Making of a Unique Birth Control Vaccine against hCG with Additional Potential of Therapy of Advanced Stage Cancers and Prevention of Obesity and Insulin Resistance

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Introduction

Human chorionic gonadotropin (hCG) is employed as a reliable index of pregnancy and is the basis of most diagnosis kits in the market. It is not normally secreted in blood (or urine) by any healthy non pregnant female or male. Bob (Robert) Edwards, who got the Nobel prize for this work reported its presence in the culture fluid of eggs fertilized in vitro [1], thus pointing to its synthesis by the early embryo prior to its transfer to the uterus. It has a crucial role in implantation of the embryo onto the uterus. Marmoset embryos exposed to anti-hCG antibodies fail to implant, whereas the same embryos exposed to normal globulins implant perfectly [2]. Besides implantation and establishment of pregnancy, hCG has a central role in maintenance of pregnancy. It is responsible for production of progesterone by the corpus luteum of the female up to about 7 weeks of pregnancy before the placental cells assume this function. Immunization against hCG causes abortion of the fetus up to 6-7 weeks of pregnancy. The above mentioned two well proven outcomes of immunization against hCG provide evidence for the critical role of hCG in establishment and sustenance of pregnancy. An immunogenic vaccine against hCG would and should prevent pregnancy to occur. Is it indeed the case? Have there been appropriate developments to support this thesis? Is a Birth Control Vaccine feasible based on anti-hCG approach, which is safe and reversible? Would this vaccine be free from derangement of menstrual regularity and bleeding profiles, which are frequent problems of many contraceptives? This article will review these issues. In addition; it will cite publications reporting the unexpected expression of hCG in advanced stage cancers, that are invariably refractory to currently available chemotherapeutic drugs and have poor prognosis. Can immunotherapy with the anti-hCG vaccine and anti-hCG antibodies prolong their lifespan? Last but not of less interest is the reported obesity & insulin resistance in transgenic hCG β mice created by Prof lipo Hutaneni at Imperial College London & Dr. Susana Rulli at Instituto de Biologia y Medicina Experimental CONICET Buenos Aires Argentina. Can the anti-hCG vaccine prevent these abnormalities? Obesity and Metabolic Syndrome of Type II Diabetes are increasing problems worldwide.

Development of a Vaccine against hCG

hCG is composed of 2 subunits : α & β. The alpha subunit is common to 3 other pituitary hormones, TSH, LH and FSH; the β subunit imparting in each case the hormonal identity. Thus logically the immunogen for a vaccine against hCG would be the β subunit and not the whole hCG. hCG and its β subunit are however immunologically tolerated entities, the mother makes a lot of it and the fetus is exposed to it during pregnancy. Thus by themselves, these would not induce any antibody response. To make the β subunit immunogenic we linked it to a carrier Tetanus Toxoid (TT). TT is an approved safe vaccine, available at low price worldwide. It is a recommended vaccine for pregnant women. Many die in economically developing countries due to sepsis following delivery taking place in aseptic conditions. The logic of using a “carrier” was to mobilize T cell help by the “carrier” to the entire conjugate, causing the formation of antibodies against both tetanus as

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Abstract

Reviewed is the work which led to the development of a unique vaccine that prevents pregnancy in sexually active women without impairment of ovulation and block of their making normally their sex steroid hormones. Being given that hCG is not expressed by non-pregnant females, immunization with the vaccine is devoid of any cross-reaction with any tissue of the body. It is totally reversible and women regained fertility on decline of antibodies. A recombinant vaccine has been developed which is highly immunogenic in mice. It is undergoing extensive Toxicology under GLP conditions in rodents and a primate species, the marmosets, before resumption of clinical trials.

Ectopic expression of hCG or its subunits takes place in a variety of cancers, particularly at advanced stage with adverse survival and poor prognosis. Anti-hCG antibodies exercise therapeutic action against such cancers as indicated by in vitro culture and in vivo studies in nude mice.

Transgenic hCG β mice put on weight and manifest insulin resistance. Immunization of these mice with the recombinant hCG β-LTB vaccine prevents obesity and insulin resistance.

