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Development of immunological methods of fertility regulation*

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A new approach to fertility regulation is the development of vaccines directed against human substances required for reproduction. Potential candidates for immunological interference include reproductive hormones, ovum and sperm antigens, and antigens derived from embryonic or fetal tissue. Several vaccines targeted at the beta chain of the human chorionic gonadotrophin molecule have reached the clinical trial stage in Australia, Finland, India and Sweden, and the preliminary results are very encouraging. A prototype vaccine using the beta subunit of ovine luteinizing hormone seems effective in female primates. Contradictory effects on spermatogenesis in male primates have been observed by different researchers using follicle-stimulating hormone as an antigen. Both this and gonadotrophin-releasing hormone require further basic research. Immunological interference could also be aimed at sperm production or maturation, or at sperm–ovum interaction in the female reproductive tract. The best characterized sperm antigen to date is LDH-C_4, an isoenzyme of lactic dehydrogenase. The identification of new sperm antigens is being aided by monoclonal antibody techniques. Another approach is to detect, and then mimic, the antisperm antibodies found in women and men with immunologically mediated infertility. Research on ovum antigens is focusing on the zona pellucida (ZP); anti-ZP antibodies dramatically depress fertility in vitro *and* in vivo *in a variety of species. Vaccines interfering with sperm function and fertilization could be available for human testing by the early 1990s.*

The world stands to gain immeasurably from the development of immunological methods of fertility regulation. Birth control vaccines would not only enlarge the choice of methods open to men and women; they would offer specific advantages. For one thing, it should prove possible to develop vaccines that are unlikely to disrupt the menstrual cycle or cause the side-effects associated with hormonal contraceptives. Fertility-regulating vaccines should be well accepted by users, given the general popularity of immunization. Finally, there would be compelling advantages for service delivery because vaccines would provide long-acting fertility regulation, could be administered by paramedical or nonprofessional personnel, and could be integrated not only with family planning services but with other health care programmes as well.

Fertility-regulating vaccines differ fundamentally from the vaccines long used in infectious disease control. The latter have the advantage of being directed against "foreign" and thus highly immunogenic pathogens. An antifertility vaccine must be capable of safely and effectively inhibiting a human substance, which would need somehow to be rendered antigenic. A fertility-regulating vaccine, moreover, would have to produce and sustain effective immunity in at least 95% of the vaccinated population, a level of protection rarely achieved even with the most successful viral and bacterial vaccines. But while these challenges looked insuperable just a few years ago, recent advances in biotechnology—particularly in the fields of molecular biology, genetic engineering and monoclonal antibody

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production—are bringing antifertility vaccines into the realm of the feasible.

In brief, a birth control vaccine acceptable for use in human beings must meet the following criteria.

(1) The antigen must be unique to the reproductive target.

(2) The antigen must have a fertility-related function that can be blocked by antibody or is susceptible to cell-mediated immunity. Alternatively, the function should be located on a cell that can be lysed by complement.

(3) An acceptable level of effectiveness ($\geqslant 95\%$) should be achieved by no more than one or two injections for the primary immunization, with booster injections at intervals of no less than six to 12 months.

(4) The vaccine must undergo sufficient testing in animals to ensure its safety for long-term use.

In addition, for the vaccine to be widely acceptable, its effects should be reversible, i.e., the vaccine should not interfere with future childbearing at desired intervals.

Assuming that a suitable antigen is identified, a major obstacle to vaccine development will be the lack of suitable animal models for evaluating efficacy and safety. Immunocontraception is a radical departure. No method of regulating fertility has ever before rested on immunological principles, nor has any vaccine ever been directed towards the inhibition of a "self-like" component or secretion. Before a fertility-regulating vaccine can be envisaged for widespread use in men or women, animal models must be found for thorough testing of toxicity, teratogenicity, effectiveness, duration and reversibility of immunity and specificity for the target.

POTENTIAL ANTIGENS FOR VACCINE DEVELOPMENT

The mammalian reproductive system is theoretically susceptible to immunological intervention at numerous points. Many reproductive hormones, as well as several antigens that could be isolated from the ovum, sperm, embryonic tissue and fetal tissue, could be candidate targets.

