Current status of a unique vaccine preventing pregnancy

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1. ABSTRACT

   The ability of a vaccine linking beta hCG to a carrier to generate antibodies against hCG, its reversibility and safety was established by Phase I clinical trials conducted in India, Finland, Sweden, Chile and Brazil. Employing a hetero-species dimer (beta hCG-αoLH) linked to tetanus toxoid further improved the immunogenicity of the vaccine. Phase II clinical trials showed that anti-hCG titres above 50 ng/ml prevented pregnancy of sexually active fertile women without derangement of ovulation and menstrual regularity. On decline of antibodies, women conceived again to give birth to normal progeny. A genetically engineered vaccine consisting of beta hCG linked to B subunit of heat labile enterotoxin of E. coli has been made. It is expressed as DNA as well as protein. Priming with DNA followed by protein version of the vaccine generates very high titres against hCG in mice. Extensive toxicology studies in 2 species of rodents, and marmosets have shown complete safety of the vaccine. The vaccine is cleared for Clinical trials by the National Review committee on Genetic Manipulation and Drugs Controller General of India.

2. INTRODUCTION

   In mid-seventies of the past century, a conversation with a family head of a large but still growing family made me realize that even though the Government of India provided a basket of methods for family planning free of charge to its citizens, very few, if any, suited the potential users. Surgical procedures of vasectomy or tubectomy (not ligations as done currently) were largely permanent procedures and despite monetary incentives, people resorted to these only after producing half a dozen or more children. Condoms are largely male options and only a few men compromise their pleasure by using these. Pills require daily motivation. In spite of their high efficacy, the side effects were not pleasant to all. IUDs provided at that time, caused extra bleeding and women who were
Contraceptive vaccine

already anaemic (hemoglobin below 9 g /100ml of most women in India) did not tolerate the long term use of IUDs. What was needed was a method which required only periodical intake, does not block ovulation and production of normal sex steroid hormones and be devoid of causing extra bleeding.

With this background, we thought of making a vaccine, which would require to be taken only periodically and would also offer privacy of use. The target of the vaccine may ideally be a molecule which does not derange ovulation nor impair normal production of reproductive hormones. It may be ideally a molecule not made by healthy non-pregnant females, so that antibodies against it do not generate undesirable auto-reactivity. On these considerations, we thought of employing the Human Chorionic Gonadotropin (hCG) as the target for the stipulated vaccine.

hCG is not normally made by any organ of the healthy female, that is why its detection in blood or urine is the basis of the pregnancy diagnosis test. Eggs fertilized in vitro and cultivated till blastocyst stage, show in the medium the presence of hCG (1). It is a product of the early embryo. Furthermore, it plays a crucial role in the implantation of the embryo onto the endometrium. Marmoset embryos exposed to anti-hCG antibodies fail to implant, whereas the same embryos exposed to normal globulins implant perfectly leading to the onset of pregnancy (2). Thus if a vaccine could be made, which generates in women antibodies neutralizing hCG, the implantation of the embryo on the endometrium would be blocked and the onset of pregnancy prevented. By choosing hCG as the target, there is expected to be no autoimmune reactivity against any organ. The only molecule that shares large molecular homologies with hCG is hLH (the human luteinizing hormone). Partial cross-reactivity with hLH may be tolerable, and not compromise its action. LH is made in large amounts and its partial neutralization may still leave enough hLH to cause ovulation and production of progesterone.

3. THE INITIAL hCGβ-TT VACCINE

Being given that the α- subunit of hCG is common to TSH (Thyroid Stimulating Hormone), FSH (Follicular Stimulating Hormone) and LH (Luteinizing Hormone), it was logical to use for the intended vaccine only the β-subunit of hCG. hCGβ was linked to a carrier, which was tetanus toxoid, to enable the mobilization of T helper cells for generating antibodies against hCGβ. Whether hCGβ-TT was competent to generate anti-hCG antibodies in women was tested in a few women who had finished their family planning, who were tubectomized and volunteered to get injected with hCGβ-TT. Each one of these 4 women immunized with hCG β-TT generated antibodies reactive with hCG (3,4). Figure 1 shows antibody titres against hCG and tetanus in two of these women ND and AM, who received four fortnightly injections of 80 µg hCGβ-TT. The women generated antibodies against both hCG and tetanus, and continued to menstruate regularly as represented by -x-x- on the upper abscissae. Their luteal phase progesterone determined in the bleeds of 2 cycles was indicative of ovulation.

