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Gonadotropin-releasing hormone/human chorionic gonadotropin β based recombinant antibodies and vaccines

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ABSTRACT

Gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (hCG) are unique targets for the control of fertility. Immunological approaches to neutralizing these hormones have additional utility in cancer treatment. Vaccines have been developed against both GnRH and hCG and these have undergone Phase I/II clinical trials documenting their safety, reversibility and efficacy. The heterospecies dimer hCG vaccine prevented pregnancy in women of proven fertility without impairment of ovulation or derangement of menstrual regularity and bleeding profiles. The protective threshold of antibody titers to achieve efficacy was determined in these first-ever trials. Recently, a recombinant vaccine against the β subunit of hCG linked to the B subunit of heat labile enterotoxin has been made and expressed as a glycosylated conjugate in *Pichia pastoris*. Experiments indicate its ability to generate antibodies above the protective threshold in all immunized Balb/c mice. Ectopic expression of hCG/hCGβ is observed in many advanced stage cancers of various origins. A chimeric high affinity and specific recombinant antibody against hCGβ linked to curcumin kills hCGβ expressing T lymphoblastic leukemia cells without any deleterious effect. Several synthetic and recombinant vaccines have been developed against GnRH. These reduce serum testosterone to castration levels causing atrophy of the prostate. Three Phase I/II clinical trials conducted in India and Austria have shown that these vaccines elicit non-surgical reduction of testosterone, a fall in prostate specific antigen and clinical improvement of prostate carcinoma patients. A multimer recombinant vaccine against GnRH has high efficacy for sterilization of pigs and other animals.

1. Introduction

The choice of gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (hCG) as targets for development of immunocontraceptive vaccines is based on a number of considerations. Their bio-inactivation can be achieved by *antibodies* without requirement for specific components of the cell mediated immune response. The two hormones travel from their production sites to their action sites via extracellular spaces, hence are accessible to intervention. The presence and titers of bioeffective antibodies can be determined easily in blood.

GnRH is a structurally conserved decapeptide in mammals and laboratory animals can serve as model species for safety and efficacy studies. GnRH is common to both males and females with the result that the vaccine against GnRH will be usable in both sexes. As GnRH acts via gonadotropins FSH and LH to control the production of gametes as well as of sex steroids, the vaccine has potential for control of fertility as well as for sex hormone-dependent male and female cancers.

hCG is made in measurable amounts only in pregnancy, and thus is a reliable indicator of pregnancy. It is not made by healthy males or non-pregnant females except for its

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ectopic expression in a variety of cancers at advanced stage (Iles, 2007). Hence, an immune response against hCG should be devoid of side reactions with other tissues. The synthesis and secretion of hCG commence at early embryonic stage and the hormone is measurable in culture fluid of *in vitro* fertilized eggs (Fishel et al., 1984). It plays a critical role in implantation; marmosets embryos exposed to anti-hCGβ antibodies do not implant (Hearn et al., 1988). The same is true for humans—women protected from becoming pregnant by circulating antibodies do not have a longer luteal phase of the cycle (Talwar et al., 1994). The antibodies against hCGβ thus prevent implantation without impairing ovulation and normal production of native reproductive hormones by the female. This property imparts a unique importance to the anti-hCG vaccine as a contraceptive.

2. Anti-hCG vaccine(s)

2.1. Rendering the "self" hormone immunogenic

Immunogenicity in hCG was achieved by linking the hormone to a carrier to mobilize T cell help. Initially the β-subunit of hCG was linked to tetanus toxoid (TT). The conjugate induced both anti-hCG and anti-tetanus antibodies in elective tubectomised women of reproductive age (Talwar et al., 1976). On *in vivo* challenge with purified hCG, the antibodies bound to hCG and reduced titers proportionate to the dose of hCG administered. This occurred without resulting in a booster response, suggesting the non-likelihood of auto-stimulation by endogenous hCG. These probing studies provided justification for formulating a vaccine employing TT as carrier. The concomitant generation of anti-tetanus antibodies was useful in preventing post delivery death of women due to tetanus, which in developing countries accounted for a substantial mortality in those years. Repeated immunization with TT however, resulted in carrier induced suppression of immune response to the ligand, hCGβ, which can be prevented by use of alternate carriers, diphtheria toxoid (DT) or cholera toxin B-subunit (Gaur et al., 1990).

