REVIEW OF LITERATURE
GONADOTROPIN RELEASING HORMONE (GnRH)

GnRH, also known as luteinizing hormone releasing hormone (LHRH), is a decapeptide released by the hypothalamus. It regulates the secretion of the gonadotropins: luteinizing hormone and follicle stimulating hormone from the pituitary; these gonadotropins in turn act on the gonads to produce gametes and sex steroid hormones. GnRH is thus a "master" molecule, regulating a cascade of events affecting reproduction and steroid hormone production. Immunization against GnRH blocks these processes and, because GnRH is made by both males and females, immunization can be used to influence either male or female fertility. Furthermore, as the molecule is essentially conserved through evolution, immunization against the decapeptide is an effective way of controlling fertility in a variety of mammalian species. This property has been used in studies determining the efficacy and safety of vaccines designed to produce antibodies against GnRH.

ANTI-GnRH VACCINES

Vaccines traditionally mobilize the body's immune system against pathogens bearing "non-self" antigens, a novel variation on this theme being the development of anti-fertility vaccines which aim to counteract a self protein/peptide critical to the success of reproduction.
GnRH being an evolutionarily conserved "self" peptide and a hapten has to be therefore linked to a carrier protein in order to elicit anti-GnRH antibodies. Current concepts of how carrier molecules help in generating anti-haptenic responses in a hapten-carrier system dates back to several decades ago, when Rajewsky et al (1969) and Mitchison (1971a) demonstrated that to obtain an augmented secondary response, the hapten must be conjugated to the same carrier in both immunizations—a phenomenon now referred to as the "carrier effect". At first, immunologists thought that the carrier effect was caused by the simultaneous recognition, by the same cell, of the hapten and part of the carrier, so that when the hapten was presented to the stimulated cells on another carrier, the cells could not recognize it. But later studies proved this explanation to be wrong. Experiments suggested that during an antibody response, the haptenic and carrier portions of the conjugate are recognized separately, but that both types of recognition must occur simultaneously for the response to be initiated. Mitchison injected the hapten-carrier, NIP-OVA (a conjugate of 3-iodo-4-hydroxyl-5-nitrophenylacetic acid and ovalbumin) into mice and then transferred spleen cells from these mice into three groups of irradiated syngeneic recipients. One group of recipients received NIP-OVA, in addition to the cells, while the second and the third groups received NIP-BSA. The third group also received spleen cells from
syngeneic mice immunized against BSA. Testing of the secondary response revealed an increased antibody response in the first and third, but not in the second group. The results obtained in the first and second groups establish the fact that the carrier effect operates following cell transfer; results from the third group demonstrate that the carrier effect can be mediated by the addition of cells sensitized to the homologous carrier. This observation suggests that the hapten and the carrier are recognized by different cells, and that the antibody response is the result of the cooperative effort of at least two cell types, one recognizing the hapten and the other the carrier. This hapten-carrier bridge mechanism suggested for carrier-specific helper activity in these early studies is as viable today as it was when first stated. Further experiments revealed that T cells from the carrier primed spleen cells are responsible for recognizing carrier determinants on the antigen and delivering help to the B cells which recognize the hapten.

In early studies (Arimura et al, 1973; Fraser and Gunn, 1973), GnRH was tagged to bovine serum albumin (BSA) to obtain antibodies reactive with GnRH and the conjugates so prepared required the use of Freund's complete adjuvant (FCA). These studies demonstrated the efficacy of immunization in blocking the fertility of both male and female animals. FCA is not suitable for
use in humans, and alternative immunization strategies therefore had to be evolved in which adequate immune responses could be obtained using adjuvants appropriate for humans. The observation that a conjugate of GnRH with tetanus toxoid (TT) as a carrier could induce anti-GnRH antibodies in mice using as an adjuvant alum, which is approved for human use, laid the basis for a potential vaccine (Shastri et al, 1981).

In the past, 1-ethyl-3(3 dimethylaminopropyl) carbodiimide hydrochloride was used for conjugating GnRH to carrier proteins. As native GnRH does not have free carboxyl or amino groups, linkage is probably effected through the hydroxyl group of serine or tyrosine, or through a carboxylated derivative of histidine. The conjugate made by this method is poorly defined and gives inconsistent results. More efficient conjugation was obtained by diazotization of the molecule, and coupling through histidine and tyrosine residues (Koch et al, 1973; Pick et al, 1978).

