilin, V. P., Golmacher, V. S. and Smirov, V. N. (1978) *Biochem. Biophys. Res. Commun.,* **85,** 983.
Tyrrell, D. A., Heath, T. D., Colley, C. M. and Ryman, B. E. (1976) *Biochim. Biophys. Acta,* **457,** 259.
Uemura, R., Nicolotti, R. A., Six, H. R. and Kinsky, S. C. (1974) *Biochemistry,* **13,** 1572.
van Houte, A. J., Snippe, H. and Willers, J. M. N. (1981) *Immunology,* **43,** 627.
van Rooijen, N. and van Nieuwmegen, R. (1977) *Immunol. Commun.,* **6,** 489.
van Rooijen, N. and van Nieuwmegen, R. (1978) *Immunol Commun.,* **7,** 635.
van Rooijen, N. and van Nieuwmegen, R. (1980a) *Cell. Immunol.,* **49,** 402.
van Rooijen, N. and van Nieuwmegen, R. (1980b) *Immunol. Commun.,* **9,** 243.
van Rooijen, N. and van Nieuwmegen, R. (1980c) *Immunol. Commun.,* **9,** 747.
van Rooijen, N. and van Nieuwmegen, R. (1981) in *Targeting of drugs,* (eds. G. Gregoriadis, J. Senior and A, Trouet) (New York: Plenum press), p. 301.
van Rooijen, N. and van Nieuwmegen, R. (1983) *Biochim. Biophys. Acta,* **755,** 434.
Weir, D. M. (1980) *Immunol. Today,* **1,** 45.
Wood, C. and Kabat, A. C. (1981) *J. Exp. Med.,* **154,** 432.
Yasuda, T., Dancy, G. F. and Kinsky, S. C. (1977) *Proc. Natl. Acad. Sci. USA,* **74,** 1234.