In each case the antibodies were reactive with hCG and were competet to neutralize its bioactivity. Figure 2 (A,B) shows an in vivo study of antibody titres after administration of 5000 IU of hCG or 6000 IU of hCG given in two lots of 2000 IU and 4000 IU on two consecutive days. The circulating antibodies bound to the administered hCG, inactivating its bioactivity. The titres declined temporarily and got restored to original level within a month.

Thus the strategy of employing β subunit of hCG linked to a useful and cheaply available carrier such as tetanus toxoid, rendered it immunogenic in women, who generated bio-effective antibodies reacting with hCG. All women kept menstruating regularly and had luteal progesterone indicative of ovulation.

It was considered necessary to determine the cross-reactivity of the antibodies generated by hCG β-TT with pituitary hormones. It was observed that the antibodies are totally devoid of cross-reaction with hFSH and hTSH, Prolactin and hGH but did react with hLH (5).

Subjects immunized with hCG β-TT vaccine were tested for possible reactivity with other human tissues. They were found to be negative for anti-nuclear, anti-microsomal antibodies and rheumatoid factor. The sera from these subjects did not give any reaction with human thyroid, pituitary, parathyroid, adrenal, testes and ovaries as examined by immunofluorescence techniques (6,7).

Studies were carried out to determine whether immunization of rhesus monkeys with the hCGβ-TT vaccine for variable time periods caused any immunological and toxic damage to various organs. The pituitary showed normal distribution of acidophils and basophils. No evidence of hypo or hyper-plasia were noted in any of the endocrine organs. The ovaries of the female monkeys showed corpora leutae and spermatogenesis was evident in the male monkeys (7).

3.1. Evaluation of the consequences of partial cross-reaction with hLH

After extensive toxicology and safety studies, the vaccine was found to be fully safe. The only cross-
reaction of antibodies generated by hCGβ-TT was with hLH. However, the degree of cross-reaction did not appear to interfere with continued ovulation by women, nor in disturbance of menstrual regularity. Investigators at the Population Council, New York carried out long chronic toxicology studies of 5-7 years duration in rhesus monkeys to determine the consequences of hyper-immunization of the rhesus with β-oLH given along with Freund’s complete and incomplete adjuvant. The antibodies were frankly cross-reactive with monkey LH and hCG. The effect on control of fertility was reversible and animals returned to normal gestation on decline of antibodies (8). Furthermore, no ill consequences of the anti LH antibodies were seen on the pituitary (8).

4. CARBOXY TERMINAL PEPTIDES (CTPs) OF hCGβ AS IMMUNOGENS

The primary structure of both hLH and hCGβ is known. While there are large homologies in these two molecules, the β-hCG has an additional carboxy terminal region of 35 amino acids. It was logical to enquire whether this additional carboxy terminal part (CTP) of hCGβ can be utilized as the antigen for the intended anti-hCG vaccine. Indeed, extensive work was done by Vernon Stevens and WHO Task Force as well as by our group. One common observation was that CTP region of hCG β was a poor immunogen and necessitated the use of strong adjuvants to evoke the production of antibodies. While the antibodies were totally devoid of cross-reactivity with hLH, surprisingly these reacted with the human pancreatic cells and pituitary as observed by Noel Rose et al at Johns Hopkins (10). Louvet et al (11) have reported the lack of hCG neutralizing effect of antibodies generated by the terminal 22 amino acid peptide vaccine, even though these are highly specific to hCG and devoid of cross-reactivity with hLH.

To improve the immunogenicity of the C-terminal peptide, we investigated longer terminal
peptides of 45 and 53 amino acids (12,13). These peptides linked to the carrier TT/DT were relatively more immunogenic than the terminal 35 amino acid C-terminal and retained the specificity of reacting with hCG but not with hLH. These peptides did not induce sufficiently high titre antibodies and the antibodies generated were not of as high affinity as those induced with β hCG. These studies brought home to us that none of these peptides would finally be a suitable candidate for anti-hCG vaccine for use by women, even though the cross-reactivity with hLH was completely avoided. Previous studies in primates as well as Phase I clinical trials in women indicate no serious harm of a partial cross-reaction with hLH. In fact, it may be useful and contribute to the efficacy of the vaccine. Phase I trials conducted with our β hCG-TT vaccine in Finland, Sweden, Chile and Brazil by eminent clinicians under the International Committee on Contraception Research (ICCR) of Population Council showed the immunogenicity, safety and reversibility of β hCG vaccine in women (14). These important studies contributed valuably to the concept and validity of safety of the vaccine.

