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The Adventure of Making New Vaccines

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Described are two vaccines, one developed against leprosy and the other for control of fertility in women. The leprosy vaccine based on a cultivable non-pathogenic mycobacteria (*Mycobacterium w*) has completed Phase III clinical trials in two urban leprosy control centres and in a rural community block in Kanpur Dehat alongwith the National Leprosy Eradication Programme. The vaccine has significant therapeutic properties. Multibacillary patients given the vaccine once every three months in addition to the standard chemotherapy, become bacillary negative in significantly shorter period and immunization renders a large majority of these patients to lepromin positivity status, which drugs alone do not bring about. No ill effects of the vaccine were noted in either urban or rural clinical trials. The background leading to the development of this vaccine is discused. A vaccine inducing antibodies against human chorionic gonadotropin (hCG) has also been developed. The strategy adopted to make this vaccine is discussed. This vaccine after extensive experimental studies and pre-clinical toxicology has completed Phase I and Phase II clinical trials in women, which have provided data on its safety, reversibility and efficacy at and above antibody titres of 50 ng/ml. The vaccine acts without disturbance of ovulation and menstrual profiles. To enable the use of this vaccine on a large scale, R&D has been carried out to cover the logistic needs. The initial lag period of about three months for the antibody titres build-up, during primary immunization is planned to be covered by a companion approach. A single intrauterine application of extracts of neem seeds blocks fertility for a few months without impairing ovulation. Biodegradable microspheres are under development for a single contact point delivery of multiple doses of the vaccine. A live recombinant βhCG vaccine has also been made that elicits high and long term antibody response against hCG. This vaccine is currently in clinical trials in Mexico in patients of lung cancer of the type that makes hCG and or its subunits. HCG or its subunits act as autocrine growth factors for the tumour cells and antibodies inactivating hCG inhibit tumour growth. The vaccine has been well tolerated and no metastasis have been detected over two years of follow-up.

Kew Words: Leprosy, *Mw* vaccine, Immunotherapy; hCG vaccine, Safety, Reversibility, Efficacy; Praneem VILCl, Biodegradable microspheres, Live recombinant vaccine, hCG-synthesizing lung cancers

Introduction

Vaccines have historically been the most cost effective agents to control communicable diseases. A vaccine enabled the eradication of small-pox from the surface of the earth. Vaccines against tetanus, diphtheria, pertussis, polio and measles have drastically reduced infant mortality and morbidity. A number of diseases are, however, still rampant in developing countries, for which vaccines are not available. Amongst these is leprosy, an affliction that does not kill but disables and disfigures the human being. I intend discussing here the development of two vaccines, one against leprosy and the other for control of fertility. Both presented special challenges,

M. leprae, in killed or live form, given by whatever route, does not imunize the lepromatous leprosy patients, the ones who really need the vaccine. The tradjtional homologous approach was thus not feasible in this case. As regards the birth control vaccines, the task was to intercept selectively without ill effects a given step in the internal physiological process of the body.

Leprosy

Leprosy is an intriguing disease. Nearly 99% of the human beings have immunity to successfully resist infection. Amongst those who develop the disease, the form in which it is manifested varies and bears a relationship to the degree of immune deficit. It is a spectral disease. At one end are the lepromatous leprosy (LL) patients, who have extreme immunodeficiency. Mycobacterium leprae, the causative organism grows abundantly in their tissues. On the other end are the tuberculoid (TT) leprosy patients, who develop only a single lesion, the infection in them is limited and circumscribed. Within this lesion, the bacilli are killed and eliminated, with hardly any solid bacillus visible. The cell mediated immune reactions towards *M. leprae* are strong in the TT patients, manifested in histopathology and strong positivity (DTH skin reaction) to lepromin (a killed suspension of *M. leprae*). On the other hand, LL patients are lepromin negative and fail to respond to *M. leprae* in various tests such as antigen driven blast transformation, production of cytokines, etc.

The challenge was to see whether the multibacillary (MB) lepromatous subjects can be converted to the TT type with ability to kill and dispose off the bacilli. In case a vaccine invigorating immunity in MB patients is developed, it would be of help to cure them quicker by a possible joint interplay of immunotherapy and chemotherapy. There were additional repercussions. Multibacillary (MB) patients serve as the reservoir of infection and spread it to others in the community. If they are rendered as inhospitable territory for proliferation of *M. leprae*, there would be hope of reducing the incidence of the disease and eventually its eradication. *M. leprae* is an obligate intracellular parasite and man is the vector in almost all countries where leprosy is endemic.

