REVIEW OF LITERATURE
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Birth Control Vaccines

Theoretically a number of vaccines are feasible for the control of fertility, since the reproductive process is interceptible at a number of points. Events leading to pregnancy starting from the maturation of gametes to fertilization are under the control of two gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones are secreted by the pituitary into the blood stream, through which they travel to the gonads. The fertilized egg and early embryo secrete factors such as chorionic gonadotropin which enable the sustenance of the corpus luteum and make possible the establishment and maintenance of pregnancy during early stages. Thus a number of sites are available for interception by antibodies, and a number of vaccines are possible for the control of fertility.

The basic principle of birth-control vaccines is to generate an immune response that can either inactivate a hormone playing an indispensable role in reproduction or counteract a gamete antigen crucial for the development of the gamete or fertilization. The primary requisite of an anti-fertility vaccine is that the antigen should be unique to the reproductive tract and should have a fertility-related function that can be blocked. The ideal vaccine would not interfere in the process of ovulation
and the production and action of sex hormones. One of the attractive features of birth-control vaccines are that they are relatively free from the risk of user-failure. Condoms for instance suffer from this risk; failure to comply results in unplanned pregnancies. In the case of vaccines, immunization involves the injection of small amounts of the immunogen at given intervals, in far fewer doses compared to contraceptive pills. Thus the body escapes constant drugging by synthetic compounds. Birth-control vaccines directed at hCG, GnRH, FSH, LH, sperm and egg antigens are currently under development.

**Vaccines Based on hCG**

hCG-based vaccines for the control of fertility are at very advanced stages of testing. The principle of these vaccines is to induce antibodies which can neutralize the bioactivity of hCG. hCG plays a crucial role in the maintenance of pregnancy by preventing menstrual shedding and preparing the uterus for implantation of the embryo. The inactivation of hCG therefore would prevent the implantation of the embryo.

Active and passive immunization studies with hCG have proved that hCG-based vaccines are indeed feasible. The administration of anti-hCG antibodies to pregnant baboons resulted in a decline in progesterone levels and in subsequent abortion (Tandon et al 1981). These passively immunized animals eventually resumed regular menstrual
cycles and regained fertility. Active immunization of rodents (Hulme et al 1980) and baboons (Talwar et al 1980) and marmosets (Hearn, 1979) against hCG prevents pregnancy. hCG is composed of two subunits, \( \alpha \) and \( \beta \), of which the \( \alpha \)-subunit is highly homologous to the \( \alpha \)-subunit of LH, FSH and TSH while the \( \beta \) subunit is highly specific to hCG. Thus, the hCG-specific \( \beta \)-subunit or subpart of it have been the immunogens used as for candidate vaccines. \( \beta \)-hCG is a "self" molecule, and is therefore poorly immunogenic in humans; however, presentation to the immune system, of the \( \beta \)-subunit linked to a non-self carrier, bypasses the necessity of T cells specific for hCG, presumably because carrier-specific T cells stimulate \( \beta \)hCG-specific B cells to proliferate and secrete antibodies.

Two series of phase I trials were conducted. In the first trial 63 women were immunized with the prototype vaccine in five countries. The prototype vaccine consisted of \( \beta \)hCG linked to TT (\( \beta \)hCG-TT) (Talwar et al 1976, Das et al 1976, 1978, Shastri et al 1978); the ability of this \( \beta \)hCG-TT conjugate to induce anti-hCG antibodies was confirmed in probing trials (Rose et al 1988, Nash et al 1980, Talwar et al 1986, Sharma et al 1986). This vaccine evoked anti-hCG antibody responses in 61 out of 63 women immunized and the antibodies generated could bioneutralize hCG in vivo and in vitro (Das et al, 1976).
Analysis of several parameters showed no side-effects after vaccination (Gupta et al 1978, Nath et al 1976, Nash et al 1980). The only limitation observed was a large variation in anti-hCG antibody levels in different subjects, those with low antibody levels becoming pregnant.

