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Fertility regulating and immunotherapeutic vaccines reaching human trials stage

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The progress and current status of vaccines which induce antibodies against human chorionic gonadotrophin (HCG) and luteinizing hormone-releasing hormone (LHRH) are reviewed. Three vaccines devised against HCG have undergone phase I clinical trials documenting their safety, and reversibility. One of these, the hetero-species dimer (HSD)–HCG vaccine has also completed phase II efficacy trials in sexually active women of proven fertility. Immunization with the vaccine prevents pregnancy, as long as the antibody titres remain ≥50 ng/ml HCG bioneutralization capacity. There is no disturbance of menstrual regularity and women continue to ovulate normally. The antibody response is predominantly againsy an epitope in the core part of β-HCG. Fertility is regained at titres <35 ng. These observations have laid the scientific foundations of a birth control vaccine. Research suggests the feasibility of making a cost-effective recombinant vaccine. The carriers tetanus toxoid (TT) and diptheria toxoid (DT) can be advantageously replaced by peptide determinants recognizing T, not B cells. In addition to optional fertility control, HCG vaccines may have tumour growth inhibition potential in lung cancers which produce HCG. The vaccine against LHRH can be used in both males and females. As it is a structurally conserved molecule, the same vaccine is applicable to both animals and humans. Antibodies against LHRH block the generation of gametes and sex steroids, with the result that the vaccine can be used for fertility control (domestic pets, prolongation of lactation amenorrhoea); as well as for sex hormone-dependent cancers. Phase I/phase II clinical trials have been conducted with the LHRH vaccine in advanced metastazing carcinoma of prostate patients with encouraging results. Bioeffective monoclonal antibodies have been developed against both LHRH and HCG. These can be 'humanized' and produced cost-effectively in bacteria and plants, thus paving the way for passive use of such antibodies for immunotherapy of cancers and fertility control.

Key words: fertility control/HCG/human trials/immunization/LHRH

Introduction

Research into birth control vaccines began with the conviction that the currently available contraceptive methods do not suit all those seeking family planning. Fertility regulating vaccine(s), if feasible, would meet with the perceived requirements of: (i) reversibility; (ii) periodic intake; (iii) freedom from the risk of user failure; (iv) impairment of menstrual regularity or increased bleeding; and (v) no blockade of ovulation or normal production of sex hormones by the woman. The use of these vaccines would avoid the pharmacological intake of synthetic steroids and would, hopefully, be admissible at all stages of reproductive life, whether nulliparous [where intra-uterine devices (IUDs) are contra-indicated] or for women aged >30 years (especially in the case of smokers, where contraceptive steroids entail a significantly higher risk of cancer). These requirements are fulfilled by vaccines raised against human chorionic gonadotrophin (HCG).

The possibility of causing a fertility block by immunization with different antigens became evident in experimental animals by a variety of studies. Clinical reports had also discussed infertility in humans ascribable to

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immunological factors. However, little was known about how these individuals developed an immunological block, nor how it could be reversed reliably. Experimental approaches to the production of birth control vaccines for the deliberate blocking of fertility had to ensure that reversibility was not compromised; today, immuno-contraception is an active field of research. There are 27 vaccines for regulating fertility under development (Talwar, 1997). Six of these, all directed against reproductive tract hormones, have undergone Phase I clinical trials (see Table I) and one, the HSD–HCG vaccine, has also completed successfully Phase II efficacy studies (Talwar *et al.*, 1994), providing evidence for the feasibility of safe, reversible and effective vaccination for control of fertility. Three of the seven vaccines are targeted against HCG, two against luteinizing hormone-releasing hormone (LHRH) and one against follicle stimulating hormone (FSH). Moudgal *et al*.(1997) discuss FSH in this issue. This article will, therefore, be restricted to a review of the current status of the vaccines against HCG and LHRH/gonadotrophin-releasing hormone (GnRH). An additional application of these vaccines is in cases of hormone-dependent cancers, e.g. carcinoma of the prostate (LHRH vaccine) and lung cancers which synthesize HCG (HCG vaccine).

Principles

The rationale of birth control vaccines is to induce the formation of antibodies and or elicitation of cell mediated immunity (CMI), competent to intercept the action of a hormone or a gamete antigen crucial to the success of reproduction. Mammalian reproduction is regulated by a cascade of hormones, beginning with LHRH (GnRH) from the hypothalamus, which controls the secretion of the gonadotrophins FSH and LH from the pituitary. FSH and LH in turn act on the gonads to generate spermatozoa or oocytes (depending on sex) as well as sex steroids: testosterone (in males) and oestrogens and progesterone (in females). The sex steroids act on accessory reproductive organs preparing the uterus for nidation of the embryo. Testosterone has a role in development and maturation of sperms. Both spermatozoa and oocytes have gamete-specific antigens, and antibodies against these can prevent sperm penetration. Fertilization of the oocyte leads to the synthesis and secretion of HCG, an early signal of pregnancy which is essential for its establishment. It is obvious that there are numerous points for interception and theoretically several vaccines can be devised for the control of fertility.