The site on GnRH, at which the carrier is attached has a bearing on the reactivity pattern of the antibodies induced. Nett et al (1973) reported the induction of an antibody directed at the amino terminal end of GnRH when the carboxy terminal end of GnRH was conjugated to BSA. Arimura et al (1975) confirmed this observation and also showed that conjugation of GnRH with the carrier
via its amino terminal amino acid results in antisera with reactivity against the carboxy terminal end. The importance of an amide group at the carboxy terminal of the GnRH molecule in the generation of a conformation recognized by polyvalent antibodies is highlighted by studies based on two antisera derived from primates (Singh et al, 1985). However, from the reactivity pattern of a bioeffective monoclonal antibody to GnRH, Talwar et al (1985) concluded that the antigenicity of native GnRH depends on the entire molecule and that the N terminal and C terminal regions are physically close.

Recently, Silversides et al (1988a,b) using analogs of GnRH substituted with cysteine at the carboxy and amino terminals to achieve conjugation to the carrier, keyhole limpet haemocyanin (KLH), have shown that the specificity of antibodies induced by GnRH/carrier conjugates is a function both of the orientation of the peptide antigen and immune regulatory mechanisms of the responding animals. These conclusions are in agreement with the multideterminant regulatory model of antigenicity for protein antigens (Benjamin et al, 1984). Jayashankar et al (1989) linked GnRH to DT through a functional group created by replacing glycine at position six by D-lysine which also increases the biological half-life of GnRH and confers conformational constraints. The ε-amino group of lysine is linked to a spacer, ε-amino caproic acid, which is then conjugated
to DT. This conjugate gives a consistent immunogenic response in rodents and monkeys, probably because the linkage is both flexible and long enough to hold the peptide away from the surface of the carrier.

Immunization of male rodents, rabbits and monkeys against GnRH leads to a block of spermatogenesis. It is also accompanied by a drastic reduction in testosterone levels, but the extratesticular requirements for androgens can be met by injection of a slow-release androgen. Shaha et al (1986) and Ladd et al (1988, 1989) reported that spermatogenesis in male rats and rabbits was suppressed upon immunization with GnRH conjugated to TT. All animals immunized with GnRH-TT were infertile. Administration of testosterone-17-trans-4-n-butyl cyclohexane carboxylate as a supplemental androgen restored libido and mating behavior but did not restore spermatogenesis (Ladd et al, 1988, 1989). Administration of the testosterone ester at the time of primary immunization and then at 10 week intervals did not restore spermatogenesis even though the serum testosterone levels in infertile, GnRH-TT immunized, androgen-treated rats were similar to those in non-immunized, androgen-treated fertile rats.

GnRH analogs that suppress or antagonize the effects of GnRH have found applications in many clinical conditions, such as sex steroid hormone-dependent cancers, endometriosis, etc. That an anti-GnRH vaccine
could be used to block GnRH action was demonstrated by Jayashankar et al (1989). Rats immunized with the GnRH-DT vaccine showed marked atrophy of the testes and the prostate. Every animal responded by making anti-GnRH antibodies. There was a concomitant decline in testosterone levels in every animal. The effect of antibodies was reversed by native GnRH, but not by an analog, indicating that the antibodies bind specifically to the native hormone. Although there was a reduction in the size of the testes and other androgen-sensitive secondary reproductive organs, the size of the prostate was more dramatically reduced.