Human chorionic gonadotrophin

A major candidate for vaccine development, and one on which research is the most advanced to date, is human chorionic gonadotrophin (hCG). This hormone, produced by the embryo, is essential for the maintenance of early pregnancy. It is a glycoprotein with a relative molecular mass of approximately 38 000 and 30% carbohydrate content. The hCG molecule consists of two chains, designated alpha and beta, whose structures have been established. The alpha chain of hCG is similar to the alpha chain of pituitary hormones that control ovarian and testicular function. However, part of the beta chain of hCG is unique to this hormone, and this has formed the basis of an attempt to develop a vaccine for use in women. Two basic approaches are being followed. WHO's Special Programme of Research, Development and Research Training in Human Reproduction (HRP), through its Task Force on Vaccines for Fertility Regulation, has been supporting research on a synthetic vaccine directed against the last 37 amino acids of the C-terminal end of the beta hCG molecule. This is the end of the molecule that is unique to beta hCG. The first phase of human testing (phase I—safety, dose and immune response) of this vaccine is currently under way at the Flinders Medical Centre in Australia with support from WHO. Preliminary results indicate that antibody levels several-fold higher than those estimated to confer efficacy have been elicited with few or no side-effects.

A second approach is being followed by the National Institute of Immunology, New Delhi, and the Population Council, New York. This involves developing a vaccine against the entire beta chain of the hCG molecule. Phase I clinical trials are currently being conducted in five centres in India using three different beta hCG preparations, and a similar phase I trial will begin shortly in Finland and Sweden with support from the Population Council. Eminent scientists from both the National Institute of Immunology and the Population Council participate in meetings of the WHO Task Force Steering Committee to help ensure interagency coordination of research in this area.

One important aspect of both approaches to developing an anti-hCG vaccine is the use of a carrier protein. Such a protein may be, for example, tetanus toxoid, diphtheria toxoid or the beta chain of cholera toxin, and serves two purposes. The carrier enhances the immunogenicity of the hCG antigen. At the same time, it confers immunoprophylaxis against a disease of public health importance, which is particularly critical in less developed countries.

Reproductive hormones

A prototype vaccine using the beta subunit of ovine luteinizing hormone (oLH-β) emulsified with Freund's complete adjuvant has been studied extensively in female primates by the Population Council. No evidence of acute or chronic health hazards was observed in this heterologous immunization model, although the vaccine did cause a shortened luteal phase with reduced progesterone levels. There was initial concern about potential immunopathology as a result of cross-reactivity between circulating antibodies and pituitary tissue, and immune-complex disease. These problems did not materialize in this model; however, further studies using both homologous and heterologous immunization models are needed to dispel this concern. While the mechanism of action of this apparently efficacious vaccine in animals has not been fully established, there is no evidence that immunized non-human primates become pregnant after mating. The Population Council has received approval from the US Food and Drug Administration to conduct phase I clinical trials for testing the immunogenicity of this vaccine in women. In addition, two of the beta hCG vaccine preparations currently undergoing phase I trials in India also include oLH antigens.

Other candidate hormones for vaccine development include follicle-stimulating hormone (FSH) and gonadotrophin-releasing hormone (GnRH). A vaccine against GnRH would block ovulation in the female and spermatogenesis in the male. However, it may have to be accompanied by estrogen/progesterone or androgen replacement therapy in the female and male, respectively. As for the anti-FSH vaccine, the underlying principle rests on the classical concept according to which FSH alone is responsible for the control of spermatogenesis in man, while LH controls Leydig cell function and hormone production. Thus, suppression or inactivation of FSH should lead to azoospermia and infertility without affecting testosterone levels and libido. Research being carried out in bonnet monkeys at the Indian Institute of Science in Bangalore continues to provide encouraging results along these lines. However, similar experiments conducted in rhesus monkeys at the Max Planck Institute in Münster (Federal Republic of Germany) showed that animals rendered infertile for several years experienced a spontaneous qualitative recovery of spermatogenesis even in the presence of high FSH antibody titres. One possible explanation is that testosterone alone can reinitiate spermatogenesis in the absence of FSH. Before vaccines of this nature can be fully exploited, further basic information is needed on the precise role of FSH in human spermatogenesis and on the possible immunopathological effects of anti-FSH and anti-GnRH antibodies.

As for vaccines against steroid hormones, there is no immediate role envisaged for them in human fertility regulation. However, research on such antigens is likely to be of

immense value in the fields of animal husbandry and veterinary medicine. It should also provide much needed data on reproductive biology and basic immunology.

Sperm antigens

The development of a vaccine based on sperm antigens will require a great deal of basic research. Interference with fertility could occur at several points: during sperm production in the testes, during sperm maturation in the epididymis, or during interaction with the egg in the female reproductive tract. Selective inactivation of those sperm components that actually participate in the process of fertilization would appear to be the best approach, thus providing a truly contraceptive vaccine. Whether vaccines involving sperm antigens would be more appropriately used in women or men remains an open question at this time. However, as it appears most likely that their use in women would involve the fewest complications and potential hazards, the best approach would probably be active immunization of the female or a passive local delivery such as vaginal administration of the anti-sperm antibody.