Efforts were made to enhance the immune response and to reduce individual variations. An adjuvant SPLPS (Sodium pthalyl derivative of lipopolysaccharide) was added to the first injection. Two new vaccine formulations with higher immunogenicity were thus developed. These to either TT or cholera toxin chain B (CHB). In monkeys, a mixture of the βoLH-TT and βhCG-CHB conjugates produced a higher antibody response than βhCG conjugates alone (Talwar et al, 1986). Taking advantage of the fact that βhCG specifically forms a heterospecies dimer (HSD) with αoLH and that this HSD possesses a higher steroidogenic activity (Strickland et al), a new vaccine formulation was developed. This formulation consisted of a mixture of HSD linked to TT or CHB (HSD-TT/CHB), which was more immunogenic in rats and monkeys (Sharma et al 1986, Talwar and Om Singh 1988) and elicited antibody responses with better bioneutralizing capacity (Talwar et al 1988, Pal et al 1990). Phase I clinical trials on these vaccine formulation showed that the HSD formulation was superior to the βhCG-TT vaccine (Talwar et al 1990, Kharat et al 1990).
No side-effects of this immunization was noted. Menstrual regularity was maintained and no correlation between the length of the cycles and anti-hCG antibody titres was observed (Kharat et al 1990). All other clinical and immunopathological parameters were normal (Talwar et al 1990).

There was some indication of local hypersensitivity reactions in some women and this was thought to be due to the use of the same carrier, TT, throughout the immunization schedule. In the subsequent extended phase I trials, diphtheria toxoid was used alternatively as carrier. None of the women in the diversified carrier study manifested hypersensitivity to the vaccine. All immunized women anti-hCG responses without any side-effects. The antibodies generated are effective in neutralizing hCG bioactivity in vitro. hCG challenge tests in vivo demonstrated the ability of these antibodies to scavenge exogenously administered hCG, thereby preventing sustained progesterone production. These results clinched the choice of the HSD formulation for Phase II clinical trials. The aoLH included in this formulation does not induce cross-reactive antibodies to hFSH and hTSH (Om Singhet et al 1989, 1989). Studies by Thau et al 1983, and Thau 1988, have shown that hyperimmunization of 63 monkeys with aoLH in
CFA for a period of five to seven years induced antibodies that cross reacted with LH and hCG, but did not cause any pathological effects on the pituitaries or other organs.
Conjugation of Ligands to Carriers

A variety of approaches have been tried for improving antibody responses to antigens. For example, chemical modification and derivatization of the antigen, e.g., diazotization (Cinader and Dubert 1955), acetylation, picrylation, arsenilation and sulphanilation (Weigle 1962, Deitrich 1966) have been used to enhance the immunogenicity of antigens. It has been shown that the cationization of bovine serum albumin (BSA), produced by substituting the anionic side-chain carboxylic groups with amino ethylene amide generates unique properties (Muckercheide et al 1987). Anti-BSA responses (both humoral and cellular) were found to be much higher when the modified BSA was used and the antibodies were evoked even in the absence of adjuvants. Interestingly enough, mice primed with the modified BSA gave a good response whereas mice primed with native BSA showed a suppressed immune response.

Another approach involves the chemical linkage of the antigen to a carrier molecule of high immunogenicity. Some bacterial polysaccharides which are themselves poorly immunogenic, but useful as vaccines, when conjugated to carriers elicit good antibody responses. This has been proved to be true in the case of Haemophilus influenza type b polysaccharides (Schneerson et al, 1980). Group A, B and C meningococcal
polysaccharides conjugated to tetanus toxoid elicited good immune responses compared to free or unconjugated antigens (Jennings and Lugowsk, 1981). Age-dependent variations in responses to a H. influenzae vaccine (Pincus et al 1982) were eliminated by the use of tetanus toxoid as a carrier protein (Chu et al 1983).