2.2. Carboxy terminal peptide (CTP) or entire β-subunit

Controversies prevailed for many years on using the entire hCGβ, as there is a significant homology at amino acid level between hCGβ and the β-subunit of human luteinizing hormone (hLHβ). Vernon Stevens supported by WHO Task Force vouched persistently for a hCGβ vaccine based on C-terminal peptide (CTP) (Steven et al., 1981). Our decision to use hCGβ was based on the following experimental facts:

(i) It induced *conformation-reading* antibodies of high affinity, Ka $10^{10}\,M^{-1}$ or more, in contrast to CTP vaccine which generated *sequence-reading* antibodies of lower affinity, Ka $10^{8-9}\,M^{-1}$. As the Ka of hCG for its receptor is of the order of $10^{9}\,M^{-1}$, the bioefficacy of the two types of antibodies are vastly different. The extension of CTP to 45 amino acids or to 53 carboxy terminal peptides improved the affinity and bioneutralization capacity but these were still inferior to those generated by the entire subunit (Ramakrishnan et al., 1979; Sahal et al., 1982). In fact to improve further the bioneutralization capacity and simultaneously enhance immunogenicity, we joined non-covalently hCGβ to the alpha subunit of ovine LH. This heterospecies dimer (HSD) was fully safe as confirmed by Phase I safety trials (Kharat et al., 1990; Talwar et al., 1990). The safety of hCGβ-TT was established by extensive studies (see the entire February 1976 issue of 'CONTRACEPTION', **13**: 129–268) and was independently confirmed by Phase I trials in Sweden, Finland, Chile and Brazil by the International Committee on Contraception Research of the Population Council, New York (Nash et al., 1980).

(ii) The CTP based vaccine required use of a strong adjuvant based on squalene and Arlacel A for production of antibodies, which causes granulomas and may not be acceptable for human use. Indeed, the trial in Sweden with the Stevens-WHO vaccine was aborted due to serious side effects noted in the first seven subjects. The expected safety of the CTP vaccine was questioned by the immuno-reactivity of the CTP induced antibodies with pancreatic cells, presumably due to its sequence-reading character, as the conformation-reading antibodies generated by hCGβ-TT were devoid of such immuno-reactivity (Rose et al., 1988).

2.3. Efficacy trials

Phase II trials with the HSD-TT/DT vaccine were conducted in 148 women of proven fertility with two living children and one or more Medical Termination of Pregnancy (MTP). Out of 148 immunized women, 110 (74%) made antibodies above a protective threshold titer of 50 ng/ml and 60% of women maintained these for three months or longer. Women became readily pregnant in the absence of boosters upon antibodies declining to 35 ng/ml or lower levels. The progeny born to such women were normal in their development and cognitive abilities as compared to their siblings (Singh et al., 1998). All women kept ovulating and had regular menstrual cycles with normal bleeding profiles (Talwar et al., 1994, 1997). Women had the option to take boosters to keep themselves protected from pregnancy. Eight women completed more than 30 cycles, nine completed 24–29 cycles, 12 completed 18–23 cycles, 15 completed 12–17 cycles and 21 women completed 6–11 cycles without becoming pregnant. The vaccine was highly protective at antibody titers above 50 ng/ml, only one pregnancy took place in 1224 cycles.

These trials, the first of their kind for any birth control vaccine have indicated the potential of the hCG vaccine for fertility control. It can prevent pregnancy without impairment of ovulation and derangement of menstrual regularity and bleeding profiles. The shortcoming of the vaccine was the non-production of antibody titers at 50 ng/ml or above in near to 100% of recipients.

2.4. Revival of the hCG vaccine

Two years ago, research on the hCG vaccine was resumed under an Indo-US project. A recombinant vac-