



Review

Molecular Targets for Immunocontraception

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Abstract. Effective contraception is necessary in countries where limiting population growth has become a public policy imperative. The main antigenic targets for contraceptive vaccine development can be listed as following: 1) sperm antigens, 2) zona pellucida antigens, 3) gonadotrophin-releasing hormone, 4) chorionic gonadotrophin, 5) other protein/peptide hormones (follicle-stimulating hormone, luteinizing hormone, luteinizing hormone-releasing hormone), and 6) gonadal steroid hormones. New techniques, such as the application of monoclonal antibodies, hybridoma and DNA recombinant technologies, have become useful in search for contraceptive candidates. Current state of development of contraceptive vaccines based on specific antigenic targets and trials in animal/human models are presented in this article.

Key words: immunocontraception; sperm; zona pellucida; hormonal antigenic targets.

Introduction

Contraception is required to be an acceptable, safe, effective and cheap procedure. The concept of a birth control vaccine is both simple and audacious. One should take a component of the reproductive system, put it into a vaccine vector, and then use this vaccine to block the component's activity by means of antibodies or other immunological effector mechanisms. The audacity exists at two levels: 1) any component employed is a normal, self component of the body and, as such should be unable to provoke an immune response: it should evoke immunological tolerance, and 2) there exists a possibility that induction of an immune response may potentially be harmful to the vaccinated organism¹⁷.

An effective hormonal contraceptive in men that could be implanted or injected as a long-acting formu-

lation (every 3–6 months) would be useful in countries where limiting population growth has become a part of public policy². The development of gametes is under the control of gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are secreted from the pituitary and reach the gonads through the bloodstream¹⁵. Neutralization of LH and/or FSH by circulating antibodies will impede their action on the target organs (ovaries and testes) and thus interfere in the maturation of oocytes and spermatozoa. Secretion of the gonadotrophins, in turn, is regulated by gonadotrophin-releasing hormone (GnRH) secreted by the hypothalamus. A neutralization of human chorionic gonadotrophin (hCG) by developed antibodies also leads to infertility. Immune responses can also be elicited against the unique surface determinants of the gametes involved in the process of fertilization⁸.

An immune attack on any component involved in

Abbreviations used: cDNA – complementary DNA, FD – fertilizing dose, FSH – follicle-stimulating hormone, GnRH – gonadotrophin-releasing hormone, hCG – human chorionic gonadotrophin, LH – luteinizing hormone, mAbs – monoclonal antibodies, rhZPB – recombinant human zona B pellucida, ZP – zona pellucida, CFA – complete Freund's adjuvant.

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the control mechanisms that regulate reproduction should lead to a suppression of fertility. The choice of potential targets is constrained by their immunogenicity, specificity, antigen density and location¹. Current targets of contraceptive vaccine development can be listed as following: 1) sperm antigens, 2) zona pellucida (ZP) antigens, 3) GnRH, 4) chorionic gonadotrophin, 5) other protein/peptide hormones (FSH, LH, luteinizing hormone-releasing hormone), and 6) gonadal steroid hormones. It is assumed that antibodies developed by any immunocontraceptive vaccine will potentially act in the oviduct, uterus, cervix or vagina¹¹, i.e. preferentially in a female local (secretory) system.

Fertilization is a complex process requiring the spermatozoon to undergo a cascade of events before it can fuse with the egg plasma membrane. This chain of events includes capacitation, acrosome reaction, binding to ZP, penetration through ZP and fusion with the plasma membrane of an oocyte, which subsequently cleaves, develops and implants²⁰. The concept of a contraceptive vaccine can be stated in general terms as follows: a formulation of certain immunogenic molecules injected or taken orally by a man or woman triggers the production of circulating antibodies or immune effector cells that interrupt reproductive processes and sustain a period of infertility without side effects⁶.

