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Antisperm Immunity for Contraception

RAJESH K. NAZ

From the Reproductive Immunology and Molecular Biology Laboratories, Department of Obstetrics and Gynecology, The West Virginia University, School of Medicine, Morgantown, West Virginia.

Presently, there are only limited options available for family planning. Contraceptive modalities available to women include hormonal contraceptives (birth control pills, contraceptive patch, and hormone injections), natural methods (abstinence near ovulation and early withdrawal before ejaculation), intrauterine devices (IUDs; copper IUD, vaginal rings, diaphragm, and spermicidal combinations), and sterilization (Contraception online, 2004; Upadhayay, 2004; Harper, 2005). The contraceptive options available to men are vasectomy, condoms, and early withdrawal. Vasectomy is the most effective male contraceptive method currently available. However, vasectomy is a permanent procedure with a limited success rate of fertility reversal even after successful surgical reanastomosis (Silber and Grotjan, 2004). Therefore, for men who wish to father a child at a later time, condoms and early withdrawal are the only contraceptive options. However, these two methods are either not readily acceptable or have high failure rates. Even with the available contraceptive methods, the world population exceeds 6.48 billion and will grow by 1 billion every 12 years at the present rate of population explosion (Anonymous World POP Clock Projection US Census Bureau, October 2005). In addition, unintended pregnancies continue to impose a major public health issue. In the United States alone, half the pregnancies are unintended, which results in more than 1 million elective abortions each year (Henshaw, 1998; Grow and Ahmed, 2000). In more than half of these unintended pregnancies, the women were using some method of contraception. The 2004 Institute of Medicine report indicates that between 1995 and 2000, more than one quarter of 1.2 billion pregnancies were unwanted (Institute of Medicine report, 2004). Thus, there is an urgent need for a better method of contraception that is reversible, nonsteroidal, nonbarrier, intercourse-independent, acceptable, and effective. The most important properties of an ideal contraceptive method desired by women are that it should be highly effective and safe, is inexpensive, has a prolonged duration of action, is rapidly reversible and easily accessible, requires infrequent administration, and can be used privately (Contraception online, 2004). A contraceptive vaccine (CV) can fulfill most of the properties of an ideal contraceptive. Since the developed nations and most of the developing nations have an infrastructure for mass immunization, the development of vaccines for contraception is an exciting proposition.

Various targets have been explored for the development of CVs. These broadly fall into three categories: vaccines inhibiting gamete production (gonadotropin-releasing hormone [GnRH]), follicle-stimulating hormone [FSH], and leuteinizing hormone [LH]), gamete function (zona pellucida [ZP] proteins and sperm antigens), or gamete outcome (human chorionic gonadotropin [hCG]; Naz, 2005a; Naz et al, 2005). Advantages of GnRH-based vaccines are that they are effective in several species and can be used for both males and females. However, they affect sex steroids, causing impotency, thus, are not acceptable for human use. GnRH-based vaccines have been taken over by pharmaceutical companies for fertility control in domestic pets, farm animals, and wild animals, and for noncontraceptive purposes, such as prostatic hypertrophy and carcinoma (Ferro and Stimson, 1999; Simms et al, 2000). Advantages of FSH-based vaccines are that they are effective in several species and can potentially provide a male contraceptive. However, FSH-based vaccines cause oligospermia rather than azoospermia. This incomplete efficacy has hindered further progress (Moudgal et al, 1997). The advantages of LH- or LH receptor–based vaccines are that they are effective in both males and females (Thau et al, 1987; Saxena et al, 2002). However, LH- or LH receptor–based vaccines affect sex steroids; therefore, they are not acceptable for humans. Disadvantages of CVs targeting gamete production are that they affect sex steroids and/
or show only a partial effect in reducing fertility. Contraceptive vaccines targeting gamete functions are better choices. Vaccines based on ZP proteins are quite efficacious in producing contraceptive effects. However, they induce oophoritis, affecting sex steroids (Tung et al, 1999; Aitken, 2002). Vaccines based on ZP proteins have been successfully used for controlling wild and zoo animals, such as deer, horses, elephants, and dogs (Kirkpatrick and Turner, 2002). Sperm antigens constitute a promising and exciting target for CVs and, at the present time, no disadvantages are known. Vaccines targeting gamete outcome primarily focus on the hCG molecule. The hCG-based vaccines have undergone phase I and II clinical trials in women, which demonstrated efficacy and lack of immunopathology (Talwar et al, 1994). However, there was a variable immune response observed among vaccinated women. The present article will focus on the development of CVs based on sperm antigens.

