ANTI-CONCEPTIVE TECHNOLOGY

PHASE V (INDIA)

(Central File No. 89-0041)

TECHNICAL REPORT

(1991 - 1996)

NATIONAL INSTITUTE OF IMMUNOLOGY

New Delhi

TECHNICAL REPORT ON
RESEARCH PROJECT ON ANTI-CONCEPTIVE TECHNOLOGY
PHASE V (April 91 - March 96)

The overall objective of the Phase V research project was to develop an anticonceptive vaccine which should evoke sufficient antibody levels against human chorionic gonadotropin in females to prevent pregnancy and which should be reversible and free of side effects.

The specific objectives of the Project were:

a. to design and conduct Phase II (efficacy) trials;
b. to develop alternative vaccine delivery systems;
c. to continue studies on live recombinant vaccine; and
d. to conduct preliminary studies towards a synthetic peptide vaccine.

Phase II Clinical Trials

After successful completion of Phase I clinical trials, phase II (efficacy) trials were conducted in three major institutions of the country, namely, the All India Institute of Medical Sciences (AIIMS) and the Safdarjung Hospital, both in New Delhi, and the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh. Prior approvals of the Drugs Controller of India, and institutional ethics committees were obtained. Women attending the Family Planning clinics in these institutions were enrolled for the study after providing informed written consent. The volunteers were of proven fertility (with at least two live children), of reproductive age (20-35 years) having regular menstrual cycles and active sexual life. Blood samples on day 18 and

24 of two pre-immunization cycles were analyzed for progesterone levels to confirm ovulation; these ranged from 16 to 51 nM/liter. Women with suspected pregnancy or breast feeding were not included in the trial. Also excluded were women with histories of recurrent abortions, secondary infertility, allergic, autoimmune or endocrine disorders, known or suspected malignancies.

The objective of the Phase II trials was to determine whether immunization with a hCG vaccine can prevent pregnancy in women, and if so, the level of antibodies necessary for efficacy. The vaccine consisted of a heterospecies dimer (HSD) of the ß-subunit of human chorionic gonadotropin (hCG) associated non-covalently with the α-subunit of ovine luteinizing hormone and conjugated to tetanus and diphtheria toxoids as carriers.

Immunization consisted of three primary injections of the vaccine at 6-week intervals. Boosters were given subsequently as and when necessary in order to maintain antibody titers above 50 ng/ml hCG bioneutralization capacity to those desiring to continue in the study. Subjects were required to report to the clinic for follow-up in the first and third week of each cycle. Blood was withdrawn on each visit for estimation of antibody titers.

A level of 50 ng/ml hCG bioneutralization capacity was fixed as the putative threshold for testing efficacy. For 95% confidence, observations were to be made for at least 750 cycles. Alternate contraception (intrauterine devices or condoms) was prescribed until antibody titers above the threshold were attained. In the event of antibody titers declining below 50 ng/ml, subjects had the option of taking a booster injection or withdrawing from the trial. In the latter case, they were followed up until antibody titers declined to near zero levels. In case the cycle was delayed, a pregnancy test was conducted, and if the test was positive, subject had the option of continuing or terminating the

pregnancy. In case medical termination was opted, it was done by the clinics free of cost.

Of 162 women interviewed, 148 completed the schedule of three primary injections. While all women made antibodies to hCG, duration of sustained titres above 50 ng/ml was variable. Booster injections to those willing to continue in the study were given at an average of 3 months. On March 31, 1992 observations were completed on 750 cycles when enrollment was stopped and boosters withheld unless specifically requested by the subjects, in accordance with trial conditions approved by institutional ethics committees. Follow-up was continued on women with circulating antibodies. As on August 1, 1993, observations had been recorded on 1224 cycles with only one pregnancy occurring in a woman having an antibody titer above 50 ng/ml (the pregnancy was terminated by vacuum aspiration upon the subject's request). Twenty three subjects completed 3-5 cycles of continuous exposure to the risk of pregnancy, twenty one 6-11 cycles, fifteen 12-17 cycles, twelve 18-23 cycles, nine 24-29 cycles and eight completed more than 30 cycles without becoming pregnant. These results show that at and above 50 ng/ml antibody titers, the vaccine was highly effective.