Table I. Birth control vaccines reaching phase I trials in women

No.	Vaccine	Adjuvant	Directed against	Status	Investigators
1	HSD–HCG *	Adsorbed on alhydrogel + SPLPS	HCG	Multicentric phase I safety trials completed*	Talwar et al. (1994)
2	bHCG–TT	Alum	HCG	Phase I clinical trials compleled in India, Finland, Chile, Brazil and Dominican Republic	Talwar *et al.* (1976a); Nash *et al.* (1980)
3	CTP–DT	Squalene Arlacel A Nor–MDP	HCG	Phase I trial completed in one centre in Australia	Stevens *et al.* (1981a,b)
4	LHRH–TT	Alum Pluronic Span Tween	LHRH	Phase I safety and immunogenicity trial in USA in orchiectomized men	Ladd et al. (1985)
5	LHRH–DT	Alum + SPLPS	LHRH	Phase I/II trials conducted in advanced stage of carcinoma prostate patients in India and Austria	Talwar *et al.* (1997b)
6	oFSH	alum (alhydrogel)	FSH	Phase I clinical trial in progress in India	Moudgil *et al.* (1997)

*HSD–HCG vaccine is the first to have also completed Phase II efficancy trials in women providing evidence for the the ability of the vaccine to prevent pregnancy in fertile women at antibody titres of ≥50 ng/ml.
HSD = HCG = human chorionic gonadotrophin; SPLPS = sodium phthalyl derivative of lipopolysaccharide from *Staphylococcus enteridis*; TT = tetanus toxoid; CTP = carboxy terminal peptide; DT = diptheria toxoid; LHRH = luteinizing hormone-releasing hormone; oFSH = ovine follicle stimulating hormone.

HCG vaccine

The choice of HCG as the preferred target of a contraceptive vaccine was based on the following considerations. HCG synthesis and secretion (summarized in Figure 1) is unique to pregnancy (excluding some cancers). Although very, very small amounts are made by the pituitary, this is inadequate to exercise any biological effect, or to cause interference in diagnosis of pregnancy based on detection of HCG. The synthesis of HCG begins in the preimplantation embryo (Fishel *et al.*, 1984). The hormone is required for the initiation of pregnancy, which begins with the attachment of the embryo to the uterus. Evidence for this contention is derived from two types of data. Marmoset embryos exposed to anti-HCG antibodies failed to implant (Hearn *et al.*, 1988). Women who were protected from becoming pregnant by virtue of anti-HCG antibodies in their circulation did not have lengthened luteal phase (Talwar *et al.*, 1997a), indicating that interception by the anti-HCG antibodies preceded implantation. The HCG vaccine is thus not an abortifacient. It imposes a preimplantation, though post-fertilization, block in the establishment of pregnancy. By targeting the immune response to HCG, the normal physiological functions of ovulation and synthesis and the secretion of native sex hormones are not interfered with. The chemistry of HCG is known, as is the primary amino acid sequence of the two subunits composing this hormone. At the time that HCG was chosen, it was available in adequate amounts from the urine of pregnant women and could be purified. This was not possible for the gamete antigens; DNA recombinant technology has today removed this limitation.

Three alternative vaccines devised

HCG, like FSH, LH and thyroid-stimulating hormone (TSH) is made up of two subunits; the α subunit is common to all and the β subunit in each case confers hormonal specificity. Either the entire β subunit of HCG or a subpart of it can be used as the immunogen. Stevens *et al.* (1981a,b) opted for the 37 amino acid carboxy terminal peptide (CTP) of β-HCG, whereas Talwar *et al.* (1976a) decided to use the entire β-HCG. CTP has the merit of being specific to β-HCG, since it is not present in β-hLH. However, it was found to be a poor immunogen and antibodies generated by CTP were not fully bioeffective *in vivo* (Ohashi *et al.*, 1980; G.Talwar, unpublished data). Attempts were made to improve on these shortcomings by lengthening of the CTP to 45 amino acids (Ramakrishnan *et al.*, 1979) and 53 amino acids (Sahal *et al.*, 1982). This improved the bioneutralization capacity of the antibodies. However,