Making of the Leprosy Vaccine

Cell mediated immune mechanisms are primarily involved in conferring resistance to *M. leprae*. The anergy of response in MB patients is specific to *M. leprae*. They respond perfectly normally to cholera and typhoid vaccine (Jha et al. 1971). They give a positive reaction to PPD but fail to develop hypersensitivity response to *M. leprae* antigens given as lepromin. The immune deficit is of two types, one is a consequence of infection and is recoverable on becoming bacillary negative, the other is long lasting, if not permanent (Nath et al. 1977). The permanent defect may be due to a "hole" in T cell receptor for reacting against key *M. leprae* antigen(s), the long lasting recoverable deficit could be due to suppression induced by either the constituents of *M. leprae* such as phenolic glycolipids (Mehra et al. 1984) or due to the suppressive factors released by the macrophages.

Strategy Adopted

In view of the fact that MB patients do not develop immunity by exposure to *M. leprae*, in contrast to tuberculoid leprosy patients or normal healthy individuals who do, we adopted a hetro-immunization approach to evoke cross reactive immunity. Immunization with homologous *M. leprae* may be of no avail. MB patients are loaded with *M. leprae* and mini immunization with lepromin does not alter their immuno-reactivity to the bacillus. The disease in MB patient is not autoregressive as is the case in TT. As the vaccine is primarily intended for MB patients, it will be little use giving them homologous immunization with *M. leprae*.

As mice are not an exact experimental animal model of human lepromatous leprosy, we made recourse to employing human peripheral blood leukocytes (PBL) of leprosy patients, which have both the lymphocytes and monocytes (besides other cells). A panel of polar TT patients was the source of PBL. The TT patients have evidence of having been infected and having successfully contained the disease and eliminated mycobacteria. The immune cells of these patients primed by prior exposure to *M. leprae*, bear thus the characteristics of defensive immunity against *M. leprae*.

Having decided for a hetero-immunization approach, the search began for a mycobacteria which shares antigens with *M. leprae* that evoke CMI reactions analogous to *M. leprae* in TT patients, but which carries also additional antigens that induce responsiveness in LL patients normally anergic in response to *M. leprae* (Talwar 1978).

A number of mycobacterial strains were procured from standard collections of Trudeau Institute and elsewhere. A number of atypical mycobacteria were also obtained from hospital collections in Delhi and Madras. These mycobacteria were cultured from throat swabs of suspected cases of tuberculosis but were distinct from *M. tuberculosis*. Many of them (including the bacillus eventually selected as candidate vaccine) turned out to be totally nonpathogenic to mice, guineapigs and monkeys.

In view of the importance of CMI, the known and the atypical mycobacteria were screened for antigen driven blast transformation and macrophage migration inhibition factor production with PBL from the panel of TT patients. *M. leprae* and BCG were run as parallel controls. Five mycobacteria evoked responsiveness similar to *M. leprae* with cells of these patients (Mustafa & Talwar 1978a). These were then examined for a number of other properties *viz* the ability to induce DTH in guineapigs to their own recall antigens and also to *M. leprae* (Mustafa & Talwar 1978b); the immunogenic potency in causing the enlargement of draining lymphnodes in mice (Mustafa & Talwar 1978c). Lepromin like preparations were made from the shortlisted mycobacteria and employed for skin tests in TT and LL patients. In the former, the aim was to score for the preparations giving reactions analogous to *M. leprae* and in the latter, for having a mycobacteria inducing lepromin like reactions in LL patients. In view of the likely modulating influence of environmental mycobacteria, these investigations were carried out in five different centres of the country (Govil & Bhutani 1978, Girdhar & Desikan 1978, Hogerzeil & Prabhudas 1978, Mustafa & Talwar 1978d, Sharma & Singh 1978, and Syed Maroof & Vellut 1978). The ensemble of these studies led to the identification of an atypical fast growing mycobacteria, investigated under the code name *w*.

Mycobacterium w resembles in its growth characteristics and metabolic properties the mycobacteria included in Runyon's Group IV. However, it differs in one respect or the other from those currently listed in this group (Saxena et al. 1978). An analysis of the base sequence in a polymorphic region of 65 kda gene shows that it is distinct from 31 other mycobacteria whose sequences have been determined (Reddi et al. 1994 and figure 1). Studies with monoclonal antibodies characterized as specific to *M. leprae* indicate that these epitopes are also present in *Mycobacterium w* (Ganju et al. 1991). The T cell clones developed from subjects immunized with BCG plus killed *M leprae* react with *Mw*. (Mustafa 1988).

