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Novel Vaccines and Adjuvants: Mechanisms of Action

Cytotoxic T Lymphocytes Induced by Liposomal Antigens: Mechanisms of Immunological Presentation

CARL R. ALVING and NABILA M. WASSEF

ABSTRACT

It is known that liposomes can deliver encapsulated substances, including drugs and antigens, to lysosomes in macrophages. Because of this it has been assumed that although liposomes might be useful for induction of humoral (class II) immunity, they would not be capable of cytoplasmic delivery of antigen for introduction into the class I pathway leading to induction of cytotoxic T lymphocytes (CTLs). However, experiments conducted by numerous laboratories, including our own, have demonstrated the ability to induce CTLs either *in vitro* with cultured cells incubated with liposome-associated antigen, or *in vivo* after immunization of mice or monkeys with liposomes containing associated antigen. Using a monoclonal antibody that recognizes repeating sequences of tetrapeptide epitopes derived from the circumsporozoite protein of *Plasmodium falciparum*, it has been shown by immunogold electron microscopy that liposomal antigenic epitopes can actually spill from endosomes into the cytoplasm of cultured macrophages. On the basis of this observation, a theoretical intracellular pathway is proposed whereby liposomal antigen is processed by macrophages through a cytoplasmic process that results in delivery of antigenic epitopes to the Golgi apparatus and the endoplasmic reticulum. The liposomal antigenic epitopes would then have the opportunity to associate with class I MHC molecules and undergo vesicular transport to the surface of the cells for presentation and induction of CTLs.

INTRODUCTION

IN THE SEARCH for useful modern adjuvants it has become evident that the immunostimulating mechanisms of adjuvants and adjuvant formulations frequently are complex and are often poorly understood. Among many mechanisms that have been identified for different immunostimulating substances are the following: depot effect for slow release of antigen, binding or adsorption of antigen, targeting of antigen to antigen-presenting cells (APCs), reconstitution of antigen and presentation of T and B epitopes, recruitment of immune cells, activation of complement, induction of cytokine production, and modulation of MHC class I or class II expression.¹⁻³

Liposome research has been a major beneficiary of a resurgence of interest in vaccine adjuvants (reviewed in Refs. 1 and 4-7). Although liposomes were originally developed as models of efferent mechanisms exhibited by the immune response, it has now become evident that antigens that are presented or reconstituted in liposomes can provide desirable properties that

promote effective humoral and cellular immune responses in many vaccines. Liposomes have been proposed as vehicles for vaccines against parasitic and viral illnesses. Experimental vaccines against malaria, HIV, hepatitis A, and influenza virus have been shown to be safe and highly immunogenic in several human trials.⁸⁻¹³

INDUCTION OF CYTOTOXIC T LYMPHOCYTES

Humoral and cellular pathways are both major elements in the generation of immune responses. Induction of cytotoxic T lymphocytes (CTLs) has been proposed as a useful strategy for developing vaccines against intracellular antigens, such as viral, parasitic, or tumor antigens.¹⁴ Exogenous antigens, such as synthetic soluble peptides or proteins, or for that matter any extracellular antigen, usually must enter the cytoplasm of an APC in order to participate in the processing pathway leading to pre-

94-35964

518

sensation with MHC class I molecules to induce CD8⁺ CTLs. This principle was illustrated by a well-known study in which purified antigen was directly introduced into the cytoplasm of cells by osmotic lysis of pinosomes.¹⁵ Studies have also indicated that macrophages can serve as APCs for generation of CTLs.¹⁶ Regardless of the cell type involved, the likely intracellular pathway by which cytoplasmic antigen normally gains access to class I MHC molecules for presentation involves partial degradation of antigen by "proteasomes,"¹⁷ delivery of immunogenic peptides to the endoplasmic reticulum via a "peptide transporter" mechanism, complexing of the peptide with MHC class I molecules, transport of the complex to the Golgi, and subsequent vesicular transport to the surface of the cell for presentation to T cells.^{18,19}

MECHANISMS OF CYTOTOXIC T LYMPHOCYTE INDUCTION BY LIPOSOMES

Numerous reports have now described class I presentation and induction of CTLs by liposomal antigens. These have in-

cluded both *in vitro* studies²⁰⁻²⁶ and *in vivo* studies²⁷⁻³⁶ with many different antigens (Table 1).

