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Liposomal Enhancement of the Immunogenicity of Adenovirus Type 5 Hexon and Fiber Vaccines

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Immunogenicity of adenovirus capsid proteins carried in liposomes was comparable to that with equivalent doses administered in Freund adjuvant, and both forms were more potent than aqueous vaccines.

Adenovirus hexon and fiber capsid proteins possess immunologically distinct antigenic determinants capable of inducing serum-neutralizing antibody (4, 6, 16, 17). Their utilization as immunogens for the prevention of adenoviral disease in pediatric age groups is currently under investigation. In unprimed children, a single dose of either highly purified adenovirus type 5 hexon or fiber aqueous vaccine was observed to be relatively nonimmunogenic (Kasel and Drake, unpublished observations), whereas in primed individuals, seroconversion rates were of a high order (3). To avoid the need for booster inoculations, other approaches are being sought with the aim of improving immunogenicity by a single administration of vaccine. Recent studies suggest that liposomes can enhance the immunogenicity of protein antigens (2, 5, 13). This report describes an assessment of the effect of liposomes on the antigenicity of hexon and fiber subunits in an animal model.

Hexon and fiber antigens were purified from soluble antigen extracts of adenovirus type 5-infected cell packs by a sequence of procedures including ion-exchange chromatography, sucrose density gradient centrifugation, crystallization, and resolubilization (10, 11).

Dipalmitoylphosphatidylcholine, cholesterol, and phosphatidic acid (Sigma Chemical Co., St. Louis, Mo.) in molar ratios of 2.0:1.5:0.2 were used to prepare multicompartment liposomes (8). For antigen entrapment, liposomes (20 mM with respect to phospholipid) were swollen in a solution containing 140 μg of hexon or fiber per ml. Untrapped protein was removed by centrifugation, and trapped antigen was determined by the fluorescamine assay (15) after lysis of the liposomes with Triton X-100 (8). The specific trapping of these hexon and fiber preparations ranged from 0.5 to 0.7 μg of protein per μmol of phospholipid.

A series of preliminary experiments established that subunits were trapped in the aqueous portion of the liposomes. The experiments performed with hexon showed that: iodine-radiolabeled hexon did not associate with preformed liposomes; the trapping efficiency was inversely related to the ionic strength of the swelling solution; reduction of the size of the aqueous compartments (e.g., omitting the phosphatidic acid) reduced the trapping efficiency by approximately threefold; and hexons were trapped in parallel with umbelliferone phosphate, a compound known to reside in the aqueous phase (14). These data fulfilled the criteria set forth by others (7, 12) for establishing that a protein resides in the aqueous portion of a liposome rather than being associated with lipid bilayers.

The immunizing capacity of 5 μg of adenovirus type 5 hexon and fiber administered in different forms was assessed in young adult New Zealand white rabbits, and the results are presented in Table 1. Each aqueous vaccine was relatively inefficient in evoking a serum-neutralizing antibody response (≥fourfold rise). Similar results were obtained with the hexon subunit and preformed liposome mixtures. However, administration of hexon or fiber entrapped in liposomes resulted in a substantial seroconversion rate to each subunit, 78 and 90%, respectively. Whereas these relative frequencies were significantly greater than those seen after vaccination by the aqueous method (namely, 17 and 30%, respectively, in each case; $P < 0.05$), they were comparable to the rates observed with the adjuvant vaccines. Among the animals that received hexon or fiber in aqueous forms, the geometric mean postimmunization antibody titers were lower than those in the liposome groups (in each instance, $P < 0.05$). Although there was no significant difference between the mean titer of hexon antibody in the liposome and adjuvant animals, the fiber response was higher in the latter group.

The serological responses after administration of a booster dose of 5 μg of aqueous vaccine are shown in Table 2. The second dose of antigen resulted in an increased frequency of responders in all groups except the hexon-adjuvant animals and a rise in the mean antibody titer. Animals that received the mixture of hexon and liposomes responded in a manner similar to those given hexon alone. The immunizing effect of two doses of aqueous hexon or fiber vaccines was comparable to that achieved with only a single dose of either immunogen administered in liposomes.

This study has shown that liposomes can enhance the immunogenicity of purified adenovirus type 5 hexon and fiber subunits. Since entrapment was a requirement, the immune response was not attributable to an adjuvant effect of lipids used in the preparation of liposomes. Responsiveness of seronegative rabbits to two injections of aqueous hexon and fiber vaccine indicates that the liposomes lowered the protein concentration necessary to elicit a response rather than stimulating antibody production to a nonimmunogenic protein.

The liposomes used in the antigenicity evaluations were prepared with lipids found in virtually all mammalian membranes and are readily biodegradable. Moreover, recent studies have show that liposomes of this same composition are nontoxic in mice at doses ten times higher than those used in the present experiments (1) and that they are nonimmunogenic in laboratory animals (9).

TABLE 1. *Serum-neutralizing antibody responses in seronegative rabbits after intramuscular immunization with adenovirus type 5 hexon or fiber administered in different forms*[a]

Vaccine form (5 μg)	Response to hexon		Response to fiber	
	Relative frequency[b]	Geometric mean antibody titer[c]	Relative frequency[b]	Geometric mean antibody titer[c]
Aqueous	2/12 (17)	2.5	3/10 (30)	3.2
Entrapped in liposomes	14/18 (78)	8.3	9/10 (90)	11.2
Emulsified in complete Freund adjuvant	10/11 (91)	10.9	8/10 (80)	47.8
Mixed with liposomes[d]	2/9 (22)	3.9	ND[e]	

[a] Antibody responses were determined as previously described (3) with serum specimens collected 4 weeks postvaccination.

[b] Number of animals with a ≥4-fold rise in titer/number immunized; number in parentheses indicates percentage.

[c] Geometric mean titers are expressed as the reciprocal of the serum dilution. For these calculations a titer of <4 was considered to be 2.

[d] Liposomes were swollen in sterile pyrogen-free water, centrifuged, and resuspended in a saline solution containing 15 μg of hexon per ml. Each animal received 5 μg of hexon and 6 μmol of liposomal phospholipid.

[e] ND, Not done.

TABLE 2. *Serum-neutralizing antibody responses in rabbits after intramuscular administration of a booster dose of adenovirus type 5 hexon or fiber*[a]

Vaccine form		Response to hexon		Response to fiber	
Primary	Booster (5 μg)	Relative frequency[b]	Geometric mean antibody titer[c]	Relative frequency[b]	Geometric mean antibody titer[c]
Aqueous	Aqueous	8/12 (67)	6.3	7/10 (70)	7.1
Entrapped in liposomes	Aqueous	18/18 (100)	20.2	10/10 (100)	48.5
Emulsified in complete Freund adjuvant	Aqueous	9/11 (82)	24.9	9/9 (100)	47.0
Mixed with liposomes	Aqueous	4/7 (57)	4.6	ND[d]	

[a] Antibody responses were determined with serum specimens collected 3 weeks after administration of the booster dose.

[b] Number of animals with a ≥4-fold rise in titer/number immunized; number in parentheses indicates percentage.

[c] Geometric mean titers are expressed as the reciprocal of the serum dilution. For these calculations a titer of <4 was considered to be 2.

[d] ND, Not done.

We believe that the liposome method of delivery of adenovirus subunits warrants further investigation.

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