Clinical Trials

After due preclinical toxicology, Drug Regulatory and ethical approvals, the *Mw* vaccine (composed of a suspension of autoclaved *Mw* in saline), has undergone successively Phase I, Phase II and Phase III clinical trials in urban and rural leprosy control centres. Large scale immunoprophylactic

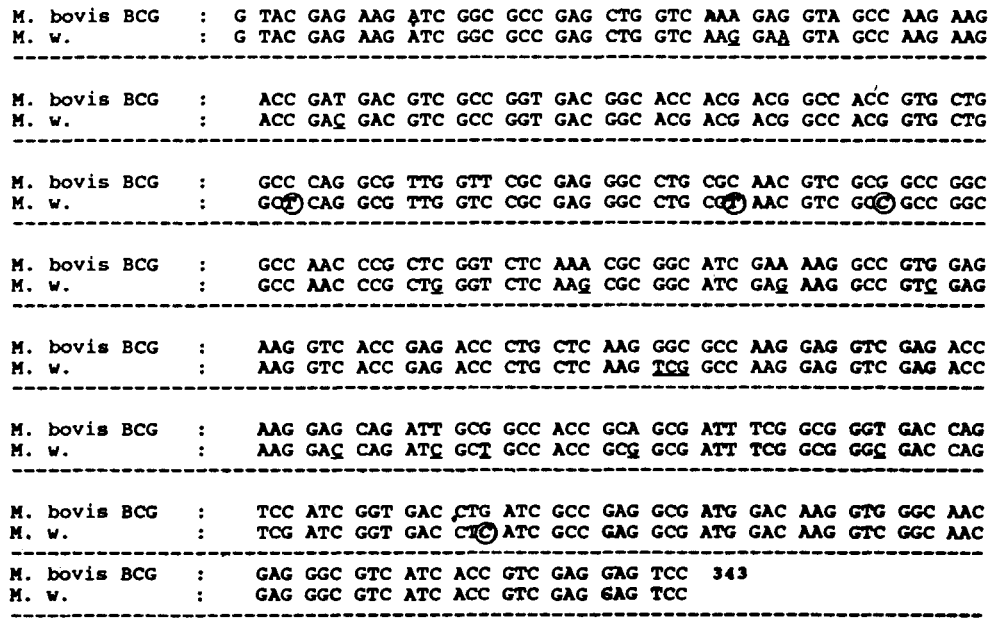

Figure 1 The nucleotide sequence of a portion of the polymorphic open reading frame (ORF) of 65 kD gene shows that *Mycobacterium w* differs from 31 other mycobacterias whose sequence in this region has been determined. The figure shows the comparison of *Mycobacterium w* with *M. bovis* BCG, the base underlined or circled differ between the two mycobacteria. The four encircled bases are signature sequences of *Mycobacterium w*. (Data from Reddi et al. 1994)

trials are also in progress in South India with the *Mw* vaccine and the Convitt vaccine consisting of live BCG plus killed *M. leprae*, under the aegis of the Indian council of Medical Research. The results of the latter trials would only be known after some years, as the latent period of the disease is upto 10 years. However; data is available on the *immunotherapeutic properties* of the *M.w* vaccine.

Two series of trials were conducted in urban leprosy control centres in Delhi. Multibacillary patients, with clinically active disease, who were bacillary positive and lepromin negative were enrolled for the study (Talwar et al. 1991). They were divided in two groups, one received the multidrug treatment (MDT) as per WHO regimen and the vaccine intradermally once every three months, the other group received MDT plus placebo injections of micronized starch. The bacterial clearance in patients receiving the vaccine and MDT was significantly better than in patients receiving drugs alone (table 1). This was accompanied by notable clinical improvement. Figure 2 illustrates the progression in a patient receiving immunotherapy in addition to chemotherapy, whereas figure 3 is representative of the rate at which lesions are cleared in patient receiving drugs alone. An important effect of the vaccine was to expedite the clearance of granulomas. Several patients manifested upgrading histopathologically and a large number receiving the vaccine attained NSI (non speciic infiltration) status (Mukherjee et al. 1992). The recovery time was shortened by combination of immunotherapy with chemotherapy (Zaheer et al.1993, Zaheer et al. 1994). Immunization with the *M.w* vaccine also converted over 64 to 66% of LL and BL patients and 94% of BB patients to lepromin positivity status, whereas in the group receiving MDT only, the conversions were 7 and 14% in LL and BL patients and 53% in BB cases.