IMMUNOGENETIC ASPECTS OF THE GnRH VACCINE

The first step in the development of any vaccine includes the demonstration that the vaccine is immunogenic, that it can induce the formation of specific antibodies and/or the generation of specific cell-mediated immunity. Then comes the demonstration that the candidate vaccine elicits a response high enough to carry out its expected function. One important objective is to obtain high titres of antibodies with
high affinity for antigen, using as few doses as possible. Since this vaccine has to be used in humans where one aims for a universal response, immunogenetic influences on the generation of immune response to GnRH needs to be investigated. Research in the field of immunoregulation and immunogenetics in the last two decades has cautioned us about potential immunogenetic considerations, as immunogenetic mechanisms may play decisive roles in immune responses to vaccines. It is now well-established that the Major Histocompatibility Complex (MHC) besides playing roles in the initiation and extent of immune responses, also influence immune responses in such a way that one obtains a high or low response depending on the MHC-type of the immunized individual (Benaceraff and Germain, 1978; Schwartz, 1978; Schwartz et al, 1976). Using inbred strains of mice, McDevitt et al (1972) demonstrated that the response to the synthetic polypeptide (T,G)-A-L is under the control of a single autosomal dominant gene cluster, which was found to be linked to the H-2 complex, the mouse MHC. Using another synthetic antigen, (H,G)-A-L, genetic mapping localized this gene to the I-A subregion of the H-2 complex. After this first demonstration of an MHC-linked immune response (Ir) gene, the response to a large number of T-dependent antigens was found to be under the control of genes located in the I-A and I-E regions of the MHC. These antigens include linear and branched chain synthetic
amino acid copolymers, other antigens such as alloantigens, the male antigen (H-Y), and protein antigens including insulin, hen egg lysozyme (HEL), cytochrome c and myoglobin. It is notable, however, that a strain that is a high responder for a given antigen could be a low responder for another antigen. Thus the MHC-linked Ir genes act in an antigen-specific fashion. It is likely that Ir genes play a role in determining the level of a response to any T-dependent antigen. Since an Ir gene may control the recognition of a given epitope on an antigen, complex antigens provide many determinants subject to Ir gene control.

Analysis of the levels at which Ir genes exert their control on cellular interactions has focussed on Ir genes controlling antigen presentation between macrophages and the T cells and Ir genes regulating T/B cooperation. Rosenthal and Shevach (1973) clearly demonstrated that a T cell proliferative response to a T dependent antigen occurred only if the T cells and APC were derived from guinea pigs of the same strain. In an immune response against an antigen (hapten-carrier) many factors determine the outcome of the antigenic challenge. Not only does the ability of the APC to process and present the antigen or of T-helper cells to recognize carrier determinants determine the level of response but also the amount of T suppressor cell activity generated by priming influence the amount,
allotype and possibly the idiotype of the antibody production. This has been demonstrated by Gammon et al (1987) in studies on HEL and its peptides, N-C, L II and L III. The response to HEL is under Ir gene control and it is possible to demonstrate T cell proliferation and generation of T helper (Th) cells to HEL in responding strains following priming by either the N-C peptide, or the L II peptide. When HEL is used for priming, it is found that Th activity is triggered predominantly by epitopes on the L II peptide. Mice of the H-2b haplotype do not develop this pattern of reactivity indicating a hole in the functional T cell repertoire. Wicker et al (1984); Yowell et al (1979) and Adorini et al (1979) have suggested that the non-responsiveness of certain mouse strains to HEL is due to the development of overriding antigen-specific T suppression. These T suppressor (Ts) cells are generated by the N-C peptide region of HEL. Responder strains primed by N-C peptide develop some Th for HEL while non-responder strains subjected to the same immunization develop Ts cells. Dissection of the molecule revealed that N-terminal was involved in suppression. Amputation of the HEL molecule was attempted to remove the amino terminal lys-val-phe. The residual molecule 4-129 could induce both proliferation and help, indicating that suppressor determinant was localized at the amino terminal end. Therefore, the pattern of overall responsiveness in a
particular mouse strain may reflect selective induction of either Th or Ts.

Gammon et al (1987) have demonstrated that in some cases genes outside the MHC regulate the level of immune response. Factors coded for on chromosome 2 probably influencing some feature of antigen processing and presentation lead to \textit{in vivo} non-responsiveness to HEL in certain H-2^b strains. Hence, a crucial step to be undertaken prior to large scale immunization with candidate vaccines is the investigation of potential immunogenetic variations in responses to that vaccine.

\textbf{CARRIER-INDUCED EPITOPE-SPECIFIC SUPPRESSION}

Hapten-carrier conjugates are somewhat more complex than conventional immunogens, because of the possibility of carrier-induced effects on anti-hapten responses. It is therefore imperative that every hapten-carrier vaccine be studied from this perspective. The epitope-specific system provides a channel through which carrier-specific mechanisms exert control over antibody responses. It plays a key role in regulating antibody responses to haptens and native epitopes on commonly used carrier molecules. The flexibility of this regulatory system and the nature of its effects on \textit{in situ} antibody responses are extraordinary. It is capable of suppressing virtually the entire primary and secondary antibody response to a given epitope. Therefore, it can completely conceal the presence of normal anti-epitope
memory B cell populations clearly demonstrable in adoptive transfer experiments. Carrier-specific and other regulatory mechanisms operative in the immunological environment when an epitope is first introduced determine which components of the antibody response will be suppressed and which will be supported.