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well as hCG. Originally a hypothesis, it was proven to be correct. Four women who had completed their desired family and had come to the clinic to get their Fallopian tubes ligated, agreed to get immunized with this test vaccine. The vaccine had by then been evaluated for its full safety and lack of undesirable side effects in laboratory animals and subhuman primates. The entire February 1976 issue of CONTRACEPTION [3] reports these studies. Quite interestingly, women did evoke antibodies, not only against tetanus, but also against hCG [4]. Figure 1 recalls the findings. A 30 year old woman, who had given birth to 4 children and had the fifth pregnancy medically terminated, received 4 intramuscular injections of the vaccine hCG β – TT at 2 weeks interval. After the third injection, antibodies reactive with hCG were detectable in circulation. They kept on increasing over time to reach a maximum around day 100, after which gradual decline was noticed. In each of the 4 immunized women with this vaccine, antibodies declined to zero level in course of time [5]. No noticeable side effects of immunization were noticeable.

The sera carrying anti-hCG antibodies were tested by immunofluorescence for possible reactivity with other human tissues : thyroid, pituitary, parathyroid, adrenal, testis & ovaries ; not only by a renowned Pathologist at the All India Institute of Medical Sciences New Delhi (India) but also at Walter & Eliza Hall Institute of Medical Research Melbourne (Australia) and WHO Immunopathology Reference Laboratory, Geneva (Switzerland). No cross- reaction was observed. The sera were also negative for anti-nuclear, antimicrosomal antibodies & rheumatoid factor [6].

An important question to ask was whether the antibody generated by the β subunit of hCG recognized the whole hCG, the bioactive molecule. The immunized subject KW was injected 5000 i.u. of hCG. It caused a decline of anti hCG titres indicating the binding of the circulating antibodies with the bioactive hormone (Figure 2). The titres started rising after 48 hrs to return eventually to near about the original levels. No effect of hCG administration was noticeable on the anti tetanus titers, thus indicating that the vaccine was engendering two independent group of antibodies against the two constituents of the conjugate, hCG β and tetanus toxoid.

The clinical study in India was conducted in only 4 women. Confirmation of the immunogenicity, reversibility & safety of the hCG β-TT vaccine was carried out by the International Committee on Contraception Research (ICCR) of the Population Council New York. This clinical study was conducted by eminent clinicians in 15 women in Sweden, Finland, Chile & Brazil with approval of their National Regulatory Authorities. Fourteen women elicited anti–hCG response with the hCG β-TT vaccine. It was reversible in all cases. According to them, "clinical surveillance, immunologic, hematologic and biochemical tests indicated excellent local and systemic tolerance to the antigen. No significant adverse effects on menstrual function, endocrine status, or health were found" [7].

Enhancement of immunogenicity

The prototype vaccine hCG β-TT was competent to overcome the immunological tolerance to hCG. The titres of the antibodies induced were however not high enough to counteract with certainty the high amount of hCG made by women in early pregnancy. The next task then was to improve the immunogenicity of the vaccine. The beta subunit of the two subunit hormones retains the ability to bind non covalently with alpha subunit of not only the species hormone but also with those of other mammals. Thus a Hetero-Species Dimer (HSD) was created by joining the hCG β to alpha subunit of ovine LH [8]. HSD linked to TT generated much higher titres of antibodies than with the previous hCG β-TT vaccines, with better neutralization capacity in primates.

hCG Vaccine by Phase I clinical trials [12,13], the crucial question was whether the vaccine can prevent an unwanted pregnancy. With due approval of the Ethics and Drugs Regulatory Authorities, we carried out Phase II efficacy trials, the first of their kind on a potential Birth Control Vaccine anywhere in the world. 148 sexually active women of proven fertility with at least two children were enrolled by written consent. Many of them were hyper-fertile and were coming to the clinics for Medical Termination of Pregnancy (MTP). Primary immunization was carried out by 3 intra-muscular injections of the HSD-TT (Tetanus Toxoid) or HSD-DT (Diphtheria Toxoid) as carrier. The alteration of the carrier in the vaccine was done to avoid very high titers of anti-carrier antibodies, which cause carrier-induced epitope specific non-responsiveness [14]. Booster immunization was done as and when the anti-hCG titers were declining below 50 ng hCG bio-neutralization capacity per ml of serum. During primary immunization, and till such time as the antibodies had not gone above the presumed protective threshold of 50 ng/ml, IUD (Intra Uterine Device) was inserted to prevent pregnancy, which was removed after the woman developed adequate antibodies. While all women made antibodies against hCG, 119 (80%) generated titers above 50 ng/ml. Only one pregnancy was recorded over 1224 cycles of observation above 50 ng/ml titres. This occurred in a woman, whose antibody titer had declined below 50 ng/ml and she was late in taking the booster. Twenty six such pregnancies occurred in women: 5 in women at titers below 5 ng/ml, 6 in women with titers of 10 ng/ml, 6 at 20 ng/ml and in 9 with titers between 20-35 ng/ml [15]. Twenty two of these 26 women had their pregnancies terminated, but 4 women carried the pregnancy to term and delivered normal babies (three boys and one girl). These observations indicated that in case a woman conceives at lower than 35 ng/ml antibody titers, the low antibodies do not interfere in normal progression of pregnancy. The vaccine was highly protective above 50 ng/ml anti-hCG titers. Figure 3 reproduced from the relevant paper [15] shows the antibody response in 4 representative women. The solid black line gives the period over which she was exposed to pregnancy. In each case boosters were given when titers were declining towards the presumed protective threshold titers. Eight women completed 30 cycles without becoming pregnant, nine were protected over 24-29 cycles, 12 for 18-23 cycles, 15 for 12-17 cycles and 21 for 16-11 cycles. The reversibility of the vaccine and regain of fertility is indicated by a case represented in Figure 4. A 30 year old subject with two children and one termination was protected from becoming pregnant for a year, so long as she was taking booster injections as and when the titers were tending to decline. She conceived in the cycle when her antibody titers were below 5 ng/ml [15].