5. THE HETERO-SPECIES DIMER (HSD)-TT/DT VACCINE

The ability of hCGβ to link non-covalently with α subunit of LH, FSH, TSH is conserved through evolution in mammals. We thought of annealing non-covalently hCGβ to α subunit of ovine pituitary hormones. The HSD thus created was linked to carrier TT (Tetanus toxoid) or DT (Diptheria toxoid) to test its immunogenicity. The HSD-TT was distinctly more immunogenic than hCGβ-TT, both in terms of immunoreactivity as well as bio-efficacy titres against hCG. Table 1 recapitulates some data obtained by these studies (15).
Table 1. hCG binding and neutralization capacity of anti-βhCG-TT or β hCG-cholera toxin B subunit and anti-HSD-TT antisera generated in bonnet monkeys and rats

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>No. of animals</th>
<th>hCG binding capacity (pg/ml) (I) (mean ± standard error of mean)</th>
<th>hCG neutralization capacity (pg/ml) (B) (mean ± standard error of mean)</th>
<th>B/Ix100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonnet Monkey</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>βhCG-TT/CHB</td>
<td>5</td>
<td>22.2 ± 2.3</td>
<td>10.1 ± 1.8</td>
<td>44 ± 3.7</td>
</tr>
<tr>
<td>HSD-TT/CHB</td>
<td>5</td>
<td>21.4 ± 1.9</td>
<td>14.0 ± 1.4</td>
<td>65 ± 1.9</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>βhCG-TT/CHB</td>
<td>6</td>
<td>27.1 ± 1.7</td>
<td>17.1 ± 1.2</td>
<td>63 ± 1.5</td>
</tr>
<tr>
<td>βhCG-TT/CHB</td>
<td>6</td>
<td>32.5 ± 1.4</td>
<td>26.1 ± 0.8</td>
<td>80 ± 2.3</td>
</tr>
</tbody>
</table>


5.1. Phase I clinical trials on HSD-TT/DT Vaccine

Appropriate toxicology studies were carried out on HSD-TT vaccine to assess its safety for usage. After obtaining the approval of the Institutional Ethics Committees and Drugs Controller General of India (DCGI), Phase I trial was carried out on HSD-TT vaccine in five centers in India. Pertinent findings were that the HSD-TT vaccine generated higher antibody titres than the hCGβ-TT vaccine (5,16). The antibodies bound to hCG and inactivated its bioactivity. No notable side effect was observed in any subject. The 300 µg dose was optimum for immunization (16).

5.2. Phase II efficacy trials

The crucial question now was whether the HSD-TT vaccine that generated adequate antibody titres of high affinity were competent for preventing pregnancy in women of proven fertility who are sexually active. Phase II clinical trials were conducted in three centres in India. A total of 148 sexually active women of proven fertility were enrolled after obtaining their written consent to undergo the Phase II efficacy trial. They were asked to wear IUD till such time as the antibody titres were well above the putative threshold of 50 ng/ml bio-efficacy titres. 110 women generated antibodies well above the putative threshold for duration of three months or longer above the threshold, although the remaining 38 also made anti-hCG antibodies but the duration of the antibody response above putative threshold was shorter than three months in these women.

It was observed that these sexually active women were indeed protected from becoming pregnant at titres above the putative threshold of 50 ng/ml. Only one pregnancy took place in 1224 cycles (17). The cycles were ovulatory as gauged by luteal progesterone and also by non-lengthening of the luteal phase of the cycles. Women kept on menstruating regularly. Figure 3 represents the typical findings in these women. 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.

6. REVERSIBILITY AND REGAIN OF FERTILITY

Four women desired to have a child. No booster immunization was given to these women, and the antibody titres returned to near zero level in course of time. All of them became pregnant to bear another child.

Figure 4 represents a woman who was protected for nearly a year after immunization with the vaccine. By not taking booster immunization, the titres came down and in the immediate cycle when antibody titres came below 20 ng/ml, she conceived and gave birth to a normal infant.

6.1. Normalcy of children born to previously immunized women

Prof. Meharban Singh at the All India Institute of Medical Sciences, New Delhi carried out an analysis of children born to the four women who were protected by the vaccine above 50 ng/ml titres, but on decline of antibody titres in absence of boosters, they conceived. Their progeny born was adjudged normal with respect to developmental landmarks and cognitive abilities with respect to their siblings (18).