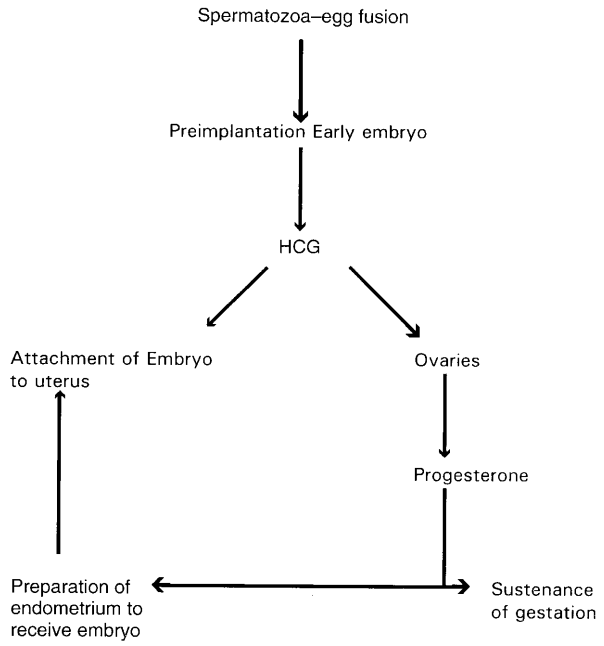

Figure 1. Human chorionic gonadotrophin (HCG) is an early chemical signal of conception. It is essential for both establishment and maintenance of pregnancy. A vaccine generating bioneutralizing antibodies against HCG would prevent implantation and the start of pregnancy. Anti-HCG antibodies given passively in the first 7 weeks of pregnancy would abrogate gestation. In addition, HCG is also made ectopically by a number of cancers.

there remained a large difference. Whereas 0.1 ml of the antiserum raised with the entire β-HCG molecule at moderate titre of 120 neutralized 200 μIU of HCG, a 726 times greater amount of anti-CTP-45 of high titre (3490) and a 267 times greater amount of the anti-CTP-53 were required for similar action.

Thus two vaccines were initially developed, derived from 37 amino acid CTP of β-HCG linked to diptheria toxoid (DT) and β-HCG linked to tetanus toxoid (TT). Linkage to a carrier was necessary to enhance immunogenicity and to overcome the immune tolerance to HCG expected in humans. The choice of TT had another advantage; competitive immunoassays using an antibody raised against the native tetanus toxoid demonstrated that it conferred simultaneous protection against tetanus. This disease accounts for a high mortality of women and newborn in India, where not all deliveries take place in aseptic maternity clinics. The CTP vaccine required the use of strong adjuvants (squalene, Arlacel A, or MDP) (Stevens *et al.*, 1981a,b), whereas the β-HCG–TT vaccine was administered adsorbed on alum.

Preliminary phase I clinical trials in women who had elective tubal ligation showed that β-HCG–TT vaccine was indeed able to generate anti-HCG and anti-tetanus antibodies

(Talwar *et al.*, 1976a,b). The response was reversible and antibodies declined to near zero concentrations in the course of time. The antibodies recognized HCG and bound to it, as shown by challenge studies in which 5000 IU of HCG was given as a single bolus or as two consecutive daily administrations of 2000 and 4000 IU, thus simulating early pregnancy. The antibody titres generated by this vaccine in the subjects tested were high enough to cope with the expected HCG concentrations at the onset of pregnancy. The antibodies consumed by challenge with HCG were replenished to the values prevailing at the time of challenge within 3 weeks, signifying firstly, that HCG by itself did not act as a booster of immune response (for that β-HCG linked to carrier was necessary), and secondly, the antibodies would be available to meet HCG challenge, were pregnancies to occur in consecutive months. No undesirable side-effects of immunization were noticeable from the endocrine, metabolic and haematological parameters (Kumar *et al.*, 1976), and no auto antibodies indicative of an immuno-pathological nature were generated (Nath *et al.*, 1976).

Phase I clinical trials with the β-HCG–TT vaccine were also carried out in Finland, Sweden, Chile and Brazil under the auspices of the International Committee on Contraception Research of the Population Council. The findings were similar and confirmed the results of the trials conducted in India (Nash *et al.*, 1980). Regular menstrual cyclicity was maintained and the women continued to ovulate. The main drawback of this vaccine was the large variability of antibody titres amongst individuals. Those with low titres would not be protected from becoming pregnant. Attempts were then made to further improve the immunogenicity of the β-HCG–TT vaccine.