Carriers are believed to stimulate T cells that go on to "help" hapten-specific B cells to proliferate and secrete appropriate hapten-specific antibodies. Indeed this was demonstrated a couple of decades ago, when Rajewsky et al (1969) and Mitchison (1971a,b) showed that carrier-primed T cells help hapten-primed B cells to give rise to adoptive secondary antibody responses. Thus, if GnRH were to be linked to say, TT, and the GnRH-TT conjugate used for immunization, the carrier TT presumably would activate helper T cells that would help GnRH-specific B cells to proliferate. A hapten like GnRH can be rendered immunogenic by this process; once a carrier is attached to it, the conjugate has two sets of determinants, one that can interact with B cells and the other with T cells. This is, however, a simplistic hapten-carrier bridge system and it does not take into consideration other intricacies such as suppressor T cells and contrasuppressor T cells.

A simplistic corollary to the concept behind hapten-carrier conjugates may then be proposed. Animals that
have been preimmunized with a carrier before receiving immunization with the same carrier conjugated to a hapten, could be expected to produce a better anti-hapten response than animals that are not preimmunized, since animals preimmunized with the carrier could be expected to have a higher level of helper T cells that would help an anti-hapten response. Indeed early results appear to support this contention (Rajewsky et al, 1969; Mitchison, 1971a,b). Interestingly, the bulk of the subsequent data, and part of the early data itself, support a contrary conclusion; carrier-primed animals often fail to produce high levels of antibodies to haptens introduced as a conjugate of the hapten with the same carrier (Gershon, 1974). Surprisingly enough, carrier-priming was found to suppress, rather than augment, responses to haptens linked to the carrier (Herzenberg et al, 1983). These findings were substantiated in several laboratories, and the underlying mechanisms were (mistakenly) attributed to impaired anti-hapten memory development (Sarvas et al, 1979) or to the presence of anti-carrier antibodies (Katz et al, 1970, Paul et al, 1970).

Gradually there emerged a clearer picture under the broad title of "carrier-induced, epitope-specific regulation". The phenomenon of epitope-specific suppression has been extensively studied in many laboratories using different haptens [DNP, TNP, SODP,
SCB₇, (NANP)₄] and different carriers (KLH, CGG, OVA, TGAL, TT). Suppression is induced by immunizing animals sequentially with a carrier molecule first and then later with the hapten-carrier conjugate. Studies done so far show that epitope-specific suppression can be induced with diverse antigens administered under widely different immunization conditions in a variety of mouse strains. Equally interesting is the finding that while the antibody response to the hapten is suppressed, the anti-carrier responses proceed normally.

Initial experiments relating to this phenomenon have been done by Herzenberg and Tokuhisa (1982) using DNP-KLH as the antigen. They demonstrate that preimmunization with KLH followed by DNP-KLH immunizations lead to an isotype-restricted anti-DNP specific suppression. IgG₂ₐ, IgG₂ₐ and IgG₃ responses are easily suppressed, whereas IgG₁ responses tend to be more resistant. They further go on to show that the individual elements in the epitope-specific system are typically bistable in that they tend to maintain their initially induced regulatory state despite antigenic stimulations that would induce them to the alternate state. That is, when induced to support an antibody response, these elements largely prevent the subsequent induction of that response and when induced to suppress a response they tend to continue to do so despite subsequent immunizations that normally induce.
substantial support for antibody production.

