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APPLICATION OF SPERM ANTIGENS IN IMMUNOCONTRACEPTION

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1. ABSTRACT

Development of a vaccine(s) based on sperm antigens represents a promising approach for contraception. The utility of an antigen in immunocontraception is contingent upon its tissue specificity, involvement in human fertility, and immunogenicity. A number of antigens have been characterized from the sperm surface. Notable among these are LDH-C_4, RSA antigens, PH-20, SP 1U, HSA-63, FA-1, FA-2 and CS-1. These antigens have been proposed as potential candidates for the development of contraceptive vaccine(s). Their current status, application, relative merits, and immunogenicity in immunocontraception are discussed in this review.

2. INTRODUCTION

With the advent of hybridoma and DNA recombinant technologies, contraceptive research has entered a new phase of development. During the last five years, significant advances have been made in this area and now it seems that realistic prospects exist for the development of contraceptive vaccine(s) for use in humans and animals (veterinary, wild and domestic), which are suitable in both female and male species. Contraceptive vaccines will be valuable supplement to the presently available methods of family planning, and due to high specificity, few to no side effects, low cost and infrequent need of administration may have larger acceptability than the currently available methods.

Reproduction starts with the unison of gametes contributed by the male and female partners. Spermatozoan has antigens on the cell surface that are unique, tissue-specific, immunogenic, accessible to antibodies. Binding of the antibodies to these antigens can inhibit gamete function and compromise fertilization. Thus, the tissue-specific antigens derived from sperm constitute interesting molecules for the development of an antisperm contraceptive vaccine. The aim of the present manuscript is to review the current status of the antigens that have been identified, characterized and proposed as potential candidates for immunocontraception, and to discuss their relative merits in the development of a contraceptive vaccine.

3. DISCUSSION

3.1. Rationale and feasibility

Development of vaccines based on sperm antigens represents a promising approach to contraception. The rationale and feasibility of this approach is based upon two lines of evidence. First line of evidence is provided by the experimental immunization studies in animals and humans. Deliberate immunization of male or female animals of various species (1-3) and humans (4-5) with preparations of

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autologous or isologous spermatozoa or mature testes results in infertility by causing fertilization failure as well as preimplantation embryonic mortality. Second line of evidence is provided by studies of involuntary immunoinfertility. Up to 70% of vasectomized men form antisperm antibodies (ASA) (6) and up to 30% cases of infertility is associated with the presence of ASA in the male and/or female partner of the infertile couple. Various studies have demonstrated that these ASA are not only associated with infertility but are the cause of infertility (7, 8).

The available data indicate that the spermatozoon has both auto- as well as isoantigenic potentials and thus can generate an immune response in both males and females that can lead to infertility in humans. However, the whole spermatozoon *per se* cannot be employed for the development of a vaccine due to several antigens present intrinsically and on the surface of the sperm cell that are likely to be shared with various somatic cells (9). Thus, only those antigens that are sperm specific can be employed for the development of an antisperm contraceptive vaccine (s). The utility of a sperm antigen for the development of a contraceptive vaccine is contingent upon its sperm specificity, involvement in human fertility, and on immunogenicity that involves raising enough antibody response locally in the genital tract that is capable of intercepting fertility.

2. Sperm antigens that have been proposed as candidates for immunocontraception

During the last decade, with the advent of hybridoma technology and the Western blot procedure, several sperm antigens have been identified that are likely to be implicated in fertility. A series of monoclonal antibodies (MCAs) have been generated that inhibit fertility in various species of animals (reviewed in 10, 11). A few of the sperm antigens recognized by these MCAs are sperm-specific and have been isolated and biochemically and immunologically characterized. The cDNAs encoding for some of these antigens have been cloned and sequenced. Notable among these are lactate dehydrogenase (LDH)-C_4, rabbit sperm autoantigens (RSAs), PH-20, SP-10, HSA-63, fertilization antigen-1 (FA-1), FA-2 and cleavage signal-1 (CS-1) protein (summarized in Table 1). These antigens have drawn special interest for immunocontraception and will be discussed below.