Improved HSD–HCG vaccine

To enhance the intrinsic immunogenecity of the vaccine, it was made partly 'foreign' by associating the β-HCG molecule with an α subunit of ovine origin (Talwar *et al.*, 1988). The ability of the subunits to bind is conserved across species. The heterospecies dimer (HSD) thus created was found superior to HCG for stimulation of steroidogenesis in a Leydig cell bioassay. Its immunogenicity was enhanced (Singh *et al.*, 1989). The bioneutralization capacity of antibodies per unit of immunoreactive antibody titres was also higher (Pal *et al.*, 1990).

After extensive toxicology studies, Drugs Regulatory Approvals and clearance from Institutional Ethics Committees, comparative Phase I clinical trials were conducted with the original β-HCG–TT and the improved HSD–TT vaccines in five centres in India. Antibodies were generated in every subject and the titres were higher with the HSD vaccine. In both cases, the titres came down near to zero levels in the course of time in absence of boosters. Blood chemistry and haematology profiles remained essentially unchanged over 0, 6 and 12 month observation periods (Talwar *et al.*, 1990). Menstrual cycle lengths were similar in control and immunized women with >85% cycles of normal duration. No correlation was detectable in longer or shorter cycles with the degree of cross-reaction of the antibodies with human luteinizing hormone (hLH) (Kharat *et al.*, 1990; Talwar *et al.*, 1990).

Phase II efficacy studies with the HSD vaccine

These were conducted in three major medical institutions in India, the All India Institute of Medical Sciences and Safdarjung Hospital, New Delhi and the Postgraduate Institute of Medical Education and Research, Chandigarh. 148 women attending the family planning clinics in these Institutions were enrolled for the study following written consent. All were of proven fertility with two live children and were sexually active. They had regular ovulatory cycles and their husbands' semen parameters were good. They were required to receive the three primary immunization injections at monthly intervals. Boosters were optional to those deciding to continue in the study. Blood samples were obtained in the third week of the cycle to determine the antibody titres and the mid-luteal progesterone concentration. A threshold of 50 ng/ml of HCG bioneutralization capacity was fixed to determine whether women were protected at this or higher titres. Once this threshold was reached, IUDs were removed and the subjects were exposed to the risk of pregnancy without the use of any other form of contraception. Observations were to be recorded for a minimum of 750 cycles. Each woman kept a menstrual diary and a record of the occurrence of intercourse. A visit to the clinic each month soon after the menstrual period was scheduled for a general check up. In cases of delayed menstruation, a pregnancy test was performed, and repeated the following week, if necessary.

All subjects immunized with the vaccine produced anti-HCG antibodies. In all, 119 women (80%) had titres >50 ng/ml, nine of these had a subsequent rapid fall in titre and were not considered appropriate for sustained observation. Only one pregnancy occurred in the women with titres ≥50 ng/ml over 1224 cycles of observation (Talwar *et al.*, 1994). The duration of protective antibody titres following a booster was for an average of 3 months (range, 6 weeks to 6 months). During the period of declining antibody titres, no lengthening of menstrual period was noted, confirming the lack of abortifacient

action of the antibody. The study was continued beyond 750 cycles at the behest of subjects who had volunteered for the study and who wished to continue. The method was thus highly effective, so long as the antibody titres remained above this threshold. The ability of antibodies generated by the vaccine to prevent pregnancy was confirmed by post-coital tests conducted in midcycle when the cervical score was high (Talwar *et al.*, 1997a).

The contraceptive effect of the vaccine was fully reversible. Women regained fertility with the decline of antibodies to <35 ng/ml. Termination of pregnancy was offered as per protocol free of charge. Four women who desired another child continued their pregnancies to full term and gave birth to healthy normal children.

Immunodominant epitopes

A panel of monoclonal antibodies (MoAb) recognizing different regions of β-HCG and whole HCG were employed to determine, by competitive immunoassay, the determinants against which antibodies were raised in women immunized with the vaccine. It was surprising to note that all sera tested had antibodies corresponding to an epitope denoted by either MoAb P_3W_{80} or MoAb 206. These two monoclonals cross-react with each other, although one of them is highly specific for HCG and the other cross-reactive with hLH. The region in which these monoclonals map is a dominant core region with differing micro-specificities. The preponderance of antibodies corresponding to this immuno-determinant in the women's sera ranged from 20 to >70% of the total anti-HCG antibodies (Deshmukh *et al.*, 1993). The proportion of such antibodies in a given subject remained constant following booster immunizations. No significant antibody activity was observed against the carboxyterminal peptide or the 38–57 synthetic fragment of β-HCG (Deshmukh *et al.*, 1994). These findings indicate the possibility of using a subpart of the β-HCG molecule as immunogen. However, the dominant determinant(s) in the response of humans to this vaccine is not in the CTP or the 38–57 region.