It is estimated that only about 1% of the proteins in sperm have been identified thus far. Among these there are very few sperm surface proteins, and not all of them are yet known to act as potential contraceptive antigens. The defined antigens in sperm include LDH-C_4, protamine, acrosin, hyaluronidase, and some plasma membrane antigens and differentiation products. With the exception of LDH-C_4, they are not readily accessible to antibody attack because they are not expressed on the surface of the sperm. Thus, unless high titres of genital tract immunoglobulin can be achieved, these antigens are not good candidates for vaccine development.

The best characterized sperm antigen is LDH-C_4, an isoenzyme of lactic dehydrogenase found only in male germ cells. Although this enzyme primarily occurs intracellularly, antibodies to it react with the surface of intact sperm. Antibodies to LDH-C_4 have been produced in a variety of species and active immunization has reduced but not completely blocked fertility in mice, rabbits and baboons. Antibodies probably inhibit fertility by causing sperm agglutination. The structure of LDH-C_4 has been determined, and vaccines based on polypeptide fragments of the molecule have likewise been shown to inhibit fertility. Recent studies have been directed towards the development of a synthetic peptide that would elicit an immune response to the native protein. Research has also focused on stimulating the secretory IgG system in primates to ensure high antibody titres in the genital tract.

The identification of new sperm-specific antigens has been aided tremendously by the use of monoclonal antibody (MAb) techniques. Some MAbs to specific regions of sperm have been prepared that are being used in turn to identify new antigens. This work could lead to a breakthrough in the sperm vaccine field by facilitating the characterization of sperm surface antigens that would be readily accessible to antibodies present in the female reproductive tract.

In June 1986 WHO and Family Health International, North Carolina, USA, co-sponsored a workshop that involved the prior worldwide distribution and testing of a coded panel of 67 mouse MAbs alleged to react with human sperm. The workshop, attended by investigators active in the field, led to a consensus on the feasibility of using MAbs to identify reproduction-specific antigens that might represent suitable candidates for the development of antifertility vaccines. Five anti-sperm MAbs demonstrated sufficient specificity to warrant further investigation as reagents for identifying potential vaccines.

Another approach to the identification of sperm antigens is the evaluation of serum samples obtained from women and men considered to have immunologically mediated infertility. This approach provides information concerning naturally (spontaneously) occurring antibodies that could perhaps be intentionally mimicked in fertile individuals.

Aside from their association with infertility, circulating antibodies in immunologically infertile individuals do not appear to cause any health hazards.

From 1974 to 1981 the WHO Task Force supported a reference serum bank for reproductive immunology at the University of Aarhus, Denmark. Collaborating investigators from around the world submitted sera from infertile couples in the hope of better characterizing the immunological factors contributing to infertility. Unfortunately, this approach to antigen identification for selecting potential vaccine candidates has not been as rewarding as anticipated; many of the test procedures used for characterizing and categorizing the sera gave inconsistent results. Recent advances in biotechnology may begin to render this approach more promising.

Ovum antigens

While the initial stages of oogenesis take place during fetal life, oocytes only undergo final maturation in women shortly before ovulation. These final maturation stages may be associated with the production of specialized antigenic substances that could be suitable targets for immunocontraception in women. Antibodies directed to the non-fertilized egg in the ovary or in the reproductive tract could prevent fertilization. Although there may be specific antigens associated with the oocyte itself, no such antigens have yet been isolated and purified.

Most research on ovum antigens is directed towards the zona pellucida (ZP), the acellular, gelatinous layer surrounding the ovum. Several research groups have recently made considerable progress in the biochemical characterization of unique glycoproteins constituting the ZP. Both *in vitro* and *in vivo* tests following active and passive immunization have shown that antibodies to ZP can have dramatic effects on fertility in a variety of species. The immunological response to ZP antigens is remarkably consistent between individual animals and between species. However, there have been indications in some species that anti-ZP antibodies also alter ovarian function and ovum development. Whether this will occur in all primates and in women is not known; more detailed studies using purified zona antigens are clearly needed to determine the respective mechanism(s) of action of immunization with different ZP antigens.

CONCLUSIONS

Multidisciplinary research and collaboration between interested investigators, institutions and funding agencies are essential if the formidable challenge of developing birth control vaccines is to be met successfully in the near future. Research is needed to identify additional antigens, particularly those that will exert an immunocontraceptive effect, and to develop methods of making available large quantities of the purified antigens selected for further study.

Given the predictable variation in individual responses to immunization with fertility-regulating vaccines, research is also needed in the field of "basic vaccinology", to find the best carrier proteins, adjuvants, vehicles and delivery systems. Such research will also have spin-off benefits for the development of vaccines against communicable diseases.

Within the next 12 months information will be available on the results of current and planned clinical trials of several different vaccines directed against human chorionic gonadotrophin. It is estimated that vaccines that interfere with sperm function and fertilization should be available for human testing by the early 1990s.