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Genetic immunization using liposome-incorporated DNA

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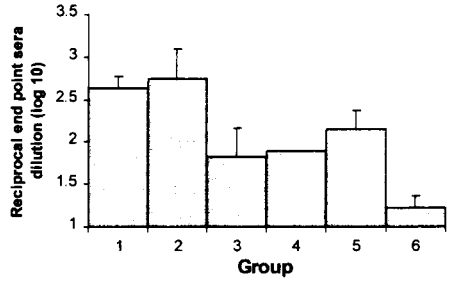
Genetic immunization using naked plasmid DNA is based on DNA uptake by and expression in skeletal muscle cells which lack vital co-stimulatory molecules. Alternatively liposomes, which target antigen-presenting cells directly and protect DNA from nuclease attack, Perrie & Gregoriadis, (1997) may be preferable. We have recently shown, Gregoriadis et al (1997); Gregoriadis et al (1998) that Balb/c and outbred mice immunized by a variety of routes with the plasmid DNA pRc/CMV HBS (encoding the S region of HBsAg, subtype ayw) entrapped in cationic liposomes mounted much higher humoral (IgG and splenic IL-4) and cell mediated (splenic IFN-γ) responses than when injected with naked DNA or DNA complexed to similar preformed liposomes. Here we report further studies on the effect of lipid composition of cationic liposomes entrapping the plasmid, on immune responses.

pRc/CMV HBS (100μg and ^{35}S-labelled tracer of the same DNA) was entrapped by the dehydration-rehydration method, Gregoriadis et al (1996); Kirby & Gregoriadis (1984); Perrie & Gregoriadis (1997) in liposomes composed of 16 μmoles egg phosphatidyl choline (PC) and a) dioleoylphosphatidylethanol amine (DOPE) and 1,2-dioleoyl-3-(trimethyl ammonium) propane (DOTAP) (4:2:1); b) phos phatidylethanolamine (PE) and DOTAP (4:2:1); c) DOTAP (4:1); d) cholesterol (CHOL) and DOTAP (4:2:1). In an additional preparation PC was replaced with distearoyl phosphatidylcholine (16 μmoles) supplemented with DOPE and DOTAP (4:2:1 molar ratio). Liposomes with entrapped DNA and preformed empty DRV as such or complexed with DNA were subjected to microelectrophoresis at 25°C in a Malvern Zetasizer 3000 to determine their zeta potential (ZP). Female Balb/c mice, 6-8 weeks old, were given 4 intramuscular (hind leg) injections of 10μg free or liposome-entrapped pRc/CMV HBS plasmid using the above formulations over an 8 week period, bled at time intervals and the sera tested for anti-HBsAg (S region; ayw subtype) IgG1 by the enzyme-linked immunoadsorbent assay (ELISA).

Incorporation of DNA into DRV was quantitative (85-96% of DNA used) with all formulations exhibiting a positive ZP (43 mV) which was higher than that (24 mV) observed with DRV-complexed DNA suggesting that much of the DNA was entrapped rather than surface bound.

Fig. 1. IgG1 response in DNA-immunized mice



1. PC:DOPE:DOTAP (16:8:4 μmoles)/DNA
2. PC:PE:DOTAP (16:8:4 μmoles)/DNA
3. PC:DOTAP (16:4 μmoles)/DNA
4. PC:CHOL:DOTAP (16:8:4 μmoles)/DNA
5. DSPC:DOPE:DOTAP (16:8:4 μmoles)/DNA
6. Free plasmid DNA only.

It can be seen (Fig. 1.) than IgG1 responses in the immunised mice 56 days after the final injection were highest with DNA entrapped in PC:DOPE:DOTAP and PC:PE:DOTAP liposomes. Omission of DOPE or substitution of DOPE with CHOL resulted in significantly lower ($p<0.005$ and 0.05 repectively) IgG1 responses. Replacement of PC with DSPC also gave a significantly lower response ($p<0.005$). All liposomal formulations of DNA gave much higher responses than plasmid DNA alone ($p<0.05$ to 0.0001). Results indicate that liposomal DNA gives a higher IgG1 response than naked DNA, with the presence of DOPE or PE being a key contributing factor.

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