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Dedicated to Professor Claude Nicolau on the occasion of his 80th anniversary

LIPOSOMAL VACCINE ADJUVANT FORMULATIONS

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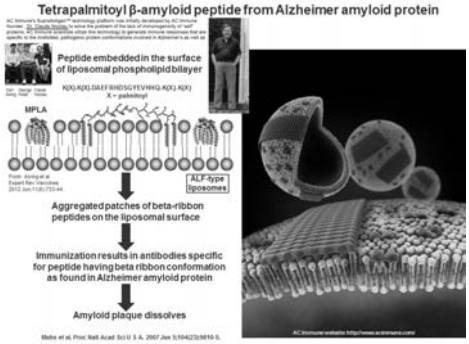
Liposomes known as Army Liposome Formulation (ALF), which are used as a potent vaccine adjuvant formulation, have a lipid bilayer bulk composed of phospholipids, usually dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol, in which the hydrocarbon chains have a melting temperature in water of ≥23°C. Cholesterol is present in the bilayer as a stabilizer, and monophosphoryl lipid A (MPLA) as an immunostimulator. Using synthetic congeners of MPLA we demonstrate that with ALF liposomes containing a cholesterol-binding saponin as an additional adjuvant, differences in the number and placement of 14-carbon hydrocarbon chains attached to the diglucosamine phosphate headgroup of the MPLA do not significantly impact the magnitude of the immune response to an HIV-1 envelope protein.



INTRODUCTION

Membrane biochemistry is a nanosized world of chemical architecture, a world of particles having structural elegance and beauty. As embodied by the utilization of liposomal model membranes it represents a creatively fecund discipline of colloid chemistry that can be manipulated to reveal a dynamic continuum of modeled normal structures and pathological changes in human membranes and cells. Into this scientific area there emerged in the 1970s Claude Nicolau, a biophysicist and biochemist whose many eclectic interests and contributions have ranged broadly from a whimsical analysis of the colloid chemistry involved in the "resurrection" of coagulated sauce bèarnaise[1] to membrane fusion with liposomes, and the use of other biophysical and biochemical methods, to introduce intracellular myo-inositol phosphate derivatives into cells to increase the oxygen-carrying capacity of erythrocytes to treat cancer and other diseases.[2-5]

Starting in 2002 Professor Nicolau turned his attention to the use of liposomes containing monophosphoryl lipid A (MPLA) (as an adjuvant) and a synthetic tetrapalmitoylated 15-amino acid peptide ($A\beta_{1-15}$) derived from β-amyloid (Aβ) (as an antigen), both embedded in the liposomal lipid bilayer, to induce antibodies in mice that could specifically dissolve Aβ plaques that are

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characteristic of Alzheimer's disease (Fig. 1).[6-8] Although it was hypothesized that the beta ribbon conformation of the peptide on the liposomal surface might be due in part to complex interactions of the peptide with the lipids of the underlying liposomal bilayer,[7] the exact mechanisms of the formation of the beta ribbons is still unknown. For success of this type of vaccine, the adjuvant formulation must facilitate both the magnitude of the immune response and the specificity of the induced antibodies. As indicated in Fig. 1, variations on this technology are being actively utilized by AC Immune to develop an immunotherapeutic vaccine for treatment of Alzheimer's disease and related disorders.

In the present context, regardless of the specificity of the induced antibodies, as noted above an important element in the immune response requires the use of liposomal MPLA as a powerful adjuvant to increase the magnitude of the immune response. The particular liposomal adjuvant formulation composition that was used to induce antibodies to Aβ was originally developed in this laboratory for creation of a vaccine to malaria by using a protein antigen.[9] This formulation has now been utilized as the adjuvant in experimental vaccines to numerous infectious diseases and cancer[10] and even to small synthetic molecules known as haptens in a candidate vaccine to heroin.[11,12] Because of its widespread use in human clinical trials, liposomes containing MPLA are now referred to as Army Liposome Formulation (ALF).[13,14] Recently, different ALF-derived formulations have been created, including ALF adsorbed to aluminum salt gel (ALFA) and ALF containing the cholesterol-binding saponin compound known as QS21 (ALFQ) [13,14] (Fig. 2).