Figure 3: Anti-hCG response to the HSD vaccine in 4 sexually active women of proven fertility. MRG 30yr old and TRW 23yr old had 2 children each; HJN 32yr & SVN 29yr old had 2 children and 1 elective termination of pregnancy. All of them remained protected from becoming pregnant over 26-32 cycles, at top edge represent the menstrual events which remained regular, solid lines denote the period over which they were exposed to pregnancy. Booster injections were given to keep antibody titres above 50 ng/ml [15].
Making of a recombinant vaccine against hCG

The merits of immunization against hCG for control of fertility without impairment of ovulation and derangement of menstrual regularity or bleeding profiles were demonstrated by Phase I & Phase II Clinical trials. The eventual large scale production of the vaccine of consistent characteristics would require a recombinant vaccine, which would (a) ensure that the “carrier” is linked to the hormonal subunit at a defined position and (b) be amenable to industrial production. This was achieved by a gene construct in which β subunit of heat labile enterotoxin of E. coli (LTB) was fused at the C-terminal of hCG β (Figure 5). hCG β-LTB was expressed in Pichia pastoris, an expression host employed by an industrial company in India to make the Hepatitis B vaccine. This vaccine is approved by The Drugs Controller General of India for human use. The cost of this vaccine is much lower than the Hepatitis B vaccine made and sold in other countries.

The recombinant hCG-β-LTB was evaluated for immunogenicity in Balb c [16] and four other genetic strains of mice [17]. It was adsorbed on alhydrogel and given intra-muscularly. Mycobacterium indicus pranii (MiP) a non-pathogenic mycobacteria was employed as adjuvant. MiP was originally developed as an immunotherapeutic vaccine for multibacillary leprosy [18]. It is approved by The Drugs Controller General of India (DCGI) and USFDA. It is licensed to Cadilla Pharma and is available for public use. Besides leprosy, MiP is being used as adjunct to Multi drugs regimen for treatment of category II (difficult to treat) tuberculosis patients. It is a potent invigorator of immune response [17]. MiP is also effective in exercising preventive and therapeutic action in development of SP2/0 myelomas in mice [19].

Figure 6 shows the antibody response to hCG β-LTB in Balb c mice. Antibodies were generated in every mouse (100% positivity) with bioefficacy titers well over the protective threshold. A booster given on day 127 ensured high titers (upto 6600 ng/ml) in mice over 8 months of observation period.

The recombinant vaccine hCB β-LTB has received approval of the National Review Committee on Genetic Manipulation (RCGM). It is undergoing Toxicology before going back for clinical trials, under the aegis of The Indian Council of Medical Research.

DNA vaccine

Being given that DNA vaccines are low cost, are thermostable and have long shelf life [20], a DNA version of hCG β- LTB was also prepared. Codon optimized gene encoding hCGβ-LTB fusion protein was PCR amplified, and cloned under the control of cytomegalovirus in eukaryotic expression plasmid VR1020 (DJ), which is an approved vector for human use by USFDA. The (DJ) version of the vector 1020 has 45 cpG motifs in the plasmid backbone for better adjuvanticity. Although DNA vaccines alone have been shown to be immunogenic in chimpanzees [21], and safe in women as per Phase I trial [22], an enhanced immune response is obtained by priming with DNA followed by protein version of the vaccine [23,24]. Moreover DNA priming followed by protein booster induces both antibody and cell mediated immunity.
immune responses, which will be particularly useful in therapy of cancers expressing hCG.

