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This thesis is directed at understanding the nature and mechanisms underlying immune responses to ligand-carrier conjugates. Haptens and "self" ligands have to be conjugated to "foreign" carriers in order to elicit immune responses to them. This work is aimed at investigating the influence of the carrier on immune responses to ligands conjugated to it. We have chosen the hCG-based anti-fertility vaccine as a model system; this vaccine consists of a heterospecies dimer (HSD) of BhCG and aoLH linked to a foreign carrier, tetanus toxoid (TT). Here we describe the conjugation of HSD to TT and DT, the immunogenicity of the conjugates and detailed investigations on the influences of immunogenetic background and the carrier on responses to the HSD.

Evaluation of the immunogenicity of two carrier conjugates of β-hCG with tetanus toxoid (TT) and diphtheria toxoid (DT).

(a) Conjugation of β-hCG with carriers.

Conjugates of β-hCG with TT and DT were made using hetero bi-functional reagents SPDP, to create -SH groups in the carrier and SMCC to activate β-hCG through free amino groups. This method primarily avoids the homopolymerization of the components used in the conjugation and yields higher BhCG:carrier ratios (upto
10:1). Using this method we have shown that the maximum extent of incorporation of βhCG to these carriers is ten moles to each mole of the carrier. The method also has the merits of ensuring a predictable and reproducible conjugation of the ligand to the carrier.

(b) Testing of different stoichiometries of β-hCG-carryer conjugates in terms of immunogenicity.

Different conjugates were made with varying stoichiometries. The carriers used were TT and DT. Immunogenicity studies showed that conjugates with higher βhCG contents (9.2:1 in the case of βhCG-TT and 8.9:1 for βhCG-DT) are substantially more immunogenic. Anti-carrier antibody levels did not appear to depend on carrier:ligand ratios.

**Regulation, by the carrier, of immune responses to the ligand**

During Phase I clinical trials, it was observed that some of the subjects failed to manifest a anti-hCG booster response upon secondary immunization with the vaccine even though they had initially developed substantial anti-hCG titres after primary immunization. Antibody responses in these women were restored by immunization with the same αoLH-βhCG dimer linked to an alternate carrier. This form of hyporesponsiveness may be attributable to the "epitope-specific suppression" effect.
initially described by Tada et al and Eardley and Sercarz then confirmed and extended by Herzenberg and colleagues and subsequently extended by others. The observed effect is that preimmunization with a carrier (such as TT) often results in an inhibitory effect on the production of antibodies to new epitopes or ligands linked to the same protein. One of the objectives of this study include the investigation of the effects of carrier-preimmunization on anti-hCG and anti-oLH levels after subsequent immunization with the oLH-hCG dimer conjugated to TT.

(a) Effect of presensitization with tetanus toxoid.

A significant reduction in anti-hCG antibody levels was observed in animals which were pre-immunized with the carrier, TT. Interestingly enough, this hyporesponsiveness is directed only at hCG and not at oLH, even though both these ligands are presented in a composite manner, conjugated to the same carrier. This effect appears to correlate with the subclass of IgG antibody response.

(b) Influence of the immunogenetic background.

In order to ascertain whether this carrier-induced hyporesponsiveness is dependent on genetic background, various strains of mice were tested for the presensitization effect with the carrier tetanus toxoid.
Two strains SJL (H-2<sup>S</sup>) and BALB/c (H-2<sup>d</sup>) did show a significant reduction in anti-ligand response upon presensitization.

(c) Delineation of T helper epitopes in tetanus toxin.

Since the carrier is believed to elicit help for the humoral response to the conjugated ligand, an attempt was made to delineate the Th epitope on tetanus toxin for use as a carrier, with the hope that such synthetic epitopes may be devoid of suppressor cell inducing elements. Using computer algorithms and predictions, some putative helper sequences were identified. Selected sequences were synthesized and evaluated in cell proliferation assays. The aminoacid residues 292-311 in the native tetanus toxin was found to be an active Th epitope in SJL mice and a cryptic one in BALB/c mice.

(d) Bypass of carrier-induced suppression by the use of

(i) an alternate carrier, and (ii) a synthetic T helper epitope as carrier.

Two strategies have been evolved in this thesis, for bypassing or circumventing carrier-induced suppression. One strategy is to use an alternate carrier, diphtheria toxoid, DT. Another is to use a Th epitope from TT as a carrier. The animals which were rendered hyporesponsive
by preimmunization with TT, when injected with HSD linked to a T helper epitope bypassed the acquired immuno suppression and give raise to anti-hCG antibodies.

In conclusion, we have shown that the carrier can play a major role in the regulation and modulation of immune responses to ligands linked to it. The various parameters that can affect immune regulation in ligand-carrier systems are the stoichiometry of the ligand to carrier, and the pre-existing immunity to the carrier at the time of immunization with the carrier-ligand conjugate. The hyporesponsiveness imparted by the pre-existing immunity to the carrier can be circumvented by the use of an alternate carrier or a T helper epitope from the same carrier molecule.