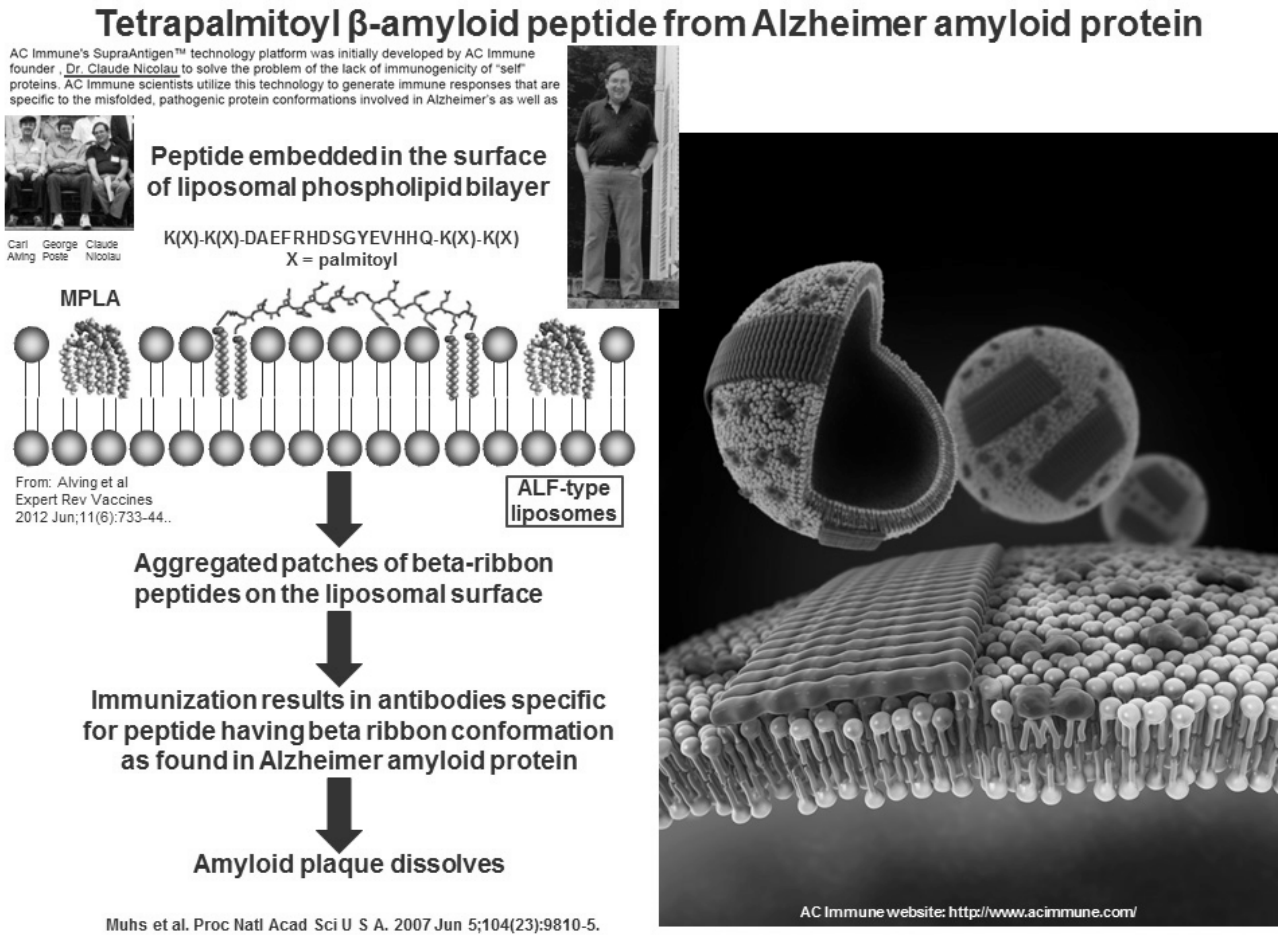

Fig. 1 – Schematic illustration of immunotherapeutic employed by Claude Nicolau and AC Immune to use liposomes containing MPLA and a synthetic tetrapalmitoylated 15-amino acid peptide (Aβ1-15) derived from β-amyloid for dissolution of amyloid plaque. Standard single letter designations of amino acids are shown in the peptide structure. Photo of CRA, GP, and CN is from a group photo at a Gordon Research Conference on Drug Carriers in 1984, and photo of CN alone was made in the 1990s by CRA at CN's home in the Loire river valley near Orléans, France. Illustration from AC Immune website used by permission.