The possibility of using synthetic immunogenic segments of proteins has opened up the potentially rich area of synthetic vaccines. This approach also necessitates the use of carriers since short synthetic peptides generally are very poor B cell immunogens per se. A segment of the bacteriophage MS-2 spanning residues 89-108, which by itself is non-immunogenic, produced virus-neutralizing antibodies in rabbits after conjugation to a synthetic carrier, poly (DL-alanine) (Langbehein et al 1976). Anti-influenza virus responses were obtained by immunization with peptide 91-108 of the haemagglutinin protein conjugated to tetanus toxoid (Muller et al 1982). Similarly, immunization with a peptide comprising residues 186-201 of diphtheria toxin conjugated to a synthetic carrier in aqueous solution produced neutralizing antibodies (Audibert et al 1982). The 35 amino acid carboxy terminal peptide of the beta subunit of hCG which is used as a potential contraceptive vaccine utilizes the complete diphtheria toxoid as a carrier to render it immunogenic. (Stevens et al 1980).
An additional problem posed by antigens such as hCG is that it is a self molecule in humans and it is necessary to break self-tolerance to elicit an anti-hCG immune response. The concept of self and non-self discrimination (Jerne, 1955) explains immunological tolerance or unresponsiveness towards self antigens. Apparently self antigens seen in the context of self MHC are tolerated, whereas self antigens when presented along with a non-self MHC overcome tolerance (Lamb and Feldmann 1984, Rammensee and Bevan 1984, Matzinger et al 1984). Several mechanisms involving the action of T cells in low zone tolerance and the action of T and B cells in high zone tolerance have been put forward. T cell help has been proved to be essential for overcoming natural and induced unresponsiveness (Leech and Mitchison 1976), and the carrier is known to induce T cell help to the ligand attached to it. It is also conceivable that the carrier confers greater affinity for the antigen-presenting cells or for the T cell receptor.

The antigen-presenting cell (APC) processes antigens and presents them to T cells in the context of MHC gene products. In ligand-carrier conjugates it is known that the T cell epitopes of the carrier are primarily important for the immunogenicity of the ligand. In recent years it has been shown that many T cell epitopes are amphipathic in nature. Although the T cell does not recognize the tertiary structure of an antigen the way
antibodies do, the secondary structure of proteins (e.g., amphipathic alpha helix) may still be important (Pincus et al, 1983). The requirement for unfolding in turn derives from the requirement for T cell recognition of antigen in association with MHC molecules on the surface of the APC. Differences in antigen-processing may lead to profound effects on T cell recognition including apparent differences in specificity (Shastri et al, 1984). In a conjugated form it is possible that the self molecule will get processed differently and will be presented successfully, thus overcoming self tolerance.

Furthermore it is possible that proteins in the native structure are in the lowest energy conformation in aqueous solution. For this reason, hydrophobic residues are mostly buried in the internal core of the molecule. However, for such proteins to be stably associated with the plasma membranes of APC, they must be in a form which is stable under a very different environment, namely the amphipathic one created by unfolding and exposing the hydrophobic residues or by conjugating to the carrier which has the amphipathic residues. While this may not be essential for processing of antigen, it may lower the energy barrier for the formation of such structures and interactions. This may help organize the antigen in a form in which it can be stably associated with the membrane of the antigen-presenting cell.
When used as a conjugate, the density of the ligand (B cell determinant) has to be optimized with respect to that of the carrier (T cell epitope). Ligand density in conjugates governs immune responses to ligands (Fernandez and Muller 1977). Overloading of the ligand often results in low responses or inactivation of the primed B cells, thus creating B cell tolerance (Klaus and Humphrey 1975). High ligand density also results in an IgM antibody response and low density leads to IgG responses (Desaymard and Ivanyi 1976). In order to obtain high levels of antibodies, ligand density should be optimal.
Carrier-induced epitope-specific suppression