Cytoplasmic delivery of antigen can be facilitated in cultured cells by so-called "pH-sensitive" or "acid-sensitive" liposomes.^{25,26,37} Although "acid-insensitive" liposomes were initially thought to be excluded from the cytoplasm of macrophages,^{25,26,37} subsequent research demonstrated that cytoplasmic delivery and class I presentation also occurred with "acid-insensitive" liposomes.²⁴ Numerous *in vivo* studies have shown that "acid-insensitive" liposomes can readily induce CTLs.²⁷⁻³⁶ Cytotoxic T lymphocyte induction was also facilitated by utilizing liposomes containing a fusion protein in order to introduce liposomal antigen directly into cells.³⁵ In an *in vivo* murine model, CTLs were even generated by encapsulation of an extremely small (15-amino acid) unconjugated peptide in liposomes containing lipid A.³⁸

The ability of liposomes to deliver antigens to macrophages as antigen-presenting cells has been presumed to be the underlying mechanism that results in potent humoral immune responses to the liposomes.^{4,6} The immune response can also be

TABLE 1. INDUCTION OF CYTOTOXIC T LYMPHOCYTES BY LIPOSOME-ENCAPSULATED ANTIGEN

Antigen	Liposome composition	Ref.
<i>In vitro</i> studies		
MHC antigens (H-2 in mice)	Egg PC/CHOL (70:30, w/w)	Hale ²⁰
MHC antigens (H-2 in mice)	Egg PC/CHOL (70:30, w/w)	Hale and McGee ²¹
Human colon tumor antigens (LS174T colon tumor cells)	PC/CHOL/PA (7:2:1)	Raphael and Tom ^{22,23}
Ovalbumin	DOPC/DOPS (4:1) and DOPE-PHC (4:1)	Harding <i>et al.</i> ²⁴
Murine hemoglobin		
Bovine ribonuclease		
Hen egg lysozyme		
Ovalbumin	DOPE/DOSG (1:1) and DOPC/PS/CHOL (5:2:3)	Reddy <i>et al.</i> ²⁵
Ovalbumin	DOPE/DOSG (1:1) and DOPC/PS/CHOL (5:2:3)	Zhou <i>et al.</i> ²⁶
<i>In vivo</i> studies		
Hemagglutinin, neuraminidase	MDP, and MDP/CHOL (1:1, w/w)	Nerome <i>et al.</i> ²⁷
Ovalbumin, β -galactosidase	DOPE/DOSG (1:1) and DOPC/PS/CHOL (5:2:3)	Reddy <i>et al.</i> ²⁸
Ovalbumin	DOPE/DOSG, DOPE/DPSG, DOPE/CHEMS, DOPC/DOPS, (all 4:1), $\pm$ 50 μ g of lipid A	Collins <i>et al.</i> ²⁹
Ovalbumin	DPPC/DPPG/CHOL (9:1:8) $\pm$ 50 μ g of lipid A	
	PC/lysoPC/CHOL (6.9:0.1:3, neutral), PC/lysoPC/SA/CHOL (6.9:0.1:1:2, positive), PC/lysoPC/DCP/CHOL (6.9:0.1:1:2, negative)	Lopes and Chain ³⁰
Multiple antigen peptide system (MAPS) from gp120 of HIV-1 (B2M-P3C)	Egg PC/CHOL/SA (7.5:1:0.25, w/w)	Defoort <i>et al.</i> ³¹
Glycoprotein B from HSV	Cationic lipids (dioleoyloxypropyl-trimethylammonium methyl sulfate, DOTAP)	Walker <i>et al.</i> ³²
Ovalbumin	Commercially available DOTAP	Chen <i>et al.</i> ³³
Repeatless <i>Plasmodium falciparum</i> CS protein	DMPC/DMPG/CHOL/lipid A (0.9:0.1:0.75:0.026)	White <i>et al.</i> ³⁴
SIV Gag protein-derived peptide (p11C)	PS/CHOL (9:1) envelope glycoproteins and lipids of Sendi virus	Miller <i>et al.</i> ³⁵
SIV Gag protein-derived peptide (M90-07A)	DMPC/DMPG/CHOL/lipid A (0.9:0.1:0.75:0.1)	Yasutomi <i>et al.</i> ³⁶

CHEMS, Cholesterol hemisuccinate; CHOL, cholesterol; CS, circumsporozoite; DCP, dicetyl phosphate; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; DOPC, dioleoyl phosphatidylcholine; DOPE, dioleoyl phosphatidylethanolamine; DOPS, dioleoyl phosphatidylserine; DOSG, 1,2-dioleoyl-sn-3-succinylglycerol; DPPC, dipalmitoyl phosphatidylcholine; DPPG, dipalmitoyl phosphatidylglycerol; DPSG, dipalmitoyl succinylglycerol; lysoPC, lysophosphatidylcholine; MDP, muramyl dipeptide; MHC, major histocompatibility antigen gene complex; PA, phosphatidic acid; PC, phosphatidylcholine; PHC, palmitoyl homocysteine; PS, phosphatidylserine; SA, stearylamine.