Rationale for Sperm Vaccine Development

There is a strong rationale for the development of a sperm-based vaccine. The sperm cell is immunogenic in both males and females. Immunization of several species of animals and humans with sperm/testis preparations develop antiserum antibodies (ASA), leading to infertility (Menge, 1970; Allardyce, 1984). The following data provides a strong rationale for the development of a sperm CV for humans. In 1932, Baskin injected 20 fertile women, who had at least 1 previous pregnancy, with their husband’s sperm (Baskin, 1932). These women developed ASA and no conception was reported for up to 1 year of observation. A US patent was issued for this spermatoxic vaccine in 1937 (US patent 2103240). More than 70% of men develop ASA after vasectomy (Liskin et al, 1983), and there is a limited success in the regain of fertility, even after successful surgical reanastomosis in vaso-vasostomy attributed to the presence of ASA (Silber and Grotjan, 2004). Up to 2% to 30% cases of infertility may be associated with the presence of ASA in the male or female partner of an infertile couple (Ohl and Naz, 1995). These ASA are causative factors of infertility, because disappearance of ASA cause regain of fertility (Bronson et al, 1984). These findings provide evidence that spermatozoa can generate an immune response in both men and women that can lead to a contraceptive state. However, the whole sperm cannot be used for the development of CV. There are numerous antigens present on the surface and internally in sperm that are shared with somatic cells. Thus, immunization with the whole sperm can cause immunopathological consequences in other tissues and organs. The usefulness of a sperm antigen is contingent on sperm-specific expression, surface expression accessible to antibody binding, and its role in fertilization/fertility. In addition, the sperm antigen, alone or after conjugation with an appropriate carrier protein, should be able to raise high titer and long-lasting antibody response in circulation and locally in the genital tract. If the sperm antigen is also involved in human immunoinfertility, then it is an especially attractive candidate. An ideal sperm antigen for immunocontraception should have tissue-specific expression on sperm surface, be involved in sperm-ZP binding, and be involved in human immunoinfertility.

Sperm Antigens

Various methodologies of genomics and proteomics have been used to delineate sperm antigens that have a role in fertilization/fertility and can be used for the CV development. Recently, using gene knockout technology, at least 93 novel testis/sperm genes/proteins have been identified that have a crucial role in various aspects of fertility (Naz and Rajesh, 2005a; Naz and Rajesh, 2005b). Some of these gene knockouts cause a defect in testis development and endocrine milieu, some in spermatogenesis, some in mating behavior, some in sperm structure/function/motility, and some in fertilization. The majority of these knockouts also demonstrated an effect on nonreproductive organs concomitant with an effect on fertility. We performed an extensive database analysis of these genes/proteins to examine how many of these have the characteristics required for the CV development, as discussed above. The knockouts of only a few genes/proteins induced a specific effect on fertility without a serious side effect. Further analysis of these genes/proteins indicated that majority are not expressed on the sperm surface, thus, are not amenable to antibody binding. Although these genes/proteins can provide ideal targets for pharmacological inhibition for contraception, they are not suitable for CV development. The gene knockout technology is a powerful approach to identify suitable novel targets, and the list of gene knockout mice is ever growing.