In a hapten-carrier conjugate system, the carrier induces T cells which provide help to B cells specific for the hapten. Thus if the immune system is first primed with a carrier before ligand-carrier conjugate immunization, one would expect that carrier-primed T cells so formed should provide appropriate help to ligand-specific B cells. Indeed early experiments with dinitrophenyl conjugates of ovalbumin (DNP-OVA) and bovine gamma globulin (DNP-BGG) have shown that carrier priming leads to a higher anti-ligand antibody response (Katz et al. 1970). This property of enhancement of anti-hapten antibody response could be adoptively transferred by T cells (Paul et al. 1970). Subsequent studies however failed to demonstrate this phenomenon universally; on the other hand, a suppression of anti-ligand antibody response was noted in some cases. Eardley and Sercarz (1976) have shown that under defined conditions one can either help or suppress a subsequent response to trinitrophenyl-β galactosidase (TNP-GZ) in CBA/J mice. Optimal helper responses were elicited by priming with 10 μg of GZ priming in 9 days whereas suppression was manifested on day 3 with 100 μg of GZ presensitization. Both helper and suppressor activities for an in vitro IgG anti-hapten p-azophenyl-B-D-lactoside (lac) response to horse erythrocytes (Lac-HRBC) were demonstrated in the same population of carrier HRBC-primed spleen cells. These activities are T cell
dependent and antigen-specific. Dilution analysis of these cells showed that help and suppression are mediated by distinct populations (Chan and Henry 1976). The studies of Herzenberg and colleagues established that carrier presensitization leads to ligand-specific suppression in the DNP-KLH conjugate system. KLH-priming prior to DNP-KLH immunization resulted in reduced production of IgG anti-DNP antibodies without interfering with the anti-KLH response and with the development of anti-DNP memory B cells (Herzenberg et al 1980). Studies designed to trace the induction of this suppression to the in situ activity indicated the presence of carrier-specific T cells (CTC) and further showed that the epitope-specific system constitutes the major, if not the only, effector mechanism through which CTC control antibody production. The characteristics of epitope-specific suppression in these carrier/hapten-carrier immunized animals revealed that they are induced by a carrier-specific mechanism and CTC duplicate this function in adoptive recipients. Thus CTC regulate antibody production by inducing typical epitope-specific suppression rather than by depleting carrier-specific help (Herzenberg and Tokuhisa 1982).

Epitope-specific suppression can be induced with diverse antigens administered under widely different immunization conditions in a variety of mouse strains (Herzenberg et al 1982). Separation of T cells on the basis of CD8
expression and recombination of T cell subsets demonstrated that a single injection of KLH concomitantly generates both helper and suppressor T cells. When these cells are transferred into irradiated recipients along with DNP-primed, purified B cells and DNP-KLH, helper activity was obtained initially but suppression was observed subsequently. Suppression required the addition of CD8+ cells and this suppressor activity existed even after nine months of priming (Langevin et al. 1984). KLH given at high doses in mice induced two sets of suppressor T cells. After the injection, two different pathways of induction, early (24 hr) and late (3 days), are characterized by its sensitivity to cyclophosphamidie (Huchet, 1986).

In the new synthetic vaccine approach only those B cell determinants giving raise to a bioneutralizing antibody production are used instead of the whole molecule. The disadvantage of this method is that the selected epitopes are often small and of low molecular weight, thus necessitating the use of a carrier to render it immunogenic. Tetanus toxoid (TT) serves as a good carrier; it is safe and widely used in humans. However, the use of TT as a carrier is vulnerable to ligand-specific suppression on pre-exposure. Moreover epitopic suppression with TT is not a generalized phenomenon; Lise et al. (1987) and Sad et al. (1991) have shown that pre-
exposure to TT does not necessarily suppress responses to all ligands attached to it. In some cases an actual enhancement, rather than suppression results from carrier-presensitization. Furthermore, epitopic suppression is strain-dependent and not all strains of mice are susceptible to hapten-specific suppression when tested with a given hapten-carrier conjugate. It is therefore quite important that every hapten-carrier conjugate vaccine be individually studied from this perspective.