Sperm-Surface Antigens

The immunization of males or females of various species, including humans (both sexes), with preparations of autologous or isologous spermatozoa causes the development of anti-sperm antibodies, leading to infertility. The whole spermatozoon *per se* cannot be employed for the development of a vaccine due to the presence of numerous antigens, both internally and on the surface, that are likely to be shared with various somatic cells. It is essential that only those antigens that are sperm-specific can be employed for immunocontraception²⁰. An "ideal sperm vaccine" may be conceived as containing sperm-specific immunogens which induce antibodies to all the accessible surface domains of spermatozoa: the head, mid-piece and tail plasmalemma, including the inner acrosomal membrane, which forms a major part of the anterior surface of the sperm head following acrosome reaction¹¹.

A series of monoclonal antibodies (mAbs) have been reported to inhibit fertility in various species of animals. So far, a few of the sperm antigens have been isolated and characterized by biochemical and immunological techniques using these mAbs, and the com-

plementary DNAs (cDNAs) encoding for some of these antigens have been cloned and sequenced²⁰.

Employing DNA recombination methods, a few novel sperm-specific antigens have been found and designated as AgX, NZ-1, FA-1, and NZ-2. Sperm-specific antigens have been identified by means of mAbs or polyclonal antisera raised against the sperm of various species, e.g. SP-10, PH-20, PH-30 and others⁶. Monoclonal Abs against sperm, selected for specificity, potency, and safety, might be used to create an improved vaginal barrier in a passive way. Mouse anti-rabbit sperm-agglutinating mAbs are effective contraceptive agents when mixed into the semen and vaginally delivered to rabbits. Since the rabbits can be inseminated with several thousand fertilizing doses (2500–5000 FD50), and a human ejaculate probably delivers no more than one FD50, a sperm-agglutinating mAb might prove to be significantly effective in humans in future⁴.

A novel dodecamer peptide sequence, designated as YLP12 on human sperm, was recently reported. It is located on the acrosomal region of the human sperm cell and is expressed only in human testis. Experimental data indicate that the YLP12 peptide sequence is involved in the sperm capacitation/acrosome reaction, what may find an application in an immunocontraceptive trial²².

Zona Pellucida Antigens

ZP, a unique extracellular translucent matrix surrounding the mature oocyte, mediates several critical steps in the fertilization process, including induction of the sperm acrosome reaction, sperm binding, and establishment of the block to polyspermy²¹. ZP antigen(s)-induced infertility can result from two modes of action: 1) an antibody-mediated inhibition of ZP sperm receptor sites or 2) the cytotoxic T cell-mediated destruction of developing ovarian follicles³. Major ZP antigenic determinants consist of amino-acid sequences, conformational or structural determinants and carbohydrate structures of these determinants that vary among species. ZP glycoproteins have been known to elicit strong immune responses in a number of species, including the rabbit, dog and non-human primates, resulting in decreased fertility, which made them suitable candidates for immunocontraception¹⁰. It is important to determine specific ZP protein(s)/peptides that induce antibodies which interfere with sperm binding with no collateral influence on ovarian follicular development^{25, 27}. In most species, ZP is composed of three (or four)

biochemically and immunologically distinct glycoproteins: ZP1, ZP2 and ZP3. On the basis of the size of mRNA transcripts, they have been classified as ZPA (the longest), ZPB and ZPC (the shortest)⁶. ZP's contain sperm receptors that restrict species-specific gamete interaction and prevent sperm binding from the already fertilized eggs of homologous species³⁰.

In a mouse model, during the fertilization process, spermatozoa initially bind to ZP3, which triggers the acrosome reaction. After induction of the acrosome reaction, ZP2 acts as the secondary receptor and helps to keep the acrosome-reacted spermatozoa bound to the egg. ZP2 is proteolytically cleaved after fertilization, and this modification, along with changes in ZP3, is postulated to play an important role in the post-fertilization block to polyspermy. ZP1 has been postulated to cross-link the ZP2-ZP3 heterodimer¹².