Status of the CTP–HCG vaccine

This vaccine is being developed by Stevens *et al.* (1981a) with the support of WHO. It consists of a 37 amino acid CTP of β-HCG linked to DT and employs squalene, Arlacel A and norMDP as adjuvants (Stevens *et al.*, 1981b). The vaccine has undergone Phase I clinical trials in a clinic in Australia. It was tested at multiple doses in 20 tubal ligated women with 10 serving as controls. No significant side-effects were reported (Jones *et al.*, 1988). Phase II clinical trials were planned with this vaccine in Sweden. However, the first group of women receiving the vaccine developed unacceptable reactions, and the trials were discontinued. Serious doubts have been expressed on the workability and efficacy of this vaccine. Dirnhofer *et al.*, 1993) have reported data discounting the ability of this vaccine to prevent pregnancy. The peak titres attained in women (during the pahse I clinical trial) with the CTP vaccine were 36–127 ng/ml; with β-HCG–TT, 120–1800 ng/ml and with HSD–TT/DT 222–6000 ng/ml. The avidity of the antibodies produced by the CTP vaccine was 10^8/M, whereas for both β-HCG-TT and HSD-TT/DT, it was 10^{10}/M. Given that the affinity of HCG for tissue receptors is of the order of 10^9, it is doubtful whether antibodies of lower avidity would prevent HCG binding to ovarian receptors.

The main reason for choosing CTP as immunogen was to avoid cross-reaction with hLH. Indeed the antibodies do not react with hLH. However, the CTP–DT-induced antibodies showed an unexpected reactivity with pancreatic cells (Rose *et al.*, 1988). This was not due to the carrier DT, but to the CTP, as it was absorbable by the CTP. It is quite possible that this vaccine generates sequence-reading antibodies and common stretches of amino acids occur in several proteins. On the other hand, antibodies generated by β-HCG–TT and HSD–TT/DT had no reactivity with pancreas or other human tissues (India, S.Sehgal unpublished data; USA, N.Rose and L.Burek, unpublished data). Nevertheless, these antibodies had partial cross-reactivity with hLH, of 10–75% (Talwar *et al.*, 1994).

Implications of cross-reactivity with hLH

Both β-HCG–TT and HSD–TT/DT vaccines produce antibodies which are cross-reactive with hLH. Several studies have been carried out at the Population Council, New York and in our laboratory at New Delhi to determine whether this cross-reaction causes any detrimental effects. Neither monkeys nor baboons ceased ovulating after immunization with these vaccines. In case it was due to a low cross-reactivity of antibodies with species LH, an analogous immunogen, β-oLH was employed, which produced antibodies which were frankly cross-reactive with monkey LH and monkey CG. Hyperimmunization was carried out employing Freund's complete adjuvant to heighten any noxious effect of such antibodies. After 7 years exposure to such antibodies, an autopsy was performed and tissues examined by three experienced pathologists. No deleterious effect on pituitary or other tissues of the animals was found (Thau *et al.*, 1987). Another study used the protocol of challenging repeatedly with HCG, monkeys previously immunized with the β-HCG–TT vaccine. The deposition of immune

complexes in sensitive tissues was then analysed. No deleterious immune complexes were detected in kidney, choroid plexus and pituitary (Gupta *et al.*, 1978).

Ovulation continued in primates over the prolonged period of the experimental studies and also in humans examined during phase I clinical trials with β-HCG-based vaccines in India, Chile, Dominican Republic and Finland, and in phase II clinical trials in India. This was in spite of low to medium cross-reactivity of antibodies with hLH, which was concluded from the luteal progesterone, ultrasonography and complete profiles of FSH, LH, oestradiol and progesterone determined in several subjects in centres abroad. It is quite likely that the LH surge taking place in mid cycle once a month produces amounts of the hormone surplus to that required for ovulation, which may be the reason for continuation of ovulation in immunized subjects. In monkeys hyper-immunized with β-oLH, ovulation was maintained since they were known to become pregnant after provision of progestational hormone cover (Thau and Sundaram, 1980). However, a deficient luteal phase was apparent, from the reduced area under the progesterone curve in hyperimmunized monkeys (in comparison with control animals). A similar observation was made in women with high antibody titres. Luteal insufficiency may contribute to infertility during the phase of high anti-HCG titres, over and above the neutralizing effect of antibodies on the bioactivity of HCG.

Where do we go from here?