Further confirmation of the protective ability of the antibodies was provided by post-coital test. The test was conducted on 8 women who volunteered for an early morning examination after intercourse in mid-cycle. Cervical scores, recorded as per the WHO Manual, ranged between 10 and 14. Given the sperm count and quality, these scores should normally result in pregnancy.

The antibodies generated by the vaccine were of high affinity (Ka $\approx 10^{10}\ M^{-1}$) for hCG. These were devoid of cross-reactivity with human FSH and TSH but were partially cross-reacive with LH. Immunization did not change the menstrual regularity; 85% of the cycles were within the normal range (22-35

days). Longer and shorter cycles occurred with similar frequencies in women with antibody titres above 50 ng/ml and in those with ≤35 ng, a level at which women could conceive in the absence of alternate contraception. Luteal progesterone estimated in a single third week bleed of the cycles ranged from 14 to 44 nM/L, indicating normalcy of ovulation.

The response to the vaccine was reversible; antibody titers declined in all cases in the absence of booster injections. Although alternative contraceptives were advised, some women became pregnant when antibody titers were below 35 ng/ml. Five women, who conceived at antibody titers of <35 ng/ml, carried their pregnancies to term and delivered normal offspring. Of the remaining pregnancies which occurred during periods of low titers, or due to non-administration of the booster injection, 12 took place at titers at or below 20 ng/ml and 9 between 21-35 ng/ml. No late abortions were observed.

Development of Alternative Vaccine Delivery Systems

It was considered necessary logistically to deliver multiple doses of the vaccine at a single contact point. Work was carried out to develop biodegradable microspheres composed of co-polymer of lactic and glycolic acid for encapsulating the vaccine. The initial standardization of the system has been done with TT as the antigen. It is cheap and available in plenty. Furthermore, there is a national need for the development of a single injection immunization modality for pregnant women to prevent neonatal tetanus.

Procedure of preparing microspheres by multiple emulsion solvent evaporation method has been standardized. It is possible to produce the microspheres in the size range of 0.5-40 μm in reproducible manner. The preparations have been evaluated in animals. Microspheres encapsulated vaccine generated antibody

response equivalent to traditional multi-injection immunization with the vaccine adsorbed on alum.

Work was initiated on the encapsulation of the hCG vaccine in microspheres prepared from copolymers of different molecular weight taken in different monomer ratio with the aim to have a preparation which can engender a protective antibody response for either six months or a year. Preliminary animal experiments have shown promising results.

Studies on Live Recombinant Vaccine

Genetically engineered vaccine in a vector such as vaccinia offers an attractive approach for a cheap, single injection capable of eliciting long duration antibody response. We developed a vaccinia virus based live recombinant viral construct. The recombinant virus VSS2 had the gene for ßhCG fused in frame with the gene for a 48 amino acid transmembrane domain of the vesicular stomatitis virus (VSV) glycoprotein. When expressed, the chimeric ßhCG got transported to the cell-surface and elicited high titer anti-hCG antibodies that were long lasting in both rats and monkeys. These antibodies had a high affinity for hCG (Ka $\approx 10^{10}$ M^{-1}). Immunization with the recombinant virus prevented pregnancy in monkeys on continuous mating with males of proven fertility.

However, due to possible complications associated with vaccinia especially in immunocompromised individuals and its ability to spread from vaccinees to unintended subjects, use of fowlpox virus as a safe viral vector was undertaken. The fowlpox virus cannot replicate in non-avian hosts but an abortive infection does occur during which early proteins are transcribed and translated in amounts sufficient to induce titers of neutralizing antibodies.