The molecules involved in sperm-oocyte membrane fusion have been actively examined for some time. Various candidates have been proposed, including DE, cluster of differentiation (CD)46, equatorin Sperad, and sperm acrosomal membrane-associated protein (SAMP). CD46 gene knockout mice do not show a defective sperm-oocyte fusion (Inoue et al, 2003). A Disintegrin and Metalloprotease (ADAM) family proteins have drawn a considerable attention because they have a putative fusion peptide (ADAM1), and disintegrin domains (ADAM2 and ADAM3; Nishimura et al, 2004). However, ADAM4, ADAM9, and ADAM11, gene knockout mice did not show a defect in sperm-oocyte membrane fusion, but did show an impairment in sperm-zona binding (Cho et al, 1998). CD9 present on the oocyte plasma membrane seems to be essential for fusion with the sperm cell (Le Naour et al, 2000). It was thought that integrins α6 and
β1 present on sperm are involved in binding to oocyte CD9 for sperm-oocyte fusion (Almeida et al, 1995). However, gene knockout of these molecules did not inhibit fertility (He et al, 2003). Recently, a gene knockout was reported that is very interesting. The gene knockout mice of a sperm gene, designated as Izumo, are healthy, but all males are sterile (Inoue et al, 2005). Izumo is named after a Japanese shrine dedicated to marriage. The male mice produce normal-appearing sperm that bind to and penetrate the ZP but are incapable of fusing with the oocyte membrane. Human sperm also express Izumo protein. Izumo protein is not detectable on ejaculated sperm but becomes detectable after sperm cell undergoes acrosome reaction. Izumo antigen seems to be an interesting molecule, and its usefulness in the CV development needs to be investigated. Because it is not exposed until the sperm cell undergoes acrosome reaction, the antibodies have to be present at that particular time and space for binding to Izumo antigen. It is not clear at the present time whether the sperm-oocyte plasma membrane fusion event is a suitable target for immuncontraception (Naz et al, 2001).

Although several sperm genes/antigens have been delineated, cloned, and sequenced; and antibodies to some of these antigens affect sperm function/fertilization in vitro, only immunization with a few of them cause a contraceptive effect in vivo in any animal model. Notable among these are lactate dehydrogenase-C\textsubscript{4} (LDH-C\textsubscript{4}; Goldberg and Herr, 1999), PH-20 (Primakoff et al, 1988), SP-17 (Lea et al, 1998), SP-10 (Herr et al, 1990), FA-1 (Zhu and Naz, 1997), and YLP\textsubscript{12} (Naz et al, 2000). Most of these active immunization studies, except those related to the PH-20 antigen, were carried out in the mouse model. At the present time, no sperm antigen has undergone a phase I/II clinical trial in humans. Two studies have examined the effect of sperm antigen vaccination in a non-human primate model. One study reported reduced fertility of female baboons after immunization with LDH-C\textsubscript{4} (O’Hearn et al, 1997). However, a study by another group found no effect on fertility in female monkeys after vaccination with LDH-C\textsubscript{4} (Tollner et al, 2004). The reason for this discrepancy is not clear. Recently, in an interesting study, male monkeys were immunized with an epididymal protein designated as epididymal protein inhibitor (Eppin; O’Rand et al, 2004). After immunization, 78% of monkeys who developed high anti-Eppin antibody titers became infertile, and 71% of those monkeys recovered fertility after immunization was stopped. To maintain high antibody titers, booster injections with Freund’s adjuvant have to be administered every 3 weeks for almost the entire duration of the study, 691 days. The potential immunopathological effects of immunization were not examined. This interesting study indicates that anti-sperm CV can also be developed for men.

Antibodies to several sperm molecules inhibit sperm-oocyte interaction/fertilization in vitro. However, the active immunization with many of these molecules does not inhibit fertility in vivo. In addition, the gene knockouts of many of these molecules do not inhibit fertility. For example, although antibodies to fertilin/PH-30 inhibit fertilization in vitro (Stein et al, 2004), active immunization with fertilin/PH-30 does not affect fertility in vivo (Hardy et al, 1997). Similarly, although antibodies to sperm integrins α6 and β1 inhibit sperm-oocyte fusion in vitro (Almeida et al, 1995), the gene knockouts of these molecules do not affect the fertility in vivo (He et al, 2003). These differences in in vitro and in vivo effects may be a result of 1) the class/subclass, valency, affinity, and kinetics of the antibodies generated in vivo; 2) the fact that antibodies have to be present in time and space to bind to the appropriate molecules; and/or 3) a possible redundancy of some of these molecules.

Another problem that the sperm vaccinologists are currently facing is finding an appropriate animal model to examine the efficacy of a sperm antigen. The most used animal model is the mouse. However, until now, no one has reported a 100% block in fertility after immunization with any single antigen in the mouse model. Even immunization with the whole sperm or their solubilized preparations does not cause a total block in fertility in mice, male or female. The maximum reduction in fertility after immunization with any antigen/sperm preparation is up to 70% to 75%. Very few, if any, knockouts of a single gene have made mice totally infertile. The recently reported Izumo gene knockout did make the male mice almost totally infertile (Inoue et al, 2005). It remains to be seen whether the 70% to 75% reduction in fertility in the mouse model translates to a 100% reduction in humans. The female mouse ovulates several (approximately 20–50) eggs every cycle and a woman ovulates mostly 1 egg every cycle. Therefore, there are differences between the mouse and human. It is possible that a 70% to 75% reduction in fertility in the mouse model translates to a 100% block in humans. It is possible that it is an inherent nature of the mouse model that it is difficult to make mice completely infertile. However, after active immunization or deleting a single gene, one does find a few mice that are totally infertile. There is no study at the present time that has examined the effect of immunization with more than 1 sperm antigen in the mouse model.