ZP of different mammalian species contains immunologically cross-reactive specificities. Antibodies generated against porcine ZP cross-react with human ZP⁵. Hetero-immunization of rabbits and monkeys with pig ZP proteins elicits the production of antibodies to self ZP antigens that can interfere with follicular development as well as with fertility, whereas the immunization of rats and mice with native pig ZP proteins has no effect on their fertility. Interestingly, allo-immunization of female rabbits with rabbit ZP proteins does not induce a significant immune response, as determined by circulatory antibody levels irrespective of the use of complete Freund's adjuvant (CFA) as an adjuvant. When rabbits are immunized with native rabbit ZP or recombinant rabbit ZP proteins expressed in bacteria, no antibodies are produced against self ZP epitopes even if CFA is used. It is of interest that some genetic strains of mice immunized with another strain of mouse ZP proteins or peptides develop antibodies against self ZP epitopes. A mAb produced in rats that is specific to murine ZP3 has been shown to reduce mouse fertilization *in vivo* and *in vitro*²⁷.

Immunizations with recombinant human ZPB (rhZPB) protein caused cynomolgus monkeys to become infertile for 9–35 months. The baboons immunized with rhZPB became infertile for 9 to 20 months. During the time when the maximum antibody titers were induced, some animals experienced disruption of the menstrual cycle, but eventually all the animals resumed normal menses¹⁴ after the antibody titers fell. Rabbit polyclonal antibodies against recombinant bonnet monkey (*Macaca radiata*) ZP glycoprotein B significantly inhibited human sperm-oocyte binding. Monoclonal Abs MA-809, -811, -813, and -825 to bonnet monkey epitopes showed a significant inhibition of

the binding of human spermatozoa to human ZP in a hemi-zona assay⁷.

Gonadotrophin-Releasing Hormone

Taking into account that GnRH stimulates pituitary secretion of gonadotrophin hormones, it was logical to predict that immunoneutralization of GnRH would prevent endogenous GnRH from binding to its gonadotrophin receptors and, thereby, delay a sexual maturation in adolescent animals, causing gonadal atrophy in adults¹⁹. The administration of carefully monitored doses of GnRH or its agonists induces ovulation, but its chronic exposure leads to an antifertility effect. Administration of GnRH agonist in males leads to suppression of circulating testosterone but azoospermia is not achieved in all cases. GnRH antagonists, however, have been shown to inhibit ovulation. Their administration during the mid-luteal phase leads to luteolysis. Studies in male sub-human primates with application of GnRH antagonists demonstrated that azoospermia can be induced. Alternatively to GnRH analogs, the mAbs obtained against GnRH can also be used for fertility regulation. Such mAbs are competent to terminate pregnancy in mice and non-human primates⁸. Furthermore, experimental data also demonstrated that GnRH vaccines are effective in inducing a reversible infertility in white-tailed deer, such infertility lasting up to two years without boosting¹⁶.

Chorionic Gonadotrophin Vaccines

Human chorionic gonadotrophin is the only well-defined protein hormone known to be absolutely essential for the maintenance of pregnancy. It is produced by the trophoblast and acts on the corpus luteum to stimulate progesterone production. It is secreted in substantial amounts only during pregnancy, so its neutralization should have no other effects than on fertility. Small amounts of this hormone are produced by the pituitary during the menstrual cycle. Soon after conception, when its concentration starts rising, hCG is found in concentrations small enough to be easily neutralized by high-affinity antibodies¹⁷.

Human chorionic gonadotrophin is composed of two subunits: the α -subunit, which is common to the other three pituitary hormones, and the β -subunit, which is hormone specific. The disadvantage in choosing of β -hCG is the partial cross-reactivity of antibodies generated against hCG with human LH. It has been

confirmed, however, that such cross-reaction does not impair the ovarian function. Superior immunogenic properties, impressive neutralization capacity of the antibodies induced and the lack of side-effects as studied by a variety of criteria, were decisive factors for selecting β -hCG and, subsequently, a hetero-specific dimer composed of α -hCG associated with the α -subunit of ovine LH as the immunogen of choice²³ for clinical trials.