Figure 2 A lepromatous leprosy patient at the time of enrollment in the trial (Left). The same patient after 4 doses of *Mw* vaccine and standard MDT for one year (Right)

Figure 3 A lepromatous leprosy patient at the time of enrollment in the trial (Left). The same patient after 8 doses of placebo injection and standard MDT over two years (Right)

Table 1 *Changes in BI values (Mean ± S.E.M.)*

Type of disease	Group	No. of patients	Initial	12 months	24 months
LL	Vaccine	84	3.54 ± 0.16	1.93 ± 0.15 ($p < 0.001$)	0.88 ± 0.12 ($p < 0.001$)
	Control	85	3.84 ± 0.14	2.78 ± 0.15	2.08 ± 0.15
BL	Vaccine	47	2.15 ± 0.18	0.56 ± 0.11 ($p < 0.001$)	0.08 ± 0.04 ($p < 0.0001$)
	Control	41	1.93 ± 0.20	1.19 ± 0.16	0.57 ± 0.09
BB	Vaccine	21	0.62 ± 0.12	0.05 ± 0.03 ($p < 0.05$)	0.00 ± 0.00
	Control	19	0.93 ± 0.19	0.39 ± 0.16	0.13 ± 0.07

All statistical analysis were done using analysis of variance. Significant decreases in BI vaccinated patients were seen in all types of leprosy. The p values are indicated in parentheses.

* S.E.M. = Standard error of mean

Immunization with the *M.w* vaccine entailed no significant side effect The frequency and the severity of reactions were in fact somewhat diminished (Kar et al. 1993).

Of interest was the effect of vaccination with *M.w* in patients who were "slow responders" or nonresponders to drugs, 14 patients showing little or no decline in skin and tissue BI despite 18 months to several years of treatment with MDT were randomly assigned to two groups, one continued to receive MDT, the other was given the *M.w* vaccine at three months interval. Initiation of immunotherapy with *M.w* led to a notable decline in the BI of the patients, whereas those continued on MDT alone manifested little change in BI over an equivalent period of observation (figure 4 a,b and Zaheer et al.1994).

These studies demonstrate the beneficial effect of combining immunotherapy with this vaccine alongwith standard chemotherapy in treatment of MB cases of leprosy (Talwar & Zaheer 1993).

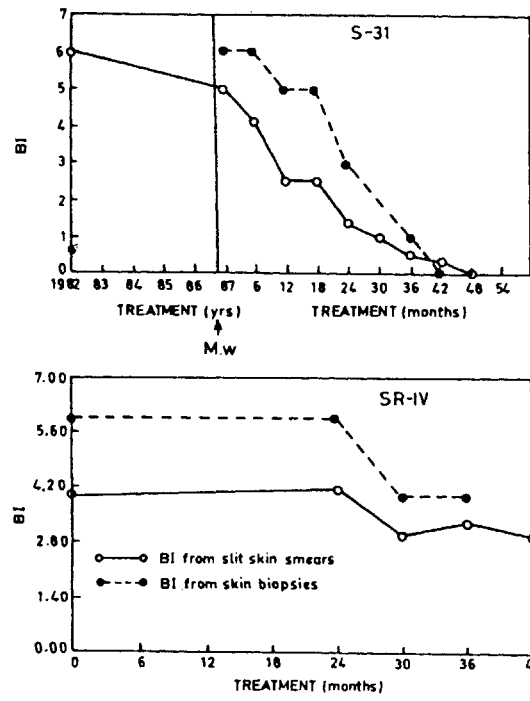

Figure 4 Representative follow-up of multibacillary patients who were "slow responders" and whose bacterial index (BI) did not fall appreciably inspite of repeated chemotherapy. The patient (S-31) after 60 months of MDT was administered the vaccine at the time point indicated by the arrow. This resulted in rapid fall of BI. The bottom figure shows the bacterial index in another "slow responder" patient who was continued on MDT and given only the placebo injection of micronized starch instead of the *Mw* vaccine

Vaccines for Control of Human Fertility

Vaccines were traditionally developed against pathogens, external to the body. We toyed with the idea of producing vaccines which could intercept selectively an internal process of the body. Mammalian reproduction is regulated by a series of hormones and inactivation of these by circulating antibodies can interrupt fertility. The sperm and the egg also carry antigens, susceptible to humoral and cell mediated intervention. Thus a number of vaccines are theoretically possible, and indeed we and others have developed several of these vaccines (Talwar et al. 1994b). Some of these vaccines may also find applications in immunotherapy of hormone dependent cancers.