Two fusion cassettes differing only in membrane anchor sequence were made: one carried the gene for ßhCG in alignment with the 48 amino acid vesicular stomatitis virus glycoprotein (VSVg) membrane anchor sequence (ßhCG-VSVg) and the other a novel 75 amino acid transmembrane anchor sequence from rabies glycoprotein (ßhCG-RGL) gene. The fusion cassette comprising of ßhCG-VSVg was taken out of the VSS2 genome by PCR amplification and this in turn was made by in frame fusion of transmembrane and cytoplasmic domains of the gene coding for VSVg to the 3' end of the ßhCG cDNA. Orientation of the anchor sequence with reference to ßhCG was checked by suitable restriction enzyme digestions. The transmembrane domain of rabies glycoprotein was taken out from the 3' end of the glycoprotein gene from LEP strain of rabies virus and was likewise fused to the 3'end of ßhCG cDNA to obtain ßhCG-RGL. The two cassettes were placed under the control of the 7.5k vaccinia promoter flanked on either side by the fowlpox thymidine kinase gene in a fowlpox transfer vector pBHCX 402 and inserted into the tk locus of viral genome by homologous recombination. The recombinant viruses were selected by their blue color in the presence of X-gal.

Chick embryo fibroblasts infected with CEVA strain of the fowlpox virus were transfected with the respective plasmid constructs to obtain the recombinant viruses. Two recombinant fowlpox viruses were obtained - VAO2 with ßhCG-RGL and VAO3 with ßhCG-VSVg. These recombinants were plaque purified. They were further enriched by immunoscreening the infected cells expressing hCG on the cell-membrane by employing the highly specific mouse monoclonal antibody against ßhCG.

The expression of ßhCG was detected in the pellet of the cells in both the chick embryo fibroblasts and CV1 cells of monkey kidney origin. These cells were infected with the viruses VAO2 and VAO3. The cell surface localization of

ßhCG was detected by immunofluorescence. The expression of ßhCG by the engineered fowlpox viral vectors (VAO2 and VAO3) on the infected cells was further demonstrated by a competitive radioimmunoassay using ßhCG specific monoclonal antibody.

Both genetically engineered fowlpox virus constructs express ßhCG on the surface of the infected cells and are totally devoid of virulence and replication in mammals tested and would, therefore, be a safe vaccine to induce anti-hCG response in humans.

Preliminary Studies Towards a Synthetic Peptide Vaccine

Present vaccine formulation employs TT and DT as carriers. Repeated immunizations with large carrier proteins often results in a low response to the attached antigen. Work is in progress in our laboratory to investigate whether synthetic T-cell helper peptides can substitute for the entire carrier for the hormonal subunit. Our preliminary results are encouraging; a 'cocktail' mixture of conjugates with three T cell peptides (one each from 38 kDa protein of *M. tuberculosis*, 1A protein of respiratory syncytial virus and CSP of *P. falciparum*) induced higher peak anti-hCG titres in mice of different haplotypes. The improvement was however not uniform in all strains; although these epitopes are promiscuous i.e. they are recognized in context of a variety of MHC/HLA alleles, they may not be equally reactive with these MHC/HLA alleles. Work has been initiated to identify additional T cell peptide(s).

Seven other peptides selected for screening are also natural epitopes from proteins of infectious agents; four from tetanus toxin and one each from fusion F protein of measles virus, hemaglutinin of influenza virus and reverse transcriptase of HIV. The rationale for selecting these is to take advantage of

any prior exposure to respective infectious agent. By selecting the appropriate cocktail of T helper cell epitopes, it may be possible to design a semi-synthetic vaccine that will be effective for all or most individuals of a genetically diverse human population. A totally synthetic hCG vaccine may however not be feasible in view of the bioneutralizing determinant(s) on ßhCG being highly discontinuous.