3.2.1. Lactate dehydrogenase (LDH)-C_4

LDH-C_4 is a sperm-specific but species-cross-reactive enzyme that has been isolated and biochemically characterized from testis of various species of animals (12). The cDNA encoding for human LDH-C_4 has been cloned and sequenced (13). Active immunization of various species of animals causes a reduction (up to 50%) of fertility by mechanism(s) primarily involving postfertilization embryonic mortality, besides causing agglutination of sperm (12). LDH-C_4 is a poor immunogen: active immunization of mice with whole sperm does not produce antibodies to LDH-C_4 (14), and sera from immunoinfertile patients (both male and female) do not have antibodies to LDH-C_4 (15). Goldberg and associates have attempted to increase the immunogenicity of this molecule by expressing it in vaccinia virus as a recombinant vector. Female baboons immunized with vaccinia virus expressing LDH-C_4 demonstrate a reduction in their fertility. Although immunization with LDH-C_4 has caused only up to 50% reduction rather than a complete block of fertility in all the species tested so far, its sperm specificity and extensive characterization makes it an interesting and model antigen for various aspects of immunocontraception.

3.2.2 Rabbit sperm autoantigens (RSA) family

O'Rand and associates have isolated and characterized from rabbit sperm at least three low molecular weight (~ 13± 2 kD) proteins, designated RSA-1, RSA-2 and RSA-3 (16). cDNAs encoding for RSA antigens have been cloned and sequenced (17). RSA antigens seem to function as lectin like molecules and to bind the spermatozoon to the zona pellucida of the oocyte in the rabbit model. Monoclonal antibodies to RSA antigens cross-react with human sperm and inhibit human sperm penetration of zona-free hamster oocytes (SPA) (18). Female mice were immunized with a synthetic peptide designated P1OG (PGGGTLPPSG) conjugated to keyhole limpet hemocyanin that provided T-cell epitope (19). This peptide was derived from the amino acids sequence of the RSA. The results of this study indicated that, by raising anti-peptide antibodies, immunization of female mice with P1OG led to cause a reduction of fertility. However, antibody response varied considerably among mice and there was only a reduction rather than a complete block of fertility.

3.2.3. PH-20 antigen

PH-20 is a glycosyl phosphatidylinositol (GP1)-anchored membrane protein with the molecular mass of 64 kD (20). This protein is present on the plasma membrane and inner acrosomal membrane of the guinea pig sperm. Immunization of male and female guinea pigs with the guinea pig sperm protein PH-20 was fully effective in contraception (21). The cDNA encoding for the PH-20 antigen has been cloned and sequenced from guinea pig, monkey and human testis (22, 23); with human PH-20 sequence showing 59% sequence homology with the guinea pig PH-20. However, besides this homology, neither the antibodies (MCA as well as polyclonal) to guinea pig PH-20 bind to human sperm, nor the sera from immunoinfertile patients have been shown to have antibodies to this antigen suggesting its non-cross-reactivity and poor immunogenicity in humans. Recently it was shown that PH-20 is structurally and functionally related to hyaluronidase (24).

3.2.4. SP-10 antigen

Herr and associates have reported a monoclonal antibody (MHS-10) against a sperm protein (SP-10) which impairs SPA by causing agglutination of sperm. The cognate antigen, designated SP-10, is an intra-acrosomal protein and has been isolated and characterized from human sperm. The purified SP-10 demonstrated a polymorphism of immunogenic peptides from 18 to 34 kD, with majority of peptides possessing an isoelectric point (ipH) of approximately 4.9 (25). The cDNA encoding SP-10 protein has been cloned and sequenced from a human cDNA expression library and contains 1117 bp sequence encoding for 256 amino acids(26). It has not been determined whether or not the sera from immunoinfertile patients have antibodies to SP-10. Active immunization trials have been performed in female baboons using the human recombinant SP-10 (having ß-galactosidase tag). These baboons developed antibodies that were reactive with the cognate antigen. However, in spite of presence of high titers of anti-SP-10 antibodies, there was only a partial reduction, if at all, in fertility in a few animals. Also, cDNA sequence encoding human SP10 was cloned on an asd+ vector and expressed to a high level in an avirulent Δ cya, Δ crp and Δ asd vaccine strain of *Salmonella typhimurium*. Female mice orally immunized with the recombinant formulation developed high titers of antibodies in serum and the reproductive tract secretions (27). However, despite a high titre of antibodies, no effect on the fertility was reported (27).