The most advanced vaccine at present is directed at the human chorionic gonadotropin (hCG). Two parallel approaches have been followed. Stevens et al. (1981) are employing the 37 amino acid carboxy terminal peptide (CTP) of βhCG, whereas we opted to use the entire βhCG as the ligand (Talwar et al. 1976b). While the CTP is not present in hLH and it has thus the advantage of generating non-LH cross reactive antibodies, it has the disadvantage of having low immunogenicity; in addition, the antibodies generated have low avidity (Ramakrishnan et al.1979) The efficacy of these antibodies to ward off pregnancy has not been confirmed by other investigators. Another unexpected side effect of the CTP vaccine was the reaction of the antibodies with somatostatin making cells of the pancreas (Rose et al. 1988), the reaction is due to the CTP peptide and is absorbable by the peptide. On the

other hand, the entire βhCG based vaccines are comparatively better immunogens, and give rise to high avidity antibodies (Om Singh et al. 1989). The antibodies being of non linear sequence reading type are not reactive with pancreatic and other cells (Sehgal 1992, Rose et al. unpublished data). These do manifest partial cross reaction with hLH but the degree of this cross reaction does not impair ovulation (Talwar et al. 1976a, 1990, Nash et al. 1980, Kharat et al. 1990). Life long chronic toxicology studies were carried out at the Population Council in monkeys hyperimmunized upto 5-7 years with βoLH to generate antibodies cross-reactive with monkey CG and monkey LH. Ovulation was unimpaired and no pathology was found in the pituitary, kidney and other organs of these animals (Thau et al. 1986, 1987). These observations are consistent with our earlier studies in monkeys immunized with βhCG-TT vaccine and challenged repeatedly with hCG. No deposits of immune complexes were observed in the kidney, pituitary and choroid plexus (Gupta et al. 1978). In addition, the antibodies generated by βhCG and its improved version, the HSD vaccine were devoid of reactivities to nuclear, DNA, parietal cell, smooth muscle, islet cell, adrenal cortex, thyroid mitochondrial, thyroglobulin, C-reactive protein and rheumatoid factor (Nath et al. 1976a,b, Sehgal 1992).

Strategy to Make βhCG Immunogenic

βhCG is not immunogenic *per se* in humans. It was coupled to tetanus toxoid to mobilize the T helper cells for overriding the immunological tolerance (Talwar et al. 1976b). The conjugate was immunogenic in women and generated simultaneously antibodies against both βhCG and tetanus toxoid (figure 5). It was a double benefit vaccine. Tetanus is a major killer of women and neonates in developing countries where deliveries often take place in non aseptic conditions. The carrier can be altered, with diversification of immunoprophylactic benefit. Conjugates with DT and cholera toxin chain B have been evaluated to be equally immunogenic, and useful. Diversification of the carriers, or their use in alternating sequence, avoids hyperimmunization to a given carrier. diminishing the chances of carrier induced epitope suppression (Gaur et al. 1990).

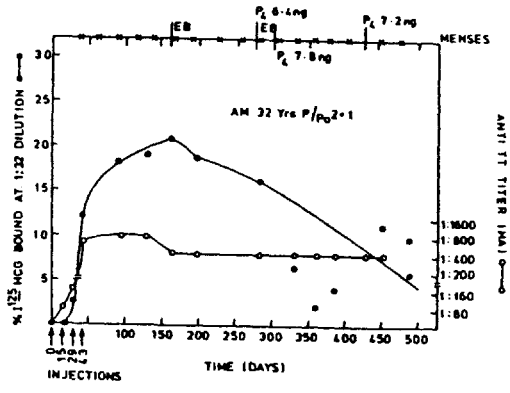

Figure 5 Anti-hCG and anti-TT antibody responses in a subject (AM, 32 years) immunized with βhCG-TT (Data from Talwar et al. 1976b)

HSD, The Improved hCG Vaccine

While the first prototype vaccine, βhCG-TT proved the soundness of the strategy for eliciting an anti-hCG response, it generated highly variable antibody titres in recipients. Its immunogenicity was improved by the following approaches:

1) An adjuvant, sodium phthalyl derivative of LPS (SPLPS), which is non pyrogenic but retains the adjuvant properties of LPS, was added in the first injection (Om Singh et al. 1982).
2) Intrinsic immunogenicity of βhCG was enhanced by associating it with the α subunit of oLH, with which it can readily combine, to generate a bioactive heterospecies dimer (HSD) (figure 6). HSD is more immunogenic than βhCG and, like the latter, also does not produce antibodies cross reactive with FSH (Talwar & Om Singh 1988). Antibodies induced by HSD have better

bioneutralization capacity (Talwar et al. 1988, Pal et al. 1990).