At present HSD–HCG vaccine is the best of the three vaccines developed against HCG. It has the highest immunogenicity; it is free from side-effects; it is reversible and competent to prevent pregnancy at and above a threshold titre. It does not disturb ovulation or menstrual regularity and causes no excessive bleeding. It provides the scientific foundations for a birth control vaccine. It is, however, not yet ready as a product to be used on a large scale. Further research and development is required to meet the following requirements.

Firstly, the three injections currently given with the present formulation at monthly intervals must be reduced to a single contact point delivery of all doses to avoid subjects receiving incomplete immunization. This seems to be technically feasible. Biodegradable delivery systems for vaccines are at an advanced stage of development. The type of polymers used for the microspheres enables delivery of the antigen for long periods ranging from 6 months to ≥1 year.

Secondly, the present formulation generated above threshold protective antibody titres in 70–80% of recepients. Further improvement is required to enhance the percentage of high responders. This may be possible by inclusion of an adjuvant. Encapsulation of the vaccine in microspheres may also increase the quantum of immune response.

Thirdly, repeated immunization with tetanus as carrier causes carrier induced immunosuppression in a percentage of subjects (Gaur *et al.*, 1990). This can be avoided by replacement of TT and DT by T non-B peptides. A cocktail of three such 'promiscuous' peptides used as carriers instead of DT has given high antibody response in several genetic strains of mice (Talwar *et al.*, 1997a).

Fourthly, to be cost effective, reproducible and manufacturable on a large scale, the vaccine has to be made by DNA recombinant technology. β-HCG has been co-expressed with the carrier in *Escerichia coli*, *V.cholera*, baculovirus and vaccinia (for references see Recombinant and Synthetic Vaccines 1994). A construct of β-HCG along with 48 amino acid membrane sequences in an attenuated strain of vaccinia is highly immunogenic in rodents (Srinivasan *et al.*, 1995). Monkeys, immunized by this vaccine and boosted at 8–9 months by an ordinary non-vaccinia booster, have generated high antibody responses of long duration, during which the animals do not become pregnant. More recent developments indicate the possibility of using just the DNA with appropriate eukaryotic promoters as an effective way of sensitizing the immune response without need of a live vector.

HCG vaccine in HCG-synthesizing cancers

While HCG is traditionally linked with pregnancy and trophoblastic tumours, several reports have appeared concerning the ectopic synthesis of this hormone in a variety of tumours (Acevado *et al.*, 1995). Metastasizing tumours in particular have been observed by Acevado and others to produce β-HCG. Some years back we carried out studies with Debajit Biswas of Harvard Medical School on a human cell line (Chago) developed from a patient with lung cancer which secreted subunits of HCG. The growth of these cells in soft agar was strongly inhibited by antibodies against HCG and α-HCG. Nude athymic mice implanted with the Chago cells experienced necrosis of the tumour on passive administration of antibodies. When the antibody treatment was instituted right from the point of implantation of cells, the tumour growth was inhibited in a dose-dependent manner (Kumar *et al.*, 1991). At present no effective chemotherapy is available for HCG-synthesizing lung cancers. Preliminary clinical trials, undertaken in three well-characterized lung cancer patients in Mexico in collaboration with Prof. Carlos Gual, showed

that the vaccine was well tolerated (unpublished data). Serum β-HCG concentrations declined to near zero levels after immunization. Patients experienced clinical well being and, although previous lesions did not regress as observed by radiography, no fresh metastases were noted during 2.5 years of follow up. These studies need to be extended to more patients to draw conclusions.

LHRH (GnRH) vaccines

This decapeptide is the master hormone controlling the secretion of the gonadotrophins FSH and LH, which in turn lead to gamete generation and sex steroids secretion (see Figure 2). Its inhibition by antibodies would thus block fertility as well as the secretion of sex steroids. LHRH is common to both males and females, so that the LHRH vaccine would have an application in both sexes. Furthermore, its structure is essentially conserved in mammals, hence the vaccine would be usable in the control of fertility in different species of animals, whether these were domestic pets or farm animals.

Design of the vaccine

Being a short peptide and also a 'self' hormone, it must be linked to a carrier in order to render it immunogenic. The carrier linkage can be performed with amino acids at the two terminals 1 and 10 or alternately in the middle by replacement of an amino acid with one having an additional functional group. Conjugates have been made of the three types and evaluated for immunogenicity. Two favoured designs have emerged: the Population Council group have linked TT at the *N*-terminal amino acid (Ladd *et al.*, 1985), whereas we prefer to substitute glycine at position 6 of LHRH with D-lysine. Linkage through the additional amino group created a spacer and a carrier, DT (Talwar *et al.*, 1992). Our design is backed by knowledge based computer graphics. The *N*- and *C*-terminals of native LHRH are proximal to each other with a bend of the molecule in the middle (Gupta *et al.*, 1993).