Experiments carried out by immunization of mice with only protein version of the recombinant hCG β- LTB vaccine, or with DNA priming followed by protein version of the vaccine indicate the near about doubling of the antibody titers by adopting the latter approach. Hence there may be advantage in adopting priming with the DNA vaccine followed by protein of hCG-LTB, for immunization with this vaccine.

Ectopic expression of hCG by advanced stage cancers

A number of reports have appeared in the literature on the expression of hCG or its subunits by a variety of cancers particularly at the advanced stage. Expression of alpha subunit of hCG was observed in lung carcinoma tumors [25]. One third of transitional cell carcinomas of bladder ectopically produce trophoblastic hormones that are specifically correlated with stage and grade of the tumor [26]. β-hCG was made ectopically and reported as a poor prognostic marker in colorectal cancer [27]. More than 40% of pancreatic exocrine tumors produce β hCG [28]. Determination of hCG in serum was considered as a potential marker in the prognostic evaluation of patients with squamous cell carcinoma of the oral cavity and oropharynx [29]. The survival of women suffering from cervical carcinoma in whom the tumor secreted β hCG was poorer(14%) as compared to those negative for β hCG(75%) [30]. Invariably at the stage that ectopic expression of hCG/subunits takes place, the cancer is advanced and refractory to the currently available drugs.

We carried out a collaborative study at Sir Gangaram Hospital New Delhi on the presence of hCG (as tested immunohistochemically) in formalin fixed sections of many tumors. The tumours were also graded. Table 1 gives the findings. The frequency of hCG positive tumors appears to be higher in high grade advanced tumors.

Being given that a variety of cancers of various origins expresses hCG ectopically, it may be relevant to enquire whether hCG plays a role in their proliferation. Indeed hCG or its subunits enhance the proliferation of tumor cells. Bladder cancer cell line T24, which does not produce hCG or its subunits, after treatment with β hCG showed a marked increase in proliferation [31]. This action may be result of its counter-acting the apoptotic effect of TGFβ-1-induced apoptosis [32]. hCG also causes the down-regulation of Fas, Fas ligand, and BAX and p53, which are major apoptotic factors [33]. Reduction in β-hCG subunit expression in cervical cancer cell lines by silencing RNA led to apoptosis of the HeLa cells [34]. Another important action of hCG or its subunits is on promotion of angiogenesis by stimulating the migration and capillary sprout formation of uterine endothelial cells. High levels of hCG and its subunits are associated with high micro vessel density in hCG expressing cancers [35]. βhCG down-regulates E-Cadherin and thus promotes migration and invasion of cancer cells [36]. A possible molecular mechanism by which hCG can promote neoplasm has been proposed recently, which suggests that hCG up-regulates the cell cycle proteins via the mammalian target of rapamycin complex 1 (mTORC1) signalling network [37].

It was relevant to determine whether antibodies directed hCG have any role in killing such cells and in preventing their proliferation. A study was conducted on A549 lung cancer cells. Their culture in vitro in presence of anti-hCG antibodies caused killing of these cells (Figure 7). To determine whether the antibodies exercised any effect on proliferation of such tumor cells in vivo experiments were carried out on Chago human lung tumour cells in nude mice [38]. Figure 8 gives the observations showing an inhibition of tumor growth as a function of antibody concentration.

Another study was conducted on MOLT-4 cells derived from an

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Table 1: Expression of α and or β hCG by early and advanced stage tumors.
acute lymphoblastic leukemia patient in relapse. A highly specific humanized chimeric recombinant antibody for β hCG (cPiPP) and another monoclonal for α hCG (P22376) were employed for studying their reactivity with intact as well as permeabilised cells. Their presence on membranes or within cells was investigated. FACS analysis indicated the expression of both α and β hCG by the MOLT-4 cells (Figure 9). In contrast, no reactivity was seen with a non-specific monoclonal antibody Moab730 that was reactive with DU 145 prostate carcinoma cells. The genuineness of reactivity of the anti-hCG antibodies with MOLT-4 cells was further confirmed by competition for binding by prior incubation of cells with bioactive hCG [39]. Culture of MOLT-4 cells with the anti-hCG antibodies did not however cause the killing of the cells. Nevertheless if the same monoclonal antibody linked to curcumin was employed, near to 100% of the cells were killed [40] (Figure 10). These observations point to the feasibility of specific delivery of drugs to cancer cells by tumor specific antibodies.