3) Alternate use was made of the HSD-TT and HSD-DT conjugates.

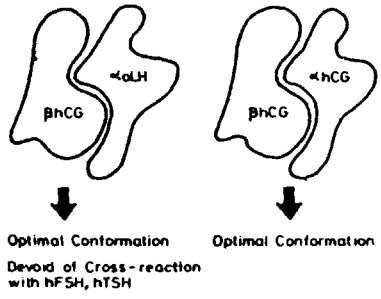

Figure 6 Association of the α-subunit of ovine luteinizing hormone with βhCG, to generate a bioactive dimer

Safety, Reversibility and Efficacy of the HSD Vaccine

After extensive toxicology studies, Drug Regulatory and ethical approvals, phase I clinical trials were undertaken with this vaccine in three major centres of the country. These trials demonstrated the lack of any significant side effect. This conclusion was reached after examination of 36 different parameters. Ovulation was maintained and menstrual regularity was unaffected. No increases in blood loss or aberrations in bleeding patterns were observed. The antibody response was reversible and declined to near zero levels in course of time in the absence of boosters (Talwar et al. 1990, Kharat et al. 1990). These studies confirmed the earlier observations on βhCG-TT vaccine in women in India, Finland, Sweden, Chile and Brazil, the international trials conducted under the auspices of Population Council (Talwar et al. 1976a, b, Nash et al. 1980, Hingorani & Kumar 1979, Shahani et al. 1982).

After ascertaining the safety and reversibility of vaccine, Phase II efficacy trials were carried out in women of reproductive age and of proven fertility with two live children. Normalcy of ovulation was confirmed in each volunteer. The protocol demanded clinical evaluation twice a month; blood samples for determination of antibody titres and luteal progesterone levels were taken at these visits. A putative threshold of 50 ng/ml was fixed for determining the efficacy of immunization. Observations were to be recorded on at least 750 cycles for drawing conclusions on efficacy. The subjects had the option to move out of the study after their antibodies declined below 50 ng/ml or to take a booster immunization for continuing in the study. This antibody titre threshold was found to be highly effective (figure 7) and only one pregnancy occurred in 1224 cycles (Talwar et al. 1993, 1994a). Immunization did not disturb menstrual regularity. Ovulation was maintained. The reversiblity of the procedure was confirmed by

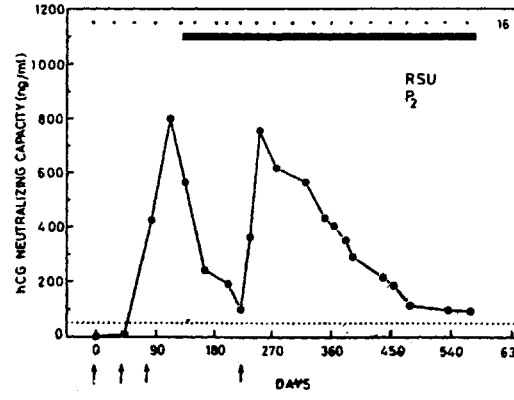

Figure 7 Efficacy of anti-hCG vaccination in a subject, RSU, immunized with the HSD vaccine during Phase II trials. The solid bar indicates duration of exposure to the risk of pregnancy (16 cycles). The small squares at the top of the graph indicate menstural events, which were by and large normal

regain of fertility on decline ot antibody titres below 35 ng/ml. Five women desirous of having a child carried their pregnancies to term and gave birth to normal babies. These babies will be followed up for 5 years and examined at periodic intervals for normalcy of physical and cognitive development.

The conclusion permissible from these studies is that it is feasible to regulate fertility of women by use of this birth control vaccine. The HSD-hCG vaccine is effective in preventing

pregnancy. The effective titres are above 35 ng/ml. The response is reversible and no significant side-effects attributable to immunization were observed in over 300 women immunized with the vaccine during Phase I & Phase II studies. The advantage of the hCG vaccine are:

1) It can be used at all stages of reproductive life by women, be it in the nuliparous stage, the mid reproductive years or at ages above 35, where the risk from some other alternate contraceptive is high, specially in smokers who use steroidal contraceptives.
2) The vaccine respects the normal physiological functioning of the pituitary and ovary, does not block ovuation, nor does it produce aberrations in menstrual episodes.

Requisite Further Development

With two important milestones achieved, namely of safety, reversibility and efficacy, further work is necessary to make the HSD vaccine logistically suitable for large scale use.