7. REVIVAL OF THE VACCINE AGAINST hCG

I retired in 1994 from the Directorship of the National Institute of Immunology (NII), New Delhi, India. On request of my successor, the vaccine was left at NII. Nothing happened for 12 years. In 2006 at the initiative of the Indo-US Committee on Contraception Research, I got a grant to revive the vaccine. We thought of developing a genetically engineered recombinant vaccine, so that it should be amenable to industrial
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 each 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. Arrows indicate the day on which vaccine was given. Booster injections were given to keep antibody titres above 50 ng/ml. Reproduced with permission from (17).

Figure 4. Regain of fertility on decline of antibodies. A 30-year-old subject (STS), with two gravidae and one elective abortion (P2+1), on immunization with the vaccine, remained protected from pregnancy for 12 cycles. In the absence of a booster injection, antibody titers declined and she became pregnant in the cycle starting on day 417. The extrapolated antibody titers at midcycle in the fertile month, shown by the dotted line, were <5 ng/ml. Arrows indicate the day on which vaccine was given. Reproduced with permission from (17).
production. The β-subunit of hCG was linked to B subunit of heat-labile enterotoxin (LTB) of *Escherichia coli* as carrier (Figure 5). The carrier chosen this time does not evoke carrier-induced-immuno-suppression which TT as carrier did on continuous repeated use for long periods (19). The fact that the suppression of immune response was carrier induced was confirmed by presenting hCG β on an alternate carrier DT (20). The B subunit of enterotoxin did not have these disadvantages. LTB has further adjuvant properties and evokes also mucosal response. Keeping all these properties in mind, the previous carriers TT and DT were abandoned and replaced by LTB.

hCG β-LTB vaccine adsorbed on alhydrogel given along with autoclaved *Mycobacterium indicus pranii* (MiP formerly known as Mw), a vaccine which we had developed originally against leprosy (21) was found to be a potent potentiator of humoral and cell mediated immune responses (22). MiP is approved by the Drugs Controller General of India as well as by US FDA. It is licensed to a Company and is available to public.

Figure 6 shows antibody response to hCG β-LTB vaccine in Balb/C mice (23). 100% of mice receiving the vaccine responded with formation of anti-hCG antibodies. The antibody titres went up to 5500 ng/ml in most of the mice and even higher upto 18000 ng/ml in some mice. The titres remained well above 50 ng/ml for more than three months. Booster immunization resulted in high boosted response to the vaccine.

It is well established that immune response varies from individual to individual. Our initial studies were in Balb/C mice, in which the vaccine hCG β-LTB was highly immunogenic. It was considered necessary to determine the immunogenicity in mice of other genetic strains. Thus studies were carried out in four other defined strains of mice namely FVB, C57Bl/6, SJL and C3H. The vaccine generated antibodies in all of these strains of mice, although the titres varied (22).

8. RECOMBINANT DNA AND PROTEIN VACCINES

Recombinant vaccines are expressed as both DNA and proteins. The DNA version is cheaper to make and is thermostable, thereby not requiring the “cold chain” for transportation and preservation. A large number of studies have been done on development of DNA Vaccines (24-27). However, a few DNA vaccines have succeeded in becoming DNA vaccines for human use (26-28).

We tried to determine whether priming of immune response with the DNA vaccine followed by protein version would be useful for immunization. It may be recalled that hCG vaccine demands on initial vaccination three primary immunizations to induce antibodies. On giving two of these primary injections with the DNA version of the vaccine followed by the protein version of the vaccine as third primary injection resulted in a distinctly elevated immune response to
hCG (29). Figure 7 (A) shows the antibody response to hCGβ-LTB vaccine along with MIP in Balb/C mice using all three primary injections with proteinic form of the vaccine and Figure 7(B), where the first two injections were given with DNA vaccine followed by third primary injection with proteinic form of the vaccine. It would be observed that priming with DNA form of the vaccine followed by proteinic form elicits a higher antibody response in Balb/C mice than the one engendered by all three primary immunizations given with the protein form of the vaccine.

Approval of the recombinant vaccine by Review Committee on Genetic Manipulation (RCGM) India, is mandatory, as all products made by genetic engineering have to seek approval of this committee for further use. Accordingly, the entire data on the making of hCGβ-LTB as also on its purification, physico-chemical attributes and immunogenicity was submitted to RCGM. After due deliberations, hCGβ-LTB received the approval of RCGM. RCGM asked us to carry out pre-clinical toxicology studies in two species of rodents on an international protocol conducted by a GLP Company. In addition, we considered it appropriate to conduct safety, immunogenicity and efficacy studies in a primate sub-human species, the Marmosets, which were carried out at the National Institute of Research in Reproductive Health, Mumbai.