Effect of immunization on prostate

Prostate is known to be exquisitely sensitive to androgens. Rats immunized with the LHRH vaccine showed a marked atrophy of the prostate and a lowering of testosterone to castration levels (Jayshankar *et al.*, 1989). The effect was reversible, and testosterone and fertility were regained on decline of antibodies over the course of time in the absence of boosters. The prostate regenerated at a somewhat slower pace than other reproductive organs. Whether the regenerated organ had the 'young' or the 'old' prostate characteristics is unknown, as no marker equivalent to α-fetoprotein for regenerating liver is available in the case of the prostate. The vaccine also caused prostatic hypoplasia in Bonnet monkeys (Giri *et al.*, 1991).

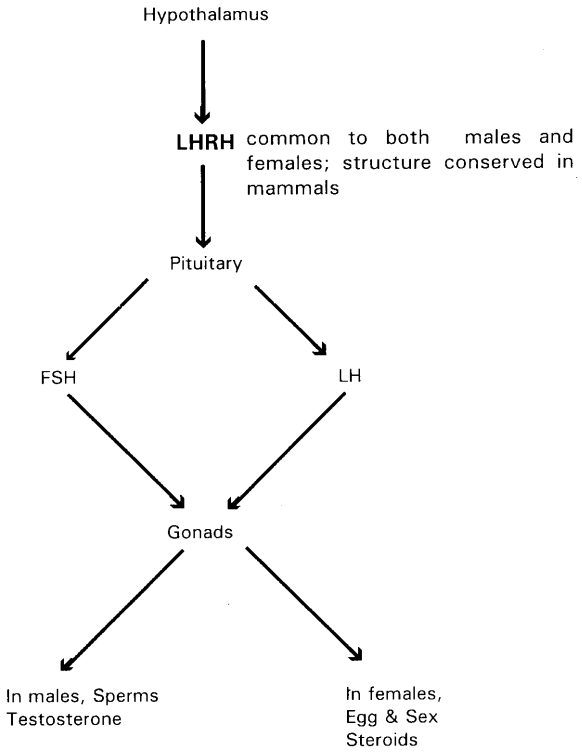

Figure 2. Luteinizing hormone-releasing hormone (LHRH) is the key hormone regulating via pituitary and gonads the formation of gametes and sex steroids. Its inactivation by antibodies would block fertility in both males and females. As well as controlling fertility in humans (and animals by virtue of essentially conserved primary structure), LHRH vaccine or hormone-inactivating antibodies can be used for immunotherapy of prostatic hypertrophy.

The reduction of prostate weight following immunization with LHRH vaccine is interpreted as being mostly due to the lowering of testosterone by eliminating the tandem action of LHRH on LH and testes. There may, however, be an additional direct action of LHRH on the prostate. J.Frick *et al.* (personal communication), carried out an experiment on an androgen independent clone of Duning tumour cells. The cells were implanted in normal, orchiectomized and LHRH vaccinated rats. Both orchiectomy and LHRH vaccine caused the decline of testosterone to castration levels. The proliferation rate of tumour cells in orchiectomized rats was similar to that in controls, as would be expected of androgen-independent cells. However, this rate was significantly lower in rats immunized with the LHRH vaccine.

Phase I/Phase II clinical trials with the LHRH vaccines

Toxicology studies have been carried out on both LHRH-1-TT (the Population Council vaccine) and on our vaccine, LHRH-6-D-Lys-DT. Permission has been granted by the Food and Drugs Authority of USA (FDA) for clinical trials with the Population Council vaccine in the USA. It has been examined for safety and immunogenicity in four orchiectomized subjects. The vaccine produced anti-LHRH antibodies with no significant side-effects. The Drugs Regulatory Authorities as well as the Institutional Ethics Committee have also given approval for clinical trials with the LHRH-6-DLys-DT vaccine in India and Austria using the Indian vaccine. Trials have been carried out on 24 patients with advanced carcinoma of prostate with metastasis (D_2 stage) at the All India Institute of Medical Sciences, New Delhi and the Postgraduate Institute of Medical Education and Research, Chandigarh, India. The same vaccine was used in four patients in Salzburg, Austria. Results from these trials have shown the safety of the vaccine when used at 200 and 400 μg of LHRH equivalent per dose. With the rise of anti-LHRH antibodies, testosterone declined to castration levels. Prostatic specific antigen (PSA) values in blood declined concomitantly. In some subjects, where serial nephrostograms and ultrascans were carried out, a shrinkage of prostatic mass was discernible. Patients on the 400 μg dose showed a better clinical response than those on the 200 μg dose. Many, but not all, patients experienced relief of symptoms (Talwar *et al.*, 1997b). The vaccine has potential for immunotherapy in clinical conditions where LHRH agonists and antagonists have been found to be effective. These include precocious puberty and endometriosis as well as cases of hormone-dependent breast and prostatic carcinoma. The vaccine would provide a much cheaper and more convenient alternative.

