

Delivery of a Mycobacterium Tuberculosis Subunit Protein using Cationic Liposomes: a Bio-distribution Profile

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Immunisation of mice with the tuberculosis subunit antigen 'Ag85B-ESAT-6' adsorbed to cationic liposomes has been shown to produce a strong immune response characterised by elevated IFN- γ and TNF- α levels upon re-stimulation with *Mycobacterium tuberculosis*. Whilst the immunological mechanism of action can, in part, be attributed to increased exposure of circulating phagocytic cells to antigen, the underlying process of tissue deposition noted with particulate adjuvant formulations is unclear. We have recently undertaken a series of bio-distribution studies investigating the retention of both antigen and adjuvant at the site of injection; the effect of liposomal surface charge and choice of cationic lipid were studied.

Liposomes composed of various lipids in combination with the synthetic bacterial cell wall glycolipid trehalose 6,6'-dibehenate (TDB) were produced using the lipid-film method [1]. Ag85B-ESAT-6 was added to a final antigen concentration of 2 μ g/dose. Both the liposome and antigen were radio-labelled using tritium and I¹²⁵ labels respectively, thereby allowing for the simultaneous detection of both components throughout the tissues. Triplicate groups of mice received a 50 μ l intramuscular injection; tissues were collected from the site of injection and local draining lymph nodes on days 1, 4 and 14 post injection.

Our results confirm the previously described 'depot-effect' attributed to cationic liposomes when used as an immunostimulatory delivery vehicle. Whilst injection of Ag85B-ESAT-6 without liposomes leads to rapid dissemination, the combination of Ag85B-ESAT-6 with liposomes formed from TDB with the cationic lipid dimethyldioctadecylammonium bromide (DDA) results in considerable levels (76 \pm 15 % of the injected dose, 1 day post injection) of antigen at the site of injection which remain detectable up to two weeks post injection. This depot-effect is attributed to the cationic nature of DDA:TDB liposomes as removal of the immunomodulatory component TDB has no effect on the antigen depot-effect. In contrast, substitution of DDA for distearoyl-glycero-phosphatidylcholine (DSPC) thereby producing liposomes with a surface charge of -9 \pm 7 mV abrogates the observed depot-effect (5-fold less antigen retention at the site of injection 1 day post injection). The antigen depot-effect observed for DDA containing liposomes is also observed when liposomes, containing other cationic immunostimulatory lipids such as [N-(N',N'-dimethylaminoethane)-carbonyl] cholesterol (DC-Chol) and N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium chloride (DOTAP) in combination with TDB, are used to deliver Ag85B-ESAT-6.

Vesicle size measurements, conducted using dynamic light scattering, show cationic liposome aggregation upon exposure to simulated blood serum conditions. DSPC:TDB liposomes do not aggregate due to their inherent anionic charge which does not interact repulsively with serum proteins. It is therefore proposed that the depot-effect observed when cationic liposomes are used as adjuvant delivery systems for Ag85B-ESAT-6 is primarily a function of the strong repulsive charges between the liposome and serum proteins, thereby causing a liposomal net which inhibits antigen dissemination. Variation in cationic vesicle size prior to serum exposure has no effect on the observed antigen depot-effect: both small DC-Chol:TDB vesicles (<400nm) and large DOTAP: TDB liposomes (approximately 1 μ m) lead to an identical antigen bio-distribution profile.

- [1] J. Davidsen, I. Rosenkrands, D. Christensen, A. Vangala, D. Kirby, Y. Perrie, E.M. Agger and P. Andersen, *Biochimica et Biophysica Acta* 1718 (2005) 22-31.