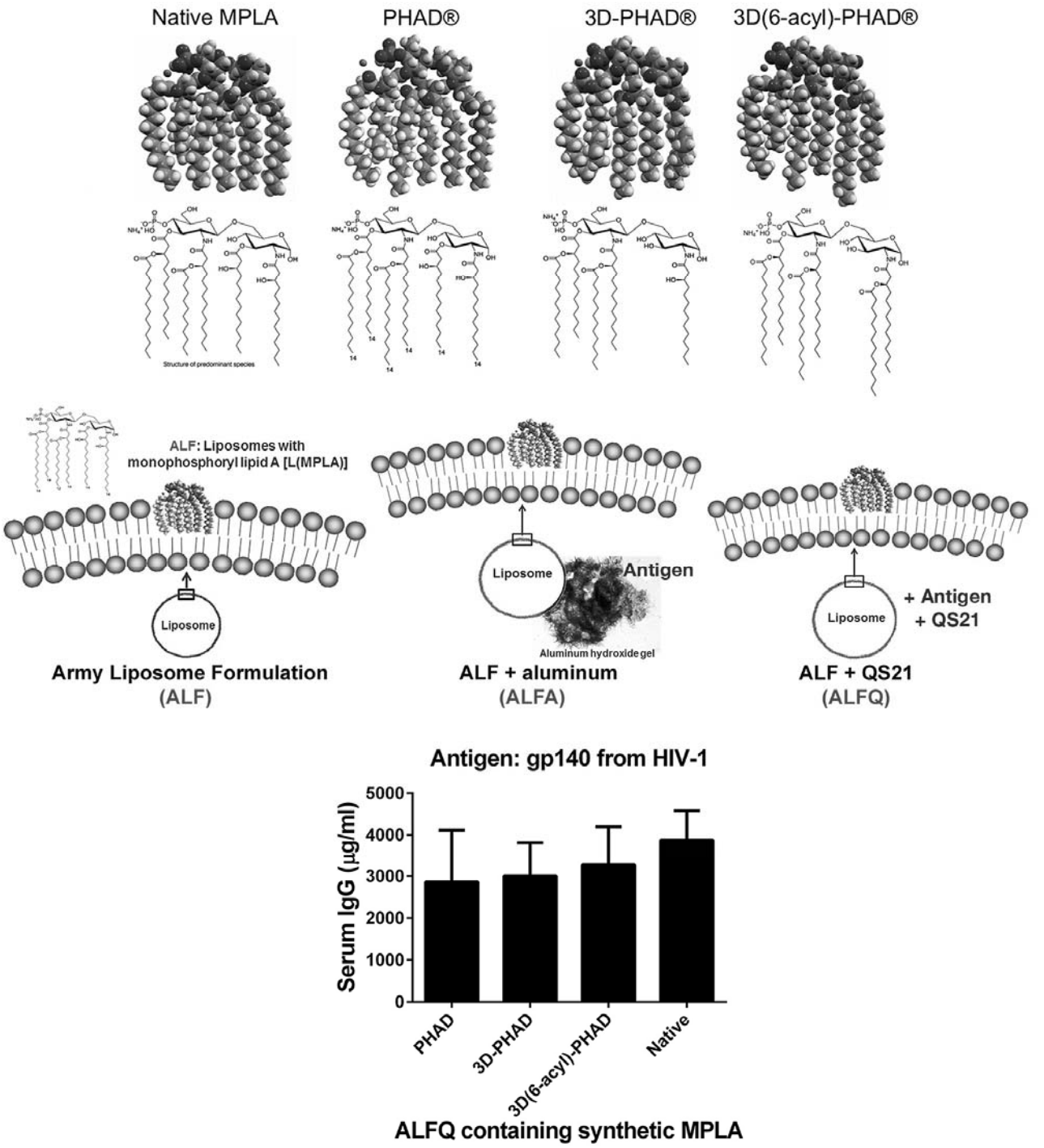

Fig. 2 – Congeners and structures of MPLA, formulations of ALF-type adjuvants, and utilization of ALFQ containing the different MPLA molecules to induce immune responses to HIV-1 gp140 envelope protein. Native MPLA and PHAD-type MPLA structures supplied by Avanti Polar Lipids.