enhanced by the presence of lipid A, the endotoxic moiety of bacterial lipopolysaccharide, as a simultaneous constituent that serves as an adjuvant in the liposomes.³⁹ In accordance with this, we have demonstrated that liposomal lipid A can serve as a stimulant for increased specific presentation of phagocytosed liposomal antigen by macrophages.⁴⁰

Proposed intracellular processing pathway for induction of cytotoxic T lymphocyte by liposomes

The same liposomes that were used for presentation studies in macrophages have also been employed for induction of CTLs with an encapsulated antigen containing a CTL epitope.³⁴ The ability of antigen contained within liposomes to enter the cytoplasmic class I pathway for induction of CTLs was anticipated by an immunogold electron microscopy study that demonstrated that liposomal antigen was disgorged in large amounts into the cytoplasm of macrophages (Fig. 1).⁴¹ On the basis of this we propose that liposomes, or lipid-peptide complexes, that escape into the cytoplasm of macrophages can gain access to class I MHC molecules in the Golgi apparatus (Fig. 2).

Investigations performed in the laboratories of Pagano⁴²⁻⁴⁴ and Ohnishi⁴⁵ have suggested that liposomes in the cytoplasm can gain direct access to the Golgi apparatus through an ATP-dependent fusion phenomenon that is mediated by a Golgi-associated protein that recognizes the liposomal lipids. We propose that the liposomes containing antigen, or partially degraded lipid peptide complexes, enter the cytoplasm of macrophages (Fig. 2). The complexes are then either taken up directly by the Golgi via the ATP-dependent fusion phenomenon described by Pagano and Ohnishi, or alternatively liposomal peptide in the cytoplasm is transported to the endoplasmic reticulum by the cytoplasmic peptide transporter (Fig. 2). The liposomal peptide would thereby gain access to the classic intracellular pathway for class I presentation of cytoplasmic antigen.

The cell biology mechanism that we propose, as shown in

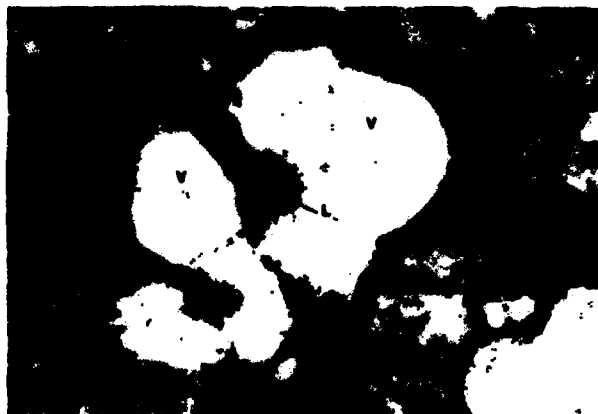


FIG. 1. Immunogold electron microscopy of cultured bone marrow-derived macrophages after phagocytosis of liposomes containing malaria antigen. The macrophages were fixed after incubation with the liposomes. The malaria antigen was detected by an antigen-specific monoclonal antibody (Pf 1B2.2), followed by treatment with a gold-labeled second antibody. Cytoplasmic antigen is indicated by four arrows. (From Verma *et al.*⁴¹)

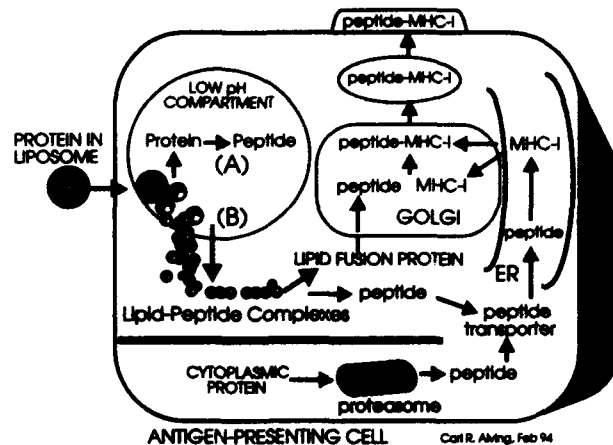


FIG. 2. Schematic representation of proposed presentation of liposomal antigen in MHC class I pathway.

Fig. 2, is amenable to experimental testing. In pursuing this, experiments are currently underway, using fluorescent-labeled liposomes and antigen, to determine whether liposomal antigenic epitopes that escape from low-pH vacuoles into the cytoplasm of macrophages are actually directly delivered to the Golgi apparatus or to the endoplasmic reticulum. If this mechanism proves to be widely applicable to different antigens it could provide a broad theoretical basis for utilization of liposomes as carriers of antigens for induction of CTLs.

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