![Image](image1.png)

**Figure 8:** Inhibition of tumour induction by anti-α-human chorionic gonadotropin (hCG) antibody. Human lung cancer Chago cells (expressing hCGα), 1 x 10^6 in 0.5 mL of PBS buffer along with different concentrations of anti-hCGα antibody, and was transplanted under the dorsal skin of athymic mice (three animals in each group). The control group was given transplants of the same number of cells and an equivalent amount of normal serum (designated as 0 ng of anti-hCGα antibody [α-HCG-ab]. Series of panels under A, B, C, D, and E show tumour sizes photographed after 2, 4, 6, 8, and 10 weeks, respectively, after transplantation of cells with indicated concentrations of antibody [38].

![Image](image2.png)

**Figure 9:** Reactivity of (a) anti-alpha-hCG (P22376) and (b) anti-beta-hCG (cPiPP) antibodies with MOLT-4 cells. FACS analysis was carried out at indicated antibody concentrations. In each case, 80-91% cells show binding with these antibodies. Histogram in both (a) and (b) shows fluorescence of cells without antibody. A non-hCG-reactive monoclonal antibody (Moab 730) demonstrated a lack of recognition (Histogram 6) in (a) [39].

![Image](image3.png)

**Figure 10:** Photomicrograph of MOLT-4 cells after incubation with cPiPP alone and cPiPP-curcumin conjugate. Cells incubated in culture medium are used as control [40].

![Image](image4.png)

**Figure 11:** Whole body scan of JEG-3 tumor bearing nude mouse injected with 121I-anti-hCG monoclonal antibody and 121I-irrelevant monoclonal after 4 days. Histology and autoradiographs of mouse tumor tissues using 121I labeled anti-hCG antibody at 10, 20 and 40X magnification. Control is the non-tumor tissue [40].

The antibodies labeled with 121I could also be used for imaging of hCG synthesizing tumors (Figure 11). Their homing primarily to the tumor, expressing hCG could also be utilized for specific delivery of radiations to such tumors.
Prevention of obesity and insulin resistance

Susana Rulli has ‘humanised’ mice by making them transgenic for hCGβ [41]. It is expressed by human ubiquitine C promoter. The transgene is microinjected into the pronuclei of fertilized oocytes of FVB/n mice, and implanted into oviducts of pseudo pregnant females. The transgenic mice make hCGβ, which combines with the alpha subunit of pituitary gonadotropins to generate bioactive hCG. The circulating hCG stimulates the production of progesterone which in turn promotes the production of large amount of Prolactin, causing hypertrophy of pituitary and mammary tumors in many animals. The transgenic mice become obese. They also manifest insulin resistance.

Immunization of transgenic hCG β mice with hCGβ-LTB vaccine was carried out starting at 3 weeks age with 3 primary injections at fortnightly interval followed by a monthly booster. Lo and behold, immunization prevented the transgenic mice to become obese, as compared to transgenic mice of the same age receiving only saline injections (Figure 12). Also the vaccinated mice did not develop insulin resistance, which was clearly manifest in non-vaccinated transgenic mice (Figure 13). Antibodies generated by the vaccine obviously inactivate the circulating hCG, with the result that progesterone and prolactin rise is prevented. Hormonal imbalance is apparently one of the causes of obesity, which is on an increase worldwide. In USA, approximately 65% of the population is overweight [42]. Also type II Diabetes, manifested by insulin resistance is a major problem in females accompanied by polycystic ovaries and hormonal imbalance. It is too early to speculate whether immunization with the anti-hCG vaccine would be of utility in such cases. No study has yet been performed on the safety and consequences of immunization with the anti-hCG vaccine in obese women or those suffering from type II diabetes, although the complete lack of any side-effects in women of reproductive age have been well established by Phase I/II clinical trials on the anti-hCG vaccines.

The present observations in transgenic mice are on prevention of obesity and related hormonal manifestations (including insulin resistance) by active immunization of growing transgenic mice with the anti-hCG vaccine. Of great relevance would be to enquire whether obesity can or cannot be reversed by active or passive immunization. Such studies are in progress.

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References