Is LHRH vaccine usable for fertility control in humans?

There is no doubt that LHRH vaccine imposes a fertility block in both males and females. However, it may be not usable in humans for contraception on the grounds that it also stops the secretion of sex steroids, although there may be a niche for it as a post-partum vaccine for lengthening of the anovulatory period and the interchild interval. LHRH secretion is physiologically suppressed following delivery of the child and remains suppressed during the lactation period so long as the breast sucking stimulus is frequent. In ancient societies this practice of breast feeding (several times during day and night) provided a ‘natural’ barrier to the woman ovulating and becoming pregnant for up to 1–2 years following the birth of a child. However, this method is not practicable for the modern woman. Can LHRH suppression achieve the same results? Fraser *et al.* (1989) carried out a trial with an LHRH agonist in nine women whose ovulation was inhibited for 11 months with no significant side-effects on the mother, lactation or nursing. The LHRH vaccine may be usable for this purpose with the advantage of low cost and infrequent intake. The anovulatory cycles are extended in monkeys immunized with the LHRH vaccine after weaning of the progeny (unpublished data). No deleterious effects of immunization were noted in mother or the progeny.

Animal fertility control with the LHRH vaccine

Given that the structure of LHRH is essentially unchanged in mammals, the LHRH vaccine can be used for the reversible control of fertility of animals. Monkeys and baboons of both sexes were rendered infertile by immunization with LHRH (Talwar *et al.*, 1984). Of practical interest may be the control of fertility of domestic pets, dogs and cats. Primary immunization with the vaccine requires at least two injections at monthly intervals. There is a time lag for the build up of antibody titres. This limitation can be removed by administration of anti-LHRH antibodies. Bioeffective monoclonal antibodies neutralizing LHRH action have been developed and these are competent to block the oestrus of female dogs (Talwar *et al.*, 1985). Passive use of preformed antibodies exerts the desirable biological effect without delay. No uncertainty exists in causation of the effect, as antibodies can be given at the appropriate dose. Active immunization does not ensure the production of high titres in every recipient. Another possible use of the preformed antibodies would be in assisted reproductive technologies where a blockade of the LH surge is necessary to maximize the output of hormonal regimes.

LHRH vaccine may also find useful applications in male animals raised for meat. Blocking androgen production at the stage when the animals have grown to near plateau point can ameliorate the quality of meat.

Conclusions

It is indeed possible to control human and animal fertility by immunological approaches. Table I lists a number of birth control vaccines that have reached phase I trials in women. Both active and passive methods of immunization are effective. The former gives a long-term response, whereas the latter has a finite duration. The advantage of passive immunization is that the effective dose of the

antibodies can be given, ensuring in each recipient the desirable biological effect, and the effect is exercised without delay. Mouse monoclonal antibodies with bioeffective properties have been developed against both LHRH and HCG. These can be 'humanized' and expressed in bacterial systems to obtain large quantities of cost-effective antibodies for therapeutic purposes.

Both LHRH vaccines developed in India and the USA have undergone phase I/phase II clinical studies in patients with advanced stage carcinoma of the prostate. They were found to be safe and acceptable. They have potential for use as immunotherapeutic vaccines in not only hormone dependent cancers, but also in other clinical states such as endometriosis and precocious puberty, where LHRH agonists and antagonists have been found to be effective. The vaccine offers a cheaper and more convenient option.

LHRH vaccine can also be used for reversible fertility control of domestic pets, dogs and cats. The vaccine reduces libido and aggressiveness as well blocking fertility. Another application is in animals raised for meat purposes.

LHRH vaccine may also have a niche as a post-partum vaccine for extension of anovulatory cycles. Women are highly motivated to prevent another pregnancy after delivery.

Three vaccines have been developed against HCG. However, only one of these (the HSD–HCG vaccine) has successfully completed phase I and phase II clinical trials documenting safety, reversibility and efficacy. This is the first birth control vaccine to have reached this stage of development. While this vaccine lays the scientific foundations of a fertility control vaccine, further research and development is necessary to make it a clinically safe product.

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