Mice presensitized to TT subsequently injected with a tandem repeat of circumsporozoite protein (NANP) or streptococcal SCB7 linked to TT {((NANP)₄-TT or SCB₇-TT) showed enhancement of anti-NANP antibodies and suppression of anti-SCB antibodies. Analysis of the isotypic pattern of the anti-peptide response showed that the IgG₂α and IgG₂β subclasses of anti-SCB were suppressed. In contrast the IgG₁ subclass anti-NANP response was enhanced by pre-immunization (Lise et al 1987). A similar effect was observed using synthetic octodecapeptide of diphtheria toxin (SODP) linked to TT (SODP-TT) (Schutze et al 1987). In vitro studies showed that epitope-specific suppression was not caused by non-specific suppressor phenomena. 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. A majority of these T cells were CD8+. The malarial parasite circumsporozoite protein repeat peptide (NANP) conjugated to TT (NANP-TT) in human volunteers was also susceptible to epitope-specific suppression due to pre-exposure to TT. Eleven volunteers were injected with an anti-malaria (Plasmodium falciparum) sporozoite vaccine candidate consisting of a synthetic peptide, Ac-Cys(NANP)₃, coupled to TT and adsorbed on alum. Anti-peptide and anti-TT antibodies increased in all volunteers with the exception of those who had the highest pre-trial anti-TT titres; these individuals failed to produce anti-Ac-Cys-(NANP)₃ or sporozoite antibody. In the three volunteers monitored after the first injection, significant T cell proliferative responses to (NANP)₃ were observed, which increased up to four weeks after immunization, when a second injection was given. Responsiveness then declined to background levels and did not reappear after immunization. In contrast, a marked TT-specific proliferation was observed for the duration of the study (Que et al 1988).

Schutze et al (1989) proposed that epitopic suppression is due to clonal dominance. Immunization with a carrier such as TT induces a clonal expansion of TT-specific B cells, thus decreasing the probability of hapten-specific B cells to react with the antigen. Increasing the density
of the hapten, TNP, on the conjugate totally prevents the induction of the epitopic suppression. Moreover, the use of low hapten-carrier concentrations to challenge carrier-primed mice enhances the induction of suppression. Priming with hapten-specific B cells before carrier/hapten-carrier immunization abrogated suppression. Thus B cells appear to be capable of exercising a strong influence on the selection of immune responses. The cellular basis of this epitopic suppression and also of the suppression induced by high dose of carrier were analyzed by Leclerc et al (1990). In vivo depletion of CD4+ or CD8+ T cells were carried out using monoclonal antibodies (MAb) at the time of carrier-priming or at the time of hapten-carrier immunization. The elimination of CD8+ T cells did not modify the anti-carrier antibody response, regardless of whether this treatment was performed at the time of KLH-priming or during TNP-KLH immunization. Moreover, in vivo treatment with anti-CD8 MAb did not modify carrier-induced epitopic suppression induced either by a low immunogenic dose of KLH or by a high dose of this antigen. The elimination of CD4+ T cells at the time of KLH immunization prevents the induction of a memory response to KLH and abrogates epitope-specific suppression.
Bypass of epitope-specific suppression

Cytokines

The antibody response following priming with a macromolecule or a peptide will depend on regulatory T cells that become activated by the antigenic determinants available. Activation of T helper (Th) and T suppressor (Ts) cells was examined by Shivakumar et al (1989) by comparing native β-galactosidase (GZ) with peptides from the immunodominant region encompassing residues 3-187 of galactosidase. Each immunogen established its own characteristic hierarchy of dominance of determinants within it; in particular GZ and residues 3-187 each induce immunodominant Th cells which could not be induced by residues 60-140. Hierarchies of suppressor determinants are also created and the outcome of immunization shifts with a change in the nature of the immunogen, and the context within which the determinant lies will crucially influence its expression. A particular context presumably determines the likely order of processing of that molecule which leads to a characteristic relationship among the Ts, Th, and B cell determinants involved. This proves that the selection of T cells by peptides is very important in terms of providing help to B cells.

Epitope-specific suppression resulting from carrier priming can be removed by treating mice with IL-1β
Mice immunosuppressed by presensitization with horse red blood cells (HRBC) and subsequently immunized with TNP-HRBC showed normal responses when treated with IL-1β. The antigen-specific immunologic tolerance induced by human gamma globulin (HGG) is abrogated by treating mice with IL-α and IL-β. Furthermore, TNF-α but not IL-6, both of which are IL-1 like in bioactivity, was found to inhibit the induction of tolerance. Although IL-2, IL-4 and IFN-Γ were incapable of directly affecting the induction of tolerance to HGG, IL-4 and IFN-Γ are capable of inhibiting the ability of IL-1 to abrogate tolerance induction. It is known that IL-4 and IFN-Γ inhibit the synthesis of IL-1, thus suggesting that the inhibition of tolerance induction to HGG by IL-1 may involve the stimulation of endogenous IL-1 synthesis (Gahring and Weigle 1990).

**Synthetic Th epitopes**

Unresponsiveness or suppression is often due to the lack of T cell help to the antigen-specific B cells. Studies with uncoupled peptides demonstrated that these peptides can realize their potential as vaccines only if they contain domains that interact with helper T-cell receptors and MHC molecules, in addition to antibody binding sites. Francis et al (1987) have shown that the addition of a helper T cell epitope to a non-immunogenic
B cell epitope determinant can override the barrier of unresponsiveness. For example, the B cell epitope peptide 141-160 from foot and mouth disease virus evokes no antibody response in H-2d mice. This peptide when synthesized with a 'foreign' Th epitope from ovalbumin (323-339) or sperm whale myoglobin (132-148), elicits high virus-neutralizing antibody response in B10.D2 (H-2d) mice. Thus it is possible to use a 'foreign' Th epitope to overcome unresponsiveness.

Tam et al have reported malarial synthetic constructs consisting of T (265-276) and B (93-108) epitopes of CSP with various arrangements. One of these constructs evoked an immune response to the B cell epitope. These epitopes, however have a limitation; they are MHC restricted. Thus they may not be universally immunogenic. For vaccines that are to be used in an outbred population, such restrictions should not exist. The malarial repeat peptide (NANP) when coupled to tuberculin purified protein derivative overcomes H-2 restriction of the antibody response and avoids the need for adjuvants. This approach also led to the production of high titres of anti-NANP antibodies in all nonresponder strains (Lussow et al, 1990). Inasmuch as products of several different MHC alleles differ in their peptide binding capacities, a given peptide can usually bind to a few of the many variants of MHC molecules. The MHC restriction of the T cell epitopes can be circumvented by using so called
"universal" epitopes. These promiscuous T cell epitopes have the ability to bind and stimulate T cell response in most of the individuals immunized with the whole antigen. Such promiscuous T cell epitopes were identified in tetanus toxin (sequences 830-844, 947-967, 580-599, 916-932) (Bordignon et al 1989, Ho et al 1990) and malarial CS protein (sequences 380-396) (Sinigaglia et al 1988). Thus it is possible to use only the T cell epitopes of a carrier to get the desired T helper cell activity in almost all haplotypes. A peptide from tetanus toxin when linked to a B cell epitope from the malarial CS protein (NANP) injected into TT-primed recipients, retained helper activity but was devoid of the suppressor activity inherent in the whole TT molecule. Furthermore, the antibody response to the B cell epitope was enhanced (Etlinger et al 1990).

Thus it may be possible to design a general method for taking advantage of previous vaccinations in the development of new vaccines.