3.2.5. HSA-63 antigen

Another antigen, designated HSA-63, has been isolated from murine and rabbit testes and partially characterized using sperm-specific MCA that inhibits *in vitro* fertilization (IVF) in mice and SPA in humans. The HSA-63 antigen is composed of three soluble glycoproteins of 50, 43 and 42 kD (28). The HSA-63 is immunogenic in female mice and rabbits, and the polyclonal antibodies against HSA-63 inhibit murine IVF and human SPA. However, like hyaluronidase and acrosin, active immunization with HSA-63 antigen does not reduce fertility *in vivo* (28). The HSA-63 antiserum reacted with four clones from murine cDNA testis library and one of these clones has been sequenced (29). Interestingly, cDNA encoding for HSA-63 antigen showed a significant sequence homology (~79%) with the SP-10 antigen (Table 1).

3.2.6. Fertilization antigen-1 (FA-1)

FA-1 has been purified and characterized from murine and human sperm and testis using a MCA that completely blocks murine IVF and human SPA (30-32). FA-1 is a glycoprotein. It exists as both a dimer (51 ± 2 kD) as well as monomer (23 kD). FA-1 develops in the testis during later stages (secondary spermatocyte onward) of spermatogenesis (33). FA-1 is an evolutionarily conserved antigen present in sperm of various mammalian species including mouse, rabbit, bull (34), rhesus monkey and humans (30-32). FA-1 seems to have similar function in these species. Anti-FA-1 MCA completely blocked IVF in these species. Also, the active immunization of female rabbits with purified FA-1 caused a significant reduction (up to complete block) in fertility (35).

The mechanism by which the anti-FA-1 antibodies inhibit fertilization is by affecting sperm-zona interaction. The human FA-1 binds to purified ZP3 of porcine zona pellucida in enzyme-linked immunosorbent assay (ELISA) and Western blotting, and completely neutralizes its sperm ligand activity in boar sperm-porcine zona pellucida attachment bioassay (36). This is an interesting finding because antibodies to porcine ZP3 antibodies have been shown to cross-react with human zona pellucida (32). The antibodies to FA-1 inhibit human sperm-human zona interaction, reinforcing the concept that FA-1 is involved in sperm-zona pellucida-ligand interaction (37). However, in the heterologous assay, the FA-1 MCA completely blocks SPA. The SPA has been reported to be an indirect measure of capacitation. Thus it is conceivable that these antibodies are be directed to an important sperm component (enzymatic or non-enzymatic) that is vital to capacitation. In fact, the immunoaffinity-purified FA-1 MCA inhibits acrosome reaction of human sperm cells in solution (but not on the zona pellucida surface) (38), suggesting a mechanism through which the antibody can inhibit the human sperm penetration of zona-free hamster oocytes. Recently, we found that the purified FA-1 antigen completely blocks human sperm-human zona binding in the hemizona assay (39). Taken together, these findings suggest that FA-1 antigen may be a human sperm receptor (ligand) for the zona pellucida, that also has a role in sperm cell capacitation and/or acrosome reaction. It was interesting to find that FA-1 antigen has autophosphorylating activity and is tyrosine phosphorylated during human sperm capacitation/acrosome reaction (40). Tyrosine phosphorylation of FA-1 antigen seems to have a vital role in capacitation/acrosome reaction and in zona pellucida binding (41).

FA-1 antigen is involved in human immunoinfertility (both men and women). The available data indicate that the anti-FA-1 antibodies that are present in vasectomized men and in infertile patients may play a causal rather than an associated role in infertility (42-46). Involvement of an antigen in human immunoinfertility indicates: a) its immunogenicity (auto- as well as iso-) in humans, and b) potential of its antibodies in causing infertility if a sufficient antibody titer is present. Since most infertile men are healthy individuals without any disease concomitant with infertility, the presence of antibodies to an antigen, is indicative, though not confirmative of its sperm specificity in humans. Thus, the involvement of FA-1 in immunoinfertility indirectly indicates its sperm-specificity, and auto- as well as isoantigenic potentials in

Table 1. Molecular and immunobiological characteristics of sperm-specific antigens prposed as candidates for contraceptive vaccines.