9. PRE-CLINICAL TOXICOLOGY STUDIES

9.1. In rodents

Pre-clinical toxicology studies in rodents, were contracted to M/s Bioneeds at their GLP Facility in Bangalore India. Studies were based on Schedule ‘Y’ guide-lines on Drugs and Cosmetics, guidelines of Institutional Animal Ethics Committees (IAEC) and biosafety issues related to Genetically Modified Organisms.

Both DNA and protein forms of the vaccine were non-sensitizing to the skin of Guinea pigs with no clinical signs of toxicity, mortality and changes in body weight. Both vaccines were non-mutagenic at the highest concentration tested by Bacterial Reverse Mutation and Mammalian Chromosome Aberration Tests. Similar observation on non-mutagenic property of the vaccines was made in vivo by Mammalian Erythrocyte Micronucleus Test in Mice.

Single dose acute toxicity study was conducted in Sprague Dawley rats. Vaccinated rats were observed for mortality, clinical signs of toxicity, body weight and gross pathological examination. No mortality, clinical signs of toxicity and treatment related changes in the body weight, were observed. No changes in gross pathology (external and internal)
were observed at even the highest dose tested. Repeat doses of the vaccines were also tested in rats, which were followed up to 90 days post immunization. These studies showed no treatment related changes in physical, physiological, clinical, hematological parameters, as also in histopathology profiles of the organs. Segment II studies conducted in rats showed that vaccines did not affect the embryo-foetal development. Body weight, food consumption, gross pathology remained normal, and no abnormal effect was observed in fetal sex ratio, fetal weight, external, visceral and skeletal norms of fetuses.

9.2. In Marmosets

Pre-clinical toxicology and safety studies in Marmosets, were carried out at the National Institute for Research in Reproductive Health, Mumbai, where the only Marmoset colony is bred and maintained in India.

Studies were carried out in 9 Marmosets at 3 different doses. Two Marmosets which were not immunized, formed the control group. Active immunization of normal fertile females by the combination of DNA and Protein vaccines did not show any adverse effect on the body weight or general activity. Profiles of steroid hormones, biochemical and haematological parameters also remained similar to those of non-vaccinated animals. Vaccinated female Marmosets did not become pregnant on cohabitation with normal male fertile Marmosets, thus demonstrating the efficacy of hCGβ-LTB to prevent pregnancy in this primate.
Thus pre-clinical toxicology studies on the hCGβ-LTB vaccine in 2 species of rodents and a subhuman primate species, the Marmosets, demonstrated the total safety of the recombinant hCGβ-LTB vaccine. RCGM reviewed and approved the pre-clinical toxicology data, and forwarded its recommendation to the Drugs Controller General of India to grant permission for conduct of trials in women. A clinical trial protocol has been developed by an expert committee of the Indian Council of Medical Research. After obtaining the approval of the DCGI and Institutional Ethics Committees, the vaccine will undergo clinical trials.

Meanwhile, technology has been transferred to M/s Bharat Biotech, Hyderabad, India for making the DNA and protein versions of recombinant vaccine under GMP conditions, which will be employed for the clinical trials to be conducted under the auspices of the Indian Council of Medical Research.

10. SUMMARY

The paper reviews the historical background, which led to the making of 3 vaccines against hCG, the β hCG-TT, the HSD-TT/DT and the recombinant hCG β-LTB. The first vaccine, hCG β-TT underwent Phase I clinical trials not only in India, but also in Finland, Sweden, Chile and Brazil under the International Committee on Contraception Research of Population Council, New York. It was found fully safe and reversible. The immunogenicity of this vaccine was enhanced by annealing non-covalently hCG β to alpha subunit of ovine LH. This hetero-species dimer (HSD) linked to TT/DT as carriers underwent both Phase I safety and Phase II efficacy trials in several centres of India. The ability of the anti-hCG titres above 50 ng/ml to prevent pregnancy in sexually active women without derangement of ovulation and menstrual regularity was clearly demonstrated. A genetically engineered vaccine hCGβ-LTB has been made, which evokes high antibody response in Balb/C and other genetic strains of mice. It has received approval of RCGM. Toxicology studies conducted in two rodent strains as per international protocol has shown its complete safety. It is also immunogenic and safe in Marmosets preventing their becoming pregnant. The technology for making the DNA and protein version of hCG β-LTB has been transferred to a Company who would make available the vaccine produced under GMP conditions for trials to be conducted under the auspices of the Indian Council of Medical Research after obtaining approval of the Drugs Controller General of India and Institutional Ethics Committees.

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