Antibodies elicited by the β -hCG vaccine inactivated hCG and prevented the action of this hormone from rescuing the corpus luteum, which resulted in progesterone fall and normal menses. A fall in the antibody response confirmed the reversibility of the approach²⁴ when no repeated doses were applied.

FSH and LH Vaccines

In females, FSH is known to help in growth of ovarian follicles, whereas in males FSH regulates growth of the seminiferous tubules and helps to maintain spermatogenesis⁸. Selective immunoneutralization of FSH was found to suppress spermatogenesis without altering the GnRH-LH-Leydig cell-testosterone axis and libido and, therefore, might be an acceptable approach toward fertility control in men¹⁹. Immunization against LH prevented pregnancy in all but one LH-immunized ewe (n=10) during two breeding seasons. It prevented ovulation presumably by inhibition of the preovulatory surge of LH, not altering its basal concentration²⁶.

Two peptides were selected from the β -subunit of FSH that were considered to be inducing the anti-FSH activity but not the anti-LH activity (sequences of β 33–53, and β 81–95 amino-acids). These peptides were subsequently used to immunize male rabbits. It was found that antisera raised against the β 33–53 tandem inhibited FSH bioactivity but not that of LH. Antisera against the β 33–53 monomer or the β 81–95 monomer or tandem did not inhibit FSH. Thus, the tandem peptide β 33–53 is an attractive candidate for use as the antigen of choice in a male contraceptive vaccine².

Gonadal Steroid Hormones Vaccines

Steroid hormones are known to be haptens and, therefore, they have to be conjugated at a non-active site to a carrier to provide essential immunogenicity. Because of their lipid solubility, steroid hormones can traverse the plasma membrane and enter the cells by

simple diffusion. Once inside, these hormones bind to specific receptors and may alter the genetic programming apparatus of the target cell¹⁹.

Data obtained in monkeys and humans treated with steroid contraceptive candidates indicated that impairment of spermiogenesis is a key to achieve azoospermia³¹.

The actively immunized BALB/c female mice with anti-idiotypic antibody (Ab2) to progesterone showed 80% reduction of their fertility rate. The duration of infertility correlated with the concentration of anti-progesterone antibody (Ab3). The Ab2 blocked pregnancy in 80% of the passively immunized mice¹³. Inhibition of implantation was attributed in part to the antibody interaction with progesterone, bound to the uterine epithelial membrane surface carrier²⁹. Progesterone immunization altered the ovarian cycles, causing anestrus and persistent infertility in sheep²⁸. Ovulation was also known to be inhibited by Ab3 in gonadotrophin-primed immature rats¹⁸.

Conclusions

Several sperm antigens, identified by using of mAbs, cDNA and hybridoma technology revealed testis-specific expression and played roles in the fertilization process: GB24, CD52, LDH-C4, PH-20, PH-30, FA-1, FA-2, NZ-1, NZ-2, and SP-10. Immunization of females with sperm antigens may lead to a block of fertility in several animal models.

Zona pellucida consists of three main glycoproteins: ZP1, ZP2 and ZP3, which elicit strong immune responses in a number of species, resulting in decreased fertility. This ability introduced them to the family of suitable candidates for immun contraceptive trials.

Neutralization of LH and FSH by circulating antibodies might impede their actions on the gonads of both sexes, interfering in this way with the maturation of the spermatozoon and oocyte. Antibodies induced in response to β -hCG can inhibit hCG-dependent progesterone synthesis, thus effectively preventing the implantation of the fertilized ovum.

A multivalent vaccine (antigenic “cocktail”) with the individual-specific epitopes of sperm cell, zona pellucida and sex hormones (FSH, LH, hCG, GnRH, gonadal steroids) may exhibit a strong immunogenic potential and efficacy at inhibition of fertilization and early embryogenesis.

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