The phage display technology is a novel and innovative tool for delineating specific binding peptide sequences to various ligands and antibodies. It was first reported by George Smith in 1985 (Smith, 1985). This technology is currently being widely used in several laboratories. The peptide sequences are presented on the surface of filamentous phage to examine their interaction with specific ligands/antibodies. The DNA encoding any peptide sequence is incorporated into the genome of the phage cap-
sid protein, and the encoded peptide is expressed and displayed on the phage surface as a fusion protein. Recently, we used this technology to delineate the peptide sequences that are involved in human immunoinfertility, and the peptide sequences present on human sperm cells that are involved in binding to human ZP.

Using the antibody-positive sera from immunoinfertile men, seven clones that had dodecamer sequences in a random order, designated #2, #22, #69, #77, #81, #84, and #95, were identified in the phage display library (Naz, 2005b). These clones did not react with the antibody-negative sera from fertile men. The clones were sequenced (#2, PSALGRFTRGPL; #22, SLIFVTISSEWG; #69, LSLSLDLLTFRT; #77, PDIRHYFIQNRG; #81, GCRIVYRRPLHL; #84, RTAGFDIKLIDT; and #95, RIQYQAISTVSL) and the peptides were synthesized based on these 7 sequences and investigated for their immunoreactivity with sera from immunoinfertile and fertile men in the enzyme-linked immunosorbent assay. All of the 7 peptides, and especially 3 peptides (#22, #69, and #95), reacted strongly with the immunoinfertile sera, compared with the fertile sera. These 3 peptides reacted with 27% to 40% of the immunoinfertile sera for the IgG class, and two of these (#69 and #95) also showed a positive reaction with 27% of the sera for the IgA class. None of the peptides reacted positively with the fertile sera. These peptide sequences are novel, without any complete identity with any known sequence in the database. The sperm cell has several proteins on the surface, some are glycosylated. Many of these peptides may constitute partial or complete peptide mimetics of the carbohydrate epitopes present on sperm that are involved in immunoinfertility. The phage display technology seems to be a powerful tool to identify immunoinfertility-associated sperm antigens, both the peptide moieties as well as the carbohydrate mimetics of the peptide epitopes. In the past, several proteomic methodologies, immunologic techniques, and the two-dimensional gel electrophoresis/matrix-assisted laser desorption mass spectrometry have been used to delineate molecular identities of the sperm antigens that are involved in antisperm antibody-mediated immunoinfertility (Auer et al, 1995; Pillai et al, 1996; Shetty et al, 1999). Although a few interesting leads have been found, very few sperm antigens have been identified that are indeed involved in immunoinfertility. The FA-1 antigen was identified in our laboratory, and the complementary DNA (cDNA) encoding for the FA-1 antigen has been cloned and sequenced from the mouse (Zhu and Naz, 1997) and human testis (Naz and Zhu, 2002). The FA-1 antigen has been clearly shown to be associated with immunoinfertility (Naz et al, 1993). Antibodies to the FA-1 antigen are present in sperm, seminal plasma, and sera of immunoinfertile men, and in follicular fluid, cervical mucus, vaginal secretions, and sera of women.

A clinical trial conducted at the University of Michigan Medical School indicates that the incubation of sperm from immunoinfertile men with the FA-1 antigen removes autoantibodies from the sperm surface, resulting in an increase in the antibody-free sperm, and enhanced acrosome reaction rates (Menge et al, 1999). The intrauterine insemination of FA-1 antigen-adsorbed antibody-free sperm caused normal pregnancies and healthy babies, indicating that the antigen treatment does not have deleterious effects on implantation or on embryonic and fetal development. Thus, the FA-1 antigen, along with other sperm antigens that are involved in immunoinfertility, may find application in immunotherapy of immunoinfertility, besides having application in the specific diagnosis.

Using the solubilized preparation of human ZP as a probe, a dodecamer sequence (YLPVGGLRRIGG), designated as YLP\textsubscript{12}, present on human sperm, was identified using the phage display library (Naz et al, 2000a). An extensive computer search in the database did not reveal any known sequence with a complete identity or significant homology with YLP\textsubscript{12}. Antibodies to synthetic YLP\textsubscript{12} peptide inhibit human sperm-human zona binding. YLP\textsubscript{12} recognizes the ZP3 component of human ZP. The YLP\textsubscript{12} peptide sequence was localized primarily on the acrosome and tail regions of humans and murine sperm (Naz et al, 2000). This peptide is also involved in human immunoinfertility. To examine the immunoncontraceptive potential, a vaccine was prepared by conjugating the YLP\textsubscript{12} peptide with the recombinant cholera toxin B subunit (rCTB; Naz and Chauhan, 2002). The YLP\textsubscript{12} peptide-rCTB vaccine caused a significant inhibition of infertility (up to 71%) in vaccinated female mice by raising a testis/sperm-specific immune response. The fertility reversed after approximately 10 months, when the antibodies disappeared from the circulation and genital tract. The fertility could also be reversed voluntarily at any given time by neutralizing the antibodies, which is done by administering the unconjugated peptide in the blood and/or in the vagina.

Besides antibodies, various cytokines can also affect sperm function and fertility, positively or negatively. For example, interferon-γ and tumor necrosis factor-α can negatively affect sperm motility and function (Naz and Kumar, 1991), and interleukin-6 can enhance sperm capacitation and acrosome reaction (Naz and Kaplan, 1994). Spermatozoa have receptors for many of these cytokines, such as interferon-γ and interferon-α (Naz et al, 2000b). These factors are present in the seminal plasma, and the levels are modulated to various degrees in infertility. Immunization with whole-sperm preparations or specific sperm antigens can raise many cytokines in addition to antibodies that can affect sperm function (Naz and Mehta, 1989). DNA vaccines have been extensively used to generate protective immunity against infectious agents and
tumors (Liu, 2003; Patil et al, 2005). DNA vaccination represents one of the recent advancements in vaccine technology. DNA vaccines have distinct advantages over the peptide vaccines because they have potential to generate both T helper cell subsets 1 and 2 immune responses. Based on these facts, a DNA vaccine was prepared by cloning YLP₁₂ cDNA into a pVAX1 DNA vector. The female mice were immunized intradermally with the YLP₁₂ DNA vaccine via a gene gun. The DNA vaccination caused a long-term contraception in female mice (unpublished data).

The progress in the development of CVs against various targets, including sperm, has been hampered by the following factors: 1) difficulty in delineating the appropriate fertility-related antigen(s), 2) variability of the immune response among the vaccinated individuals, 3) attainment and maintenance of high titers of antibodies for bioefficacy, 4) time lag to achieve reasonably good antibody titers after the first injection, and 5) uncertainty regarding how long the antibody titer will remain in the circulation to exercise the contraceptive effects. The last 4 concerns are associated with the active immunization studies involving CVs. It is envisaged that these 4 concerns may be taken care of using the passive immunization approach (Naz and Rajesh, 2004). The passive immunization approach has been successful for protection against various immunologic and infectious diseases (Casadevall, 1999; Zeilin et al, 2000). These studies have indicated that administration of homologous antibody preparations, especially the Fabs, are devoid of any side effect and immunopathological consequences. The bioefficacy of the passive immunization depends on the class/subclass of immunoglobulins and the route of antibody administration. Human IgG has a half-life of 21 days. Recently, there has been a growing interest to humanize the murine monoclonal antibodies or to raise human monoclonal antibodies against various sperm contraceptive epitopes (Isojima et al, 1987; Clayton et al, 1998; Norton et al, 2001). These antibodies, and additional antibodies that are being engineered, await trials in animal models and in humans for demonstration of efficacy, duration, and reversibility of the antifertility effects. The data from antibody therapies, including clinical trials in infectious diseases, indicate that it is an exciting, practical, viable, and durable proposition ready for experimentation.

In conclusion, a CV targeting sperm is an exciting proposition. The strong rationale and the progress made during the last several years indicate that it can culminate in a viable and successful reality. The immunologic approaches to contraception have been a priority of the World Health Organization since 1973, and were highly recommended by the Institute of Medicine in its 1996 report.

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