1) At present three injections at six weeks interval are required to complete primary immunization, similar to the requirements for anti-tetanus immunization. Experience in the Expanded Programme of Immunization shows that many subjects do not turn up for second and third injections. To avoid incomplete immunization, it will be desirable to deliver the multiple doses of the vaccine required for either six months or one year efficacy at a single contact point. This is achievable and two parallel tracks are being followed in our laboratory. In the first, the vaccine is being encapsulated in biodegradable microspheres (figure 8a,b) which deliver the antigen over the requisite period at a single contact point (Singh et al. 1992). The second track is the use of a live recombinant vaccine. The gene for βhCG in alignment with a transmembrane fragment has been inserted in vaccinia to obtain a vaccine with capability of generating long-term (two years) sustained antibody response in monkeys after a single immunization with the recombinant vaccine followed by one conventional booster at about nine months (Srinivasan et al. 1993).
2) At present there is a lag period of about three months in the build up of antibody titres during primary immunizations. This period is potentially vulnerable to pregnancy and requires to be covered by a reliable companion, compatible approach. This lacunae is planned to be filled by the combined use of Praneem VILCI, purified preparation of neem (*Azadirachta indica*) seed extract. These extracts contain immunomodulators activating locally cell mediated immune reactions. A single instillation of Praneem VILCI (PV) in the uterus blocks fertility of rodents (figure 9) and monkeys (Upadhyay et al. 1990, 1994) without impairment of ovulation. After due toxicology studies, Phase I clinical trials were conductred with PV which have shown the safety of the procedure in humans (Talwar et al. 1994c). Phase II studies are intended with PV and PV in combination with the HSD vaccine in the near future.
3) It will be appropriate to have a simple to use colour test for determining the effective antibody titres. By mapping of the determinants to which the antibodies are raised in women, a dominant epitope (recognizable by a monoclonal antibody) has been delineated (Deshmukh et al. 1993, figure 10). A prototype competitive immunogold assay using this monoclonal has been made. It will be evaluated for its workability in the next series of clinical trials with the HSD vaccine.

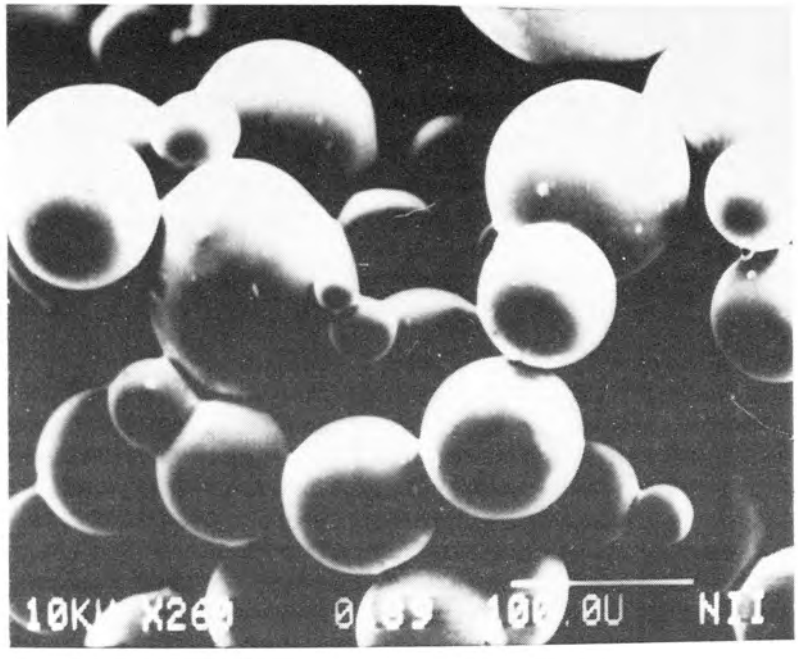

Figure 8a Scanning electron micrograph of biodegradable microspheres (X 260)