The phenomenon of epitope-specific suppression has been subsequently demonstrated by others. Jacob et al (1985) demonstrated that TT presensitization in rabbits had an inhibitory effect on the subsequent antibody response to the attached hapten CTP$_3$ (synthetic peptide derived from B subunit of cholera toxin). Schutze et al (1985) using TT as a carrier and SODP (a synthetic octadecapeptide of DT) as the hapten observed that sequential immunizations in vivo first with TT and then with SODP-TT resulted in a strong suppression of the IgG antibody response to the hapten SODP. However, the antibody response to the carrier molecule was not suppressed. Lise et al (1987) observed the same phenomenon using TT as the carrier and SCB$_7$ (34 amino acid long peptide representing a repetitive fragment of type 24 streptococcal M protein) as the hapten. Equally interesting is the demonstration by Lise et al (1987) that TT presensitization does not suppress the antibody response to (NANP)$_4$ (four repeats of the tetra peptide sequence found in the circumsporozoite protein of P. falciparum); it actually enhances the anti-peptide antibody response. Hence responses to some epitopes are suppressed, responses to some others are not affected and responses to yet others are actually enhanced upon carrier presensitization.
The priming dose of the carrier has a bearing on the extent of epitopic suppression. Herzenberg and Tokuhisa (1982), Jacob et al (1985) and Schutze et al (1985) using different hapten-carrier systems have demonstrated that suppression in rodents is maximal at 100 μg, lesser at 10 μg and nil at 1 μg dose of the carrier. Herzenberg et al (1983) have also shown that epitope-specific suppression is a very strong and a persisting phenomenon which is inducible in all strains of mice. In their antigen system DNP-KLH, they have observed that the epitopic suppression is detectable even up to one year after carrier presensitization. Furthermore, suppression is demonstrable with the use of a wide range of adjuvant formulations, even in the presence of strong adjuvants like FCA.

Sun and Waltenbaugh (1986) have demonstrated that this regulatory system not only suppresses antibody responses against low molecular weight molecules, but also suppresses responses against macromolecular immunogens. Injections of responder mice with poly (Glu^{60} Ala^{30} Tyr^{10}) (GAT) followed by immunization with GAT-methylated bovine serum albumin (GAT-MBSA) selectively suppresses anti-MBSA plaque forming cell (PFC) and delayed type hypersensitivity (DTH) reactions. Conversely, MBSA injection followed by GAT-MBSA immunization suppresses anti-GAT PFC and DTH, while anti-MBSA responses remain intact. These results
demonstrate that epitope-specific regulation is reciprocal, is not limited to humoral responses, and is not limited to molecules of low molecular weight. The studies of Arunan et al (submitted), on human chorionic gonadotropin (hCG) based birth control vaccine (a conjugate of βhCG and DT or βhCG and TT) also demonstrate that carrier presensitization has an inhibitory effect on the antibody response to hCG, a self macromolecule. The phenomenon of carrier induced epitope-specific suppression has also been studied from the perspective of its effect on a T lymphocyte mediated autoimmune disease, experimental allergic encephalomyelitis (EAE) that develops about 2 weeks following intradermal challenge with purified myelin basic protein (MBP) in FCA (Rauch et al, 1981). A peptide from MBP corresponding to the bovine MBP sequence 114-122, which is highly encephalitogenic in guinea pigs was conjugated to BSA as a carrier and used for EAE challenge. A prechallenge treatment with the BSA carrier alone could significantly inhibit the induction of EAE by (114-122)-BSA conjugate.

Schutze et al (1989a) have also shown that epitope-specific suppression influences the in vivo development of cytotoxic T lymphocytes directed against allogeneic cells. Alloantigen pretreatment induces a down regulation of the in vivo subsequent development of cytotoxic T lymphocytes direct against linked
alloantigens.

Studies done on epitope-specific suppression have demonstrated clearly that this regulatory process affects not only antibody responses in mice but also in other species such as rabbits (Jacob et al, 1985) and even humans (Etlinger et al, 1988; John et al, 1989).