The membrane biochemistry and biophysics of ALF particles are quite complex and can be influenced by factors such as relative epitope density of MPLA in the bilayer membranes, surface charge, the epitope density of liposomal cholesterol, and the structures, compositions, and orientations of the polar or nonpolar regions of the bulk phospholipids and other lipids.[13,14] In this study we address whether the nonpolar hydrophobic structures of different liposomal MPLA molecules play a role in influencing differences in the magnitude of the immune response to a model protein antigen. The ALF-type particles utilized in the original studies with $A\beta_{1\text{-}15}$

peptide contained native MPLA derived from gram-negative bacterial lipopolysaccharide as supplied by List Biological Laboratories (Campbell, CA USA),[6] while subsequent studies used MPLA synthesized and sold under the brand name PHAD® by Avanti Polar Lipids (Alabaster, AL USA)[7,8]. In this preliminary study we utilized either native MPLA or three types of synthetic MPLA having different numbers and placement of fatty acid chains, all obtained from Avanti Polar Lipids. (Fig. 2). Each of these was formulated with a protein (gp140) from HIV-1 envelope protein that served as an antigen for induction of antibodies to gp140.

MATERIALS AND METHODS

Reagents. Dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), cholesterol (Chol), native MPLA (detoxified Lipid A from Salmonella minnesota R595), synthetic MPLA compounds, including PHAD®, 3-Deacyl MPLA (3D-PHAD®), and synthetic hexa-acyl 3-Deacyl MPLA (6A3D PHAD®) were from Avanti Polar Lipids (Alabaster, AL, USA). DMPC and Chol were dissolved in freshly distilled chloroform, and DMPG and all form of MPLA were dissolved in chloroform:methanol (9:1). Purified QS21 (Desert King International San Diego, CA, USA) was dissolved in Sorensen PBS at 1 mg/mL at pH:5.6. Horseradish peroxidase (HRP)-linked-sheep anti-mouse IgG was purchased from The Binding Site (San Diego, CA, USA).

Preparation of liposomes and vaccines. ALF containing DMPC, DMPG, Chol, and MPLA were prepared by the lipid deposition method, and ALFQ were formulated by adding QS21 to ALF, with a MPLA:phospholipid ratio of 1:88 as previously described[14]. For immunizations a total injection dose of 1 μg of soluble HIV-1 clade C gp140 CN54 envelope antigen (Polymun Scientific Inc., Klosterneuberg, Austria) was used.

Immunization of mice. Female BALB/c mice (Charles River Laboratories, Indianapolis, IN, USA) were immunized with ALFQ plus HIV gp 140 formulations IM with 0.05 ml of the vaccines by injection in alternate rear thighs at 0, 3, and 6 weeks or at 0, 2, and 4 weeks, respectively.

ELISA. To determine the serum concentration of IgG antibodies, ELISA was performed as described previously.[14]

RESULTS

As shown in the experiment in Fig. 2, liposomes containing MPLA and QS21 (*i.e*., ALFQ) served as a potent adjuvant for induction of antibodies to the gp140 protein. There were no significant differences between adjuvant potencies of the different types of liposomal MPLA.

DISCUSSION

The general structure of MPLA utilized in this study comprised a phosphorylated diglucosamine headgroup to which were attached five or six acylated or amidated 14-carbon saturated hydrocarbon chains (Fig. 2). It is well-known that the detailed chemical and structural compositions of ALF-type adjuvants can have profound effects both on the surface patterns of the lipid bilayer, and on the quantity and quality of induced antibodies to gp140.[13,14] For example, the surface exposure of cholesterol was different in either ALF or ALFQ formulations containing dimyristoyl, dipalmitoyl, or distearoyl phosphatidylcholine.[13] In this Note, we demonstrate that the number and placement of the MPLA hydrocarbon chains of the various types of native or synthetic MPLA did not significantly affect the magnitude of antibodies induced to the gp140. Studies are currently underway to determine whether the quantity and quality of antibodies to peptides derived from HIV-1 envelope protein are influenced by the MPLA composition in these ALF-type liposomes. The data suggest that there might be considerable leeway in the selection of acceptable hydrophobic compositions of different types of native or synthetic MPLA derivatives for use in ALF-type liposomal adjuvant formulations for induction of antibodies to proteins or peptides.

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