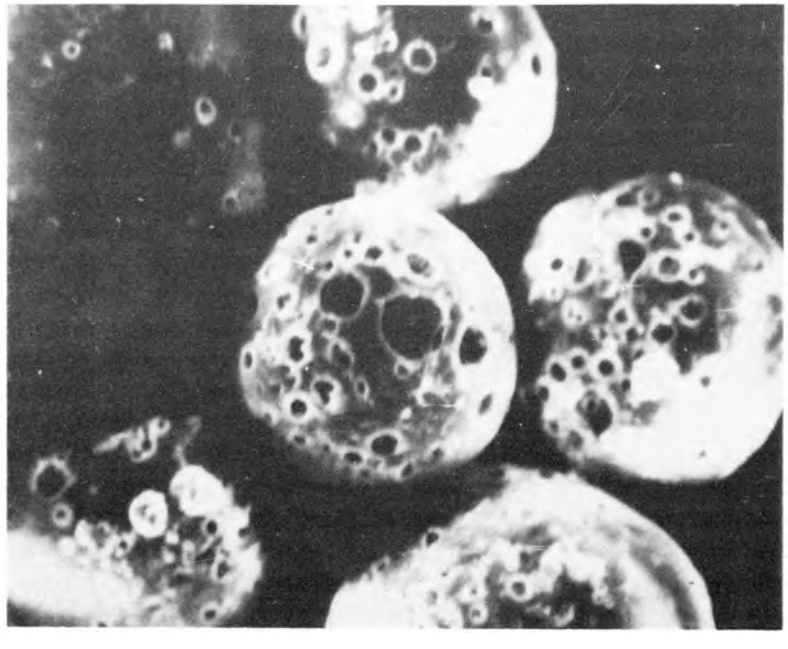

Figure 8b Scanning electron micrograph of biodegradable microspheres after 21 days *in vitro* erosion, exhibiting bulk erosion and leaching (X 720)

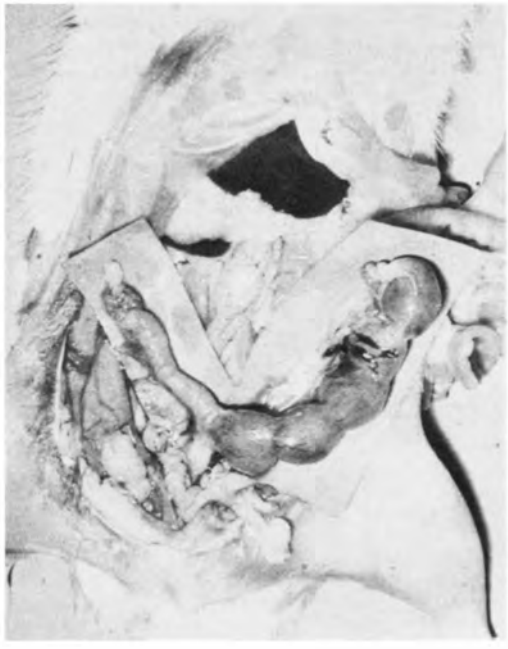

Figure 9 Fertility blocking effect of intrauterine treatment with Praneem VILCI (PV) in rats. The right uterine horn was injected with Praneem VILCI. The left uterine horn was injected with an equivalent volume of peanut oil and used as control. No implantation sites are visible in PV treated horn (Data from Talwar et al. 1994b)

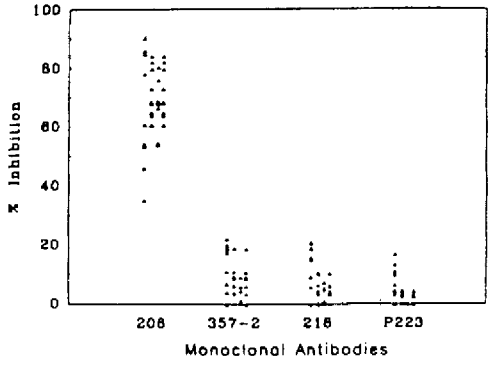

Figure 10 Inhibition of binding of human anti-hCG antibodies to hCG by monoclonal antibodies 206, 357-2, 218 and P223 which recognize different epitopic domains on hCG. The monoclonal antibody 206 caused a significant inhibition in all sera tested (Data from Deshmukh et al. 1993)

Applications of Anti-hCG Vaccination in Cancers Making hCG

The ectopic synthesis of hCG has been reported in a variety of cancers (Odell et al. 1985). Recent studies on a human lung cancer cell line (ChaGo) have indicated that hCG or its subunits act as autocrine growth promoters of these cells (Rivera et al. 1989). The inactivation of the hormonal subunits by antibodies prevents the formation of a tumor mass *in vitro* and *in vivo*. Administration of antibodies to nude (athymic) mice carrying tumors causes necrosis of the tumor. If, in addition, the ChaGo cells are also exposed to antibodies before implantation, a dose dependent inhibition in the growth of the tumor is noticed (Kumar et al. 1992). In consideration of the fact that no effective chemotherapy is presently available for such cancers, clinical trials with the hCG vaccine have been approved in Mexico in patients bearing hCG synthesizing lung cancers. Preliminary observations on three patients are encouraging and the trial is being extended to more patients.

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