LIPOSOMAL INTERLEUKIN-2 AUGMENTS IMMUNOADJUVANT ACTION OF LIPOSOMES FOR CO-ENTRAPPED PROTEIN AND PEPTIDE ANTIGEN

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Previous studies (Gregoriadis 1990) have shown that liposomes act as immunological adjuvants for a wide variety of entrapped antigens. Such action can be improved by manipulating the structural characteristics of liposomes (Davis and Gregoriadis 1987) and the presence of a mannosylated ligand on their surface (Garcon et al 1988). Here we report on the possibility of further augmenting adjuvanticity by the use of interleukin-2 which is known to act as an immunomodulator. Dehydration-rehydration vesicles (DRV liposomes) composed of equimolar egg phosphatidylcholine (PC) or distearoyl phosphatidylcholine (DSPC)and cholesterol were prepared (Kirby and Gregoriadis, 1984) to contain 125I-labelled tetanus toxoid (PC DRV) or polio-virus peptide 3-VP2 (DSPC DRV) either alone or together with recombinant IL-2 (des-Ala_1-Ser_{125} mutein)($3x10^{16}$ Cetus units/mg; Cetus Corp. Emeryville, CA, USA). Other preparations contained IL-2 only. In immunization experiments, male Balb/c mice (20-25g) in groups of five were injected intramuscularly twice (on day 0 and day 42) with 0.1 ml DRV containing 1μg toxoid only or 1μg toxoid and IL-2 co-entrapped or with a mixture of two DRV preparations containing the toxoid (1 μg) and IL-2 respectively. Animals were bled 14 days later and blood plasma assayed (Davis and Gregoriadis, 1987) by ELISA for anti-toxoid IgG_1, IgG_{2a} and IgG_{2b}. Results (ELISA median readings) in Table showing secondary response indicate that the higher (10^4 units; all subclasses) and the lower (10^3 units, IgG_1) dose of IL-2 co-entrapped with the toxoid in the same DRV significantly augment antibody response to the antigens. On the other hand, separately entrapped IL-2 significantly reduces antibody response to the toxoid at the low dose (IgG_1 and IgG_{2b}) and for one of the subclasses (IgG_{2a}) at its high dose. In other experiments mice were injected with 5μg of 3-VP2 entrapped in DSPC DRV either alone or together with 72,000 units IL-2, using an identical protocol. ELISA median readings in sera were 0.123 (IgG_1), 0.144 (IgG_{2a}) and 0.195 (IgG_3) for the liposomal peptide and 0.341 (IgG_1), 0.334 (IgG_{2a}) and 0.323 (IgG_3) for the peptide co-entrapped with IL-2. Kruskall-Wallis statistical analysis revealed significant differences between the two groups ($P<0.09$, IgG_1; $P<0.049$, IgG_{2a}; $P<0.009$, IgG_3). There was no significant difference between the groups when the subclass IgG_{2b} was tested. Results suggest that liposomal IL-2 can either improve or diminish antibody response to the liposomal antigen, depending on the mode of presentation of the mediator and antigen in liposomes and on the IgG subclass tested.

Table: Effect of liposomal interleukin-2 on the immune response to entrapped tetanus toxoid.

Liposomes	IgG_1	IgG_{2a}	IgG_{2b}
A: Entrapped toxoid only	0.359	0.661	0.352
B: Co-entrapped toxoid and IL-2 (10^3 units)	1.591 P<0.01(T=15)	0.242 N.S.(T=21)	0.776 N.S.(T=29)
C: Co-entrapped toxoid and IL-2 (10^4 units)	1.755 P<0.01(T=15)	1.484 P<0.05(T=17)	2.000 P<0.05(T=17)
D: Separately entrapped toxoid and IL-2 (10^3 units)	0.237 N.S.(T=24)	0.130 P<0.05(T=17)	0.073 P<0.05(T=17)
E: Separately entrapped toxoid and IL-2 (10^4 units)	1.234 N.S.(T=18)	1.495 P<0.05(T=16)	0.986 N.S.(T=25)

The Mann-Whitney test was used to compare groups B, C, D, and E with Group A (control). N.S., not significant. T, lower sum of the ranks in pairs of groups compared.

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