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Biodegradable nanoparticles stabilized with block co-polymer surfactants and encapsulating *Yerisinia pestis* rF1 antigen for oral vaccination against plague

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Although the importance of systemic and local immunity induction at susceptible mucosal surfaces is recognised, parenteral immunisation often fails to provide local imunity as shown by the failure of the Cutter USP vaccine to induce immunity against the pneumonic form of plague (*Yersinia pestis*). Novel strategies, such as particulate antigen delivery systems are required to recruit mucosal associated lyphoid tissue into the immune response and give mucosal protection. The limited success of orally-delivered poly(lactic acid) PLA microspheres to provide mucosal delivery is probably due to the poor bioavailability of particles, one of the important factors controlling particle uptake across the gastrointestinal tract. Nanoparticles undergo more rapid and efficient uptake compared to microparticles (Jani et al., 1990); surface hydrophobicity is also important (Alpar & Almedia 1996). The aim of this study was to assess the efficacy of F1 subunit microencapsulated into lipophilic (HLB=1) nonionic-block co-polymer (NIBC) poly DL-lactide nanospheres. Nanospheres were prepared by a solvent diffusion - evaporation method using mild processing conditions. Briefly, the polymer (poly DL-lactide, Mr 124 kDa) and NIBC surfactants with known adjuvantic characteristics such as Pluronic L101 and L121 were dissolved in acetone. The organic phase homogenised with water at 100rpm to produce an o/w emulsion. Subsequently, the solvent was removed under reduced pressure. The SEM and PCS analysis showed this method yielded nanospheres with 150nm for L101 formulation and 800nm diameter for L121 formulations. The polydispersity index for L101 formulation indicated a narrow particle size distribution (<0.2 v.s. 1.21). With similar theoretical loading levels, L101 formulations showed lower protein loading for L101 compared to L121 formulations (0.72% w/w v.s. 1.58 % w/w) as determined by BCA assay. SDS-PAGE analysis indicated that F1 subunit integrity was maintained during microencapsulation. Both preparations demonstrated very high hydrophobicity. Six groups of BalbC mice (n=4 *per* group) dosed on day 1 (i.e.once) or day 1 and 3 (i.e. twice) with either 100μg free F1 or 100 μg F1 encapsulated in PLA particles with L101 or L121. Sera was collected at different time points up to day 238 and GIT washes were collected on day 238. The samples were analysed for antibodies by ELISA. IgG sera response to oral F1 vaccines were greatly eleveated for L101/F1 microspheres, peaking at day 32. Double priming L101/F1 microspheres elicited higher serum IgG than single priming groups over the time course; day 32 titres were 1:842 (single prime) and 1:4224 (double prime) (Fig. 1). High serum titres, elicited with F1/L101 microspheres may be attributed to the lower antigen loading and relatively smaller size of these microspheres, where a larger mass of lower-loaded microspheres are required to make up the 100 μg F1 dose. The low IgA titres observed for GIT washes at day 234 was not unexpected as the mucosal immune responses are generally short lived.

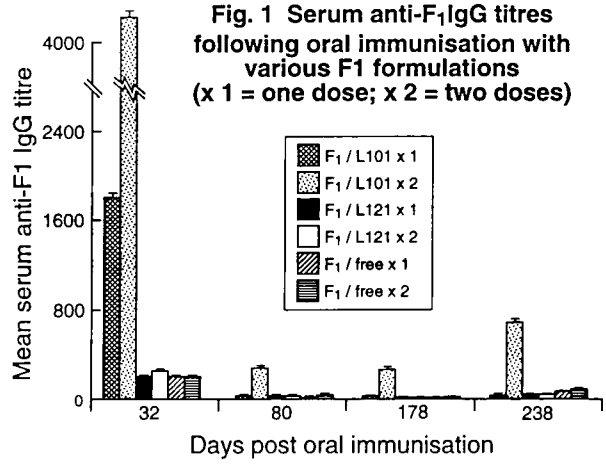

Fig. 1 Serum anti-F_1IgG titres following oral immunisation with various F1 formulations (x 1 = one dose; x 2 = two doses)

Our earlier studies with conventional PLA microspheres encapsulated with F1 subunit failed to demonstrate sufficiently high serum and mucosal responses. Present results show the effectiveness of NIBC nanospheres as a means of delivering antigens by the oral route to achieve substantial systemic immune responses.

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