In quest of low cost vaccine production attempts are also being made to genetically engineer and express ßhCG along with T helper epitopes in the form of well defined fusion protein. This method offers the flexibility to alter the orientation of epitopes, position of one determinant in relation to the other and also to express ßhCG containing variable copy of T cell epitope(s).

Publications (1991-1996)

1. Vaccines for control of fertility. GP Talwar, Om Singh, R Pal. In: Perspectives in Primate Reproductive Biology (eds NR Moudgal, K Yoshinaga, AJ Rao, PR Adiga). Wiley Eastern, New Delhi, 1991, pp 269-276.

2. Immuno-contraception: Concept to reality. R Pal, Om Singh. In: Immunology: Perspectives in Reproduction and Infection (ed SK Gupta). Oxford & IBH, New Delhi, 1991, pp 183-187.

3. Advances in contemporary research: Vaccines for control of fertility. GP Talwar, Om Singh, R Pal, N Chatterjee, AK Suri, C Shaha. Ind J Expt Biol 1992;30:947-950.

4. Immunogenicity studies on diphtheria toxoid loaded biodegradable microspheres. M Singh, Om Singh, A Singh, GP Talwar. Int J Pharmaceutics 1992;85,R5-R8.

5. Anti-hCG vaccines are in clinical trials. GP Talwar, Om Singh, R Pal, N Chatterjee. Scand J Immunol 1992;36 (Suppl.11):123-126.

6. Vaccines for fertility control and hormone dependent cancers. GP Talwar, Om Singh, R Pal, N Chatterjee. Int J Immunopharmacol 1992;14:511-514.

7. Immunological control of fertility and hormone dependent cancers GP Talwar, Om Singh, R Pal, N Chatterjee, J Srinivasan, K Arunan, S Sad, H Gupta, P Sahai. In: Advances in Physiological Sciences (ed SK Manchanda). Macmillan, New Delhi, 1992, pp 453-457.

8. Vaccines for birth control. Are they feasible? How distant are they for clinical use? GP Talwar, R Pal, K Arunan, Om Singh, S Sad, A Suri, C Shaha, SK Gupta, SN Upadhyay. In: Contraception: Newer Pharmacological Agents, Devices, and Delivery Systems (eds R Sitruk-ware, W Bardin). New York, Marcel Dekker, 1992, pp 161-192. (Clinical Gynecology Series).

9. Vaccines for control of fertility. GP Talwar, Om Singh, R Pal, S Sad, K Arunan, N Chatterjee, C Kaushic, SN Upadhyay, P Sahai, M Singh, S Chandrasekhar, H Gupta, D Salunke. In: Progress in Immunology Vol 8 (eds J Gergely, M Benczúr, A Erdei, A Falus, Gy FÜst, G Medgyesi, Gy Petrányi, Éva RajnavÖlgyi). Springer Verlag, Budapest, 1993, pp 489-496.

10. A recombinant anti-fertility vaccine. J Srinivasan, Om Singh, R Pal, L Lall, S Chakrabarti, GP Talwar. In: Local Immunity in Reproductive Tract Tissues (eds PD Griffin, PM Johnson). Oxford University Press, New Delhi, 1993, pp 477-481.

11. A birth control vaccine is on the horizon for family planning. GP Talwar, Om Singh, R Pal, N Chatterjee, SN Upadhyay, C Kaushic, S Garg, R Kaur, M Singh, S Chandrasekhar, Anushree Gupta. Annals of Medicine 1993;25:207-212.

12. Antibody response against epitopes on hCG mapped by monoclonal antibodies in women immunized with an anti-hCG vaccine and its implication for bioneutralization. US Deshmukh, Pal R, GP Talwar, SK Gupta. J Reprod Immunol 1993;25:103-117.

13. Vaccines against LHRH and hCG. GP Talwar, Om Singh, R Pal, N Chatterjee, K Arunan, S Sad, V Raghunathan, P Sahai, H Gupta, D Salunke. In: Immunology of Reproduction (ed RK Naz). CRC Press Inc. Boca Raton, 1993, pp 235-249.