MECHANISMS OF EPITOPE-SPECIFIC SUPPRESSION

Various investigators have tried to unravel the mechanism underlying this phenomenon. Tagawa et al (1984) present evidence to support the induction of suppression by KLH priming to the in situ activity of carrier specific suppressor T cells. Adoptive transfer experiments demonstrate the generation of KLH-specific suppressor T cells which regulate responses by inducing typical isotype-specific suppression for anti-DNP responses when the recipients are immunized with DNP-KLH while the anti-carrier responses proceed normally. Herzenberg et al (1982) go on to prove that anti-hapten memory B cells and carrier-specific helper T cells develop normally in carrier/hapten-carrier immunized mice. Adoptive transfer experiments show that splenic B cell populations from KLH/DNP-KLH immunized mice give rise to adoptive anti-DNP memory responses equivalent in magnitude, affinity and isotype representation to control responses produced by memory B cell populations from mice immunized only with DNP-KLH. The defect
induced in carrier-presensitized mice is speculated to be at the level of expression rather than at development of anti-hapten memory B cells. In other words, failure is caused by the induction of active suppression rather than by a deficit of the requisite cells or by depleting carrier specific help. Galelli and Charlot (1990) examined the status of TNP-specific memory B cells from KLH suppressed mice. Highly enriched populations of TNP-specific memory B cells were purified from the spleen of TNP-KLH (control) or KLH/TNP-KLH (suppressed) immunized mice and tested in vivo for their ability to respond to thymus dependent (TNP-KLH) or thymus independent (TNP-LPS) antigenic challenge in presence of a KLH specific Th cell line. TNP-specific B cells from suppressed mice could be triggered to IgG production by TNP-LPS but had an impaired ability to differentiate into IgG-secreting cells in response to TNP-KLH. The data supports the view that the effector mechanism of epitope specific regulation does not interfere with the development of hapten-specific memory B cells but that these cells have an intrinsic defect that prevents their differentiation into active IgG antibody-secreting cells.

Schutze et al (1987) showed that in in vitro experiments using SODP-TT antigen system, epitope-specific suppression did not arise due to non-specific suppressor phenomenon. Coculture experiments demonstrated that
epitopic suppression was partially mediated by suppressor T cells which specifically inhibited the anti-hapten but not the anti-carrier antibody response. The majority of these cells were shown to possess the CD8\(^+\) phenotype. Apart from the T suppressor population they also demonstrate a deficiency at the B cell level which contributes to the total suppressive effect. B cells from TT/SODP-TT immunized mice were unable to collaborate with carrier-specific helper T cells which suggests that the development of hapten-specific memory B cells has been blocked or that their subsequent differentiation into antibody-secreting cells has been suppressed. Schutze et al (1989b) further go on to rule out the involvement of suppressor T cells and support the view that epitopic suppression arises from antigenic competition through clonal dominance. Immunization with a carrier (TT) induces the expansion of clones specific for the carrier epitopes. Therefore, the hapten-specific clones would have a decreased probability of reacting with the conjugate. Upon increasing the density of the hapten (TNP) on the carrier, epitopic suppression was abrogated. Moreover, priming hapten-specific B cells before carrier/hapten-carrier immunization also abrogates suppression. Furthermore, the in vivo elimination of CD4\(^+\) or CD8\(^+\) T cells using monoclonal antibodies ruled out the involvement of CD8\(^+\) cells in mediating epitopic suppression (Leclerc et al 1990). The in vivo elimination of CD4\(^+\) T cells was found to prevent
the induction but not the expression of epitopic suppression. Based on these findings a regulatory role has been proposed for B cells, where through their capacity to process and present antigen, they would exercise a strong influence on the selection of immune responses.

**BYPASS OF EPITOPE-SPECIFIC SUPPRESSION**

Etlinger et al (1990) have devised a strategy to overcome carrier-induced epitopic suppression using a dominant, "promiscuous" T cell epitope spanning residues 73-99 in the carrier TT. This peptide when linked to a B cell epitope (NANP)₃ and injected in TT-primed and anti-(NANP)₃ suppressed recipients, helped in bypassing suppression and an excellent anti-haptenic antibody response resulted. This peptide was selected because it contains a T helper epitope but lacks B cell stimulatory activity, a potential factor in mediating epitopic suppression. Vaccinologists are beginning to consider the use of "promiscuous" T cell epitopes as a tangible substitute to larger carrier molecules with which are associated problems of epitopic suppression. Panina-Bordignon et al (1989) have reported promiscuous T cell epitopes from the tetanus toxin molecule. These peptides (residues 830-844, 947-967) have been found to be recognized by all primed donors, irrespective of their MHC haplotype. The analysis of specific clones indicate that both the peptides are promiscuous in their
capacity to bind to class II MHC molecules. Another promiscuous T cell epitope has been identified on circumsporozoite protein of *P. falciparum* by Sinigaglia et al. (1988). This peptide (378-398) also has been shown to be recognized by all the primed donors, irrespective of their MHC. Peptides like these could pave the way for totally synthetic B cell determinant-plus-T cell epitope vaccines which would be devoid of epitopic suppression, potentially a major obstacle to "hapten-carrier" vaccines.