Antigen[References]	Molecular Identity		Immunobiological Activity	Cross-reaction with human sperm	Involvement in human immuno-infertility[b]
	Weight	cDNA[a] (species)			
1. LDH-C_4[12-15]	140 kD; 4 subunits of 35 kD each	1171 bp, 331 aa(human)	Active immunization causes a reduction (up to 50%) in fertility in various species.	Yes	No
2. RSA family[16-19]	13 ± 2 kD	2186 bp, 680 aa (rabbit)	Active immunization trials not conducted; antibodies inhibit murine IVF and human SPA.	Yes	No
3. PH-20[20-24]	64 kD	1010-2156 bp, 468 aa(guinea pig); 1683 bp, 509 aa(human) (59% homology between species	Active immunization causes a complete block in guinea pigs.	No[1]	No
4. SP-10[25-27]	18 to 34 kD; ipH 4.9	1117 bp, 256 aa[c] (mouse)	Active immunization in baboons causes a partial reduction of fertility.	Yes	No
5. HSA-63[28, 29]	3 proteins of 50, 43, and 42 kD	1067 bp, 261 aa[c] (mouse)	Active immunization did not significantly affect fertitlity; antibodies inhibit murine IVF and human SPA.	Yes	No
6. FA-1[30-46]	Monomer of 23 kD; dimer of 51± 2 kD	2 putative human clones presently being sequenced; decapeptide bioeffective epitope isolated	Active immunization did not significantly affect fertitlity; antibodies completely block murine IVF, bovine IVF, monkey IVF, human IVF and IVF	Yes	Yes[3]
7. FA-2[47, 48]	95 kD	Presently being cloned and sequenced	Active immunization studies not conducted ; antibodies completely block human SPA.	Yes[2]	Not done
8. CS-1[50, 51]	Double band of 14 &18 kD	1828 bp, 249 aa (human)	Active immunization studies not conducted ; antibodies inhibit early cleavages of zygotes.	Yes	No

[a] cDNA clone represents the species in which it was cloned; base pairs (bp) and deduced amino acids sequence (aa).
[b] No means either not reported or not involved.
[c] These clones have significant sequence homology.
[1] Antibodies to guinea pig PH-20 do not cross-react with human sperm.
[2] Mat be related to tyrosine kinase substrate proteins reported in mice.
[3] Antibodies seem to be causative factor of infertility.

humans. Thus, if an antigen, such as FA-1 is involved in human immunoinfertility, the extensive phase I clinical trials to investigate its toxicity in actively-immunized subjects may not be absolutely necessary.

The cDNA encoding for the FA-1 antigen has been cloned and sequenced from murine testis. We also have isolated two putative clones from the human testis - λgt11 cDNA expression library which react with FA-1 MCA (32). The functional epitope of FA-1 antigen, recognizing the FA-1 MCA, has been isolated, sequenced

and synthesized. Interestingly, the sera from immunoinfertile patients (men and women) and not from fertile humans show a strong reaction with this synthetic decapeptide epitope, and the decapeptide-reactive immunoaffinity-purified antibodies inhibit human SPA. Northern blot analysis of mRNA isolated from various human tissues, confirmed the testis-specific expression of the FA-1 antigen in humans. Active immunization trials using the synthetic decapeptide epitope as well as purified recombinant antigen obtained by expression using the pGEX system are being planned in a non-human primate model.

3.2.7. FA-2 antigen

Recently, we have purified a 95 kD antigen designated FA-2, from human sperm using a MCA (Vic-1) that was described as a high-priority antibody at the World Health Organization Task Force on Contraceptive Vaccines meeting held in Rome (August, 1992) (47). The high priority was based on its sperm-specificity and high bioactivity. Vic-1 MCA completely blocks human SPA, reduces acrosome reaction and the release of acrosin activity from human sperm cells; and does not affect percent sperm motility but significantly affects various motility characteristics such as linearity, amplitude of lateral head displacement (ALH) and beat frequency; motility parameters involved in the hyperactivation phenomenon leading to capacitation and acrosome exocytosis. It remains to be determined whether the FA-2 antigen is one of the 95 kD sperm proteins that have been reported to bind ZP3 and serve as tyrosine kinase substrates in response to zona binding in mice (48) and/or is related to the 94 kD, human sperm protein, that undergoes autophosphorylation after exposure to zona pellucida proteins (39-41, 49).