14. Biodegradable delivery system for single step immunization with tetanus toxoid. Raghuvanshi RS, Singh M, Talwar GP. Int J Pharm 1993;93:R1-R5.

15. A vaccine that prevents pregnancy in women. GP Talwar, Om Singh, R Pal, N Chatterjee, P Sahai, K Dhall, J Kaur, SK Das, S Suri, K Buckshee, L Saraya, BN Saxena. Proc Natl Acad Sci USA 1994;91:8532-8536.

16. Current status and future of immunological approaches to fertility control. GP Talwar, R Pal, S Dhawan, Om Singh, C Shaha. In: Population-The Complex Reality (ed G Smith). The Royal Society, London, 1994, pp 287-302.

17. Approaches to recombinant hCG birth control vaccine. T Krishnan, S Chandrasekhar, AG Ghorpade, P Sridhar, Om Singh, LC Garg, SE Hasnain, GP Talwar. In: Recombinant and Synthetic Vaccines (eds GP Talwar, KVS Rao, VS Chauhan). Narosa Publishing House, New Delhi, 1994, pp 207-215.

18. Bioodegradable delivery system for two vaccines. M Singh, RS Raghuvanshi, Om Singh, GP Talwar. In: *Recombinant and Synthetic Vaccines* (eds GP Talwar, KVS Rao, VS Chauhan). Narosa Publishing House, New Delhi, 1994, pp 403-406.

19. IgG subclass distribution of anti-hCG and anti-diphtheria toxoid antibodies in women immunized with ßhCG based immunocontraceptive vaccine. US Deshmukh, GP Talwar, SK Gupta. J Clin Immunol 1994;14:162-168.

20. Antibody response against three epitopic domains on hCG in women and rodents immunized with ßhCG based immunocontraceptive vaccine. US Deshmukh, GP Talwar, SK Gupta. J Reprod Immunol 1994;26:65-72

21. Targeting vaccinia virus-expressed secretory ß subunit of human chorionic gonadotropin to the cell surface induces antibodies. J Srinivasan, Om Singh, S Chakrabarti, GP Talwar. Infect Immun 1995;63:4907-4911.

22. Development of contraceptive vaccines for humans using antigens derived from gametes (spermatozoa and zona pellucida) and hormones (human chorionic gonadotropin): current status. RK Naz, A Sacco, Om Singh, R Pal, GP Talwar. Human Reproduction Update 1995;1:1-18.

23. Controlled release of hCG birth control vaccine using biodegradable microspheres. S Ganga, Om Singh. In: Proc Int Symp Control Rel Bioact Mater 1996;23:353-354.

24. Stabilization of tetanus toxoid against dichloromethane. RS Raghuvanshi, S Ganga, Amit Misra, S Mehta, Om Singh, AK Panda. In: Proc Int Symp Control Rel Bioact Mater 1996;23:859-860.

25. Vaccines against GnRH and FSH. R Pal, SS Majumdar, Om Singh, GP Talwar. In: Reproductive Immunology (eds RA Bronson, NJ Alexander, DJ Anderson, DW Branch, WH Kutteh). Blackwell Science Inc., Cambridge, 1996, pp 665-672.

26. The hCG vaccine. GP Talwar, Om Singh, R Pal. In: Reproductive Immunology (eds RA Bronson, NJ Alexander, DJ Anderson, DW Branch, WH Kutteh). Blackwell Science Inc., Cambridge, 1996, pp 673-682.

27. Expression of ß-subunit of human chorionic gonadotropin on the membrane surface of non-permissive mammalian cells by recombinant fowlpox virus. Anushree Gupta, S Chandrasekhar, GP Talwar, Om Singh (Mmanuscript to be submitted).

28. Studies towards development of a semi-synthetic hCG vaccine: use of T helper peptides as carriers. Aparna Mondokhot, Rahul Pal, GP Talwar, Om Simgh (Manuscript to be submitted).