3.2.8. Cleavage signal protein (CS-1)

The signal which is required for the mammalian fertilized oocyte to cleave is not identified as yet. In human embryo, transcription starts at 4- to 8- cell stage. Thus, the signal to the first cleavage has to be provided by an extra-nuclear preformed message/molecule. Our laboratory reported a sperm surface antigen, the cleavage signal protein (CS-1) that may provide the first signal for initiation of oocyte cleavage (50). The CS-1 is present on the sperm surface of various mammalian species including human and mouse. Interestingly, the immunoinfertile patients have antibodies to CS-1. These antibodies inhibit cleavage of the pro-nuclear stage zygotes. The sequence of CS-1 from the human testis did not indicate any homology with any known sequence and thus it represents a novel molecule (51).

3.3 Increasing the immunogenicity of antisperm contraceptive vaccine(s)

Usually, most protein and peptide immunogens produced by the recombinant DNA technology or chemical synthesis are weak immunogens. Some of the sperm antigens described above are strong (*e.g.* FA-1) whereas others are weak immunogens (*e.g.* LDH-C_4). The sperm proteins, especially those developed during later stages of spermatogenesis and involved in human immunoinfertility may be strong immunogens.

The production of an immune response to an antigen depends on a series of events that include: delivery of the antigen to a site accessible to lymphoid cells; activation of antigen-presenting cells (APCs); recognition of the antigen-MHC complex by T lymphocytes; binding of the antigen epitopes to lymphocyte receptors and internalization; and production of the appropriate cytokines by APCs, T cells and B cells that are needed for clonal expansion of antibody producing and/or cytotoxic cells. In addition, sustained humoral immunity requires persistent exposure of B cells to the antigen, either by retention of non-degraded epitope sequences, or by the continuous release of antigen. The persistence of antigen is essential for maintaining a high level of antibody production without having to use frequent booster immunizations for contraceptive vaccines. For contraceptive vaccines based on sperm antigens, persistent high levels of antibodies must be present in the local genital tract secretions to exercise an effect.

Once an immunogen has been identified as a candidate for contraceptive vaccine, an appropriate delivery system needs to be devised (52). The antigen most likely will contain only B-cell epitopes of the reproduction-associated target molecule, and its conjugation to a foreign carrier protein or peptide will provide the T-cell help. Numerous delivery systems that have been proposed and are being investigated for various vaccines include alum adsorption, water-oil emulsions, liposomes, immunostimulating complexes (ISCOMS), non-ionic block polymers and live vectors (Vaccinia virus/ Salmonella/Shigella/BCG). However, for sperm antigens, these delivery systems have not been extensively explored as yet, since at the present time the major thrust has been on delineating the appropriate antigens and on isolation, characterization, cloning and sequencing of these delineated antigens. However, these systems have been extensively investigated for enhancing the efficacy of prototype anti-fertility vaccines based on human chorionic gonadotropin (hCG). Of all these systems, the success in enhancing and prolonging the immune response against hCG has been achieved using microspheres of polylactide-polyglycolide (PLPG) co-polymers, and by conjugating to diphtheria and tetanus toxoids. Although the mechanisms responsible for the immunostimulating property of the PLPG delivery system is not fully elucidated, a single injection of the hCG prototype vaccine with the co-polymer microsphere caused a production of high titer of antibodies that persisted for almost one year in rabbits (52). The hCG vaccine comprised of β-hCG annealed to α subunit of ovine luteinizing hormone (oLH) and conjugated to tetanus/diphtheria toxoid, designated heterospecies dimer (HSD) vaccine has undergone phase I, II and early phase

III clinical trials in women. The results of these trials have been extremely promising indicating enhanced antibody titers to hCG, that were able to neutralize the activity of hCG and prevent pregnancy (53).

We obtained some interesting data on immunoregulation of antigenicity of sperm cell in women that may have implication in the development of antisperm contraceptive vaccine. This data was obtained using sera from fertile, immunoinfertile and virgin women, and anti-FA-1 MCA (54). The study was conducted using Fab' portions of the antibodies to avoid non-specific effects mediated through Fc portion of the antibody. Our findings indicated presence of anti-idiotypic antibodies (ab-2) in the sera of fertile women that were absent in the sera of infertile women. That ab-2 formation is a result of exposure to sperm cells is suggested by their absence in the sera of virgin women (54). Presence of ab-2 in fertile women was confirmed using Western blotting, immunoprecipitation and immunoaffinity purification procedures. The ab-2 had biological activity. The antibodies isolated from sera of fertile women neutralized the activity of anti-FA-1 MCA in inhibiting human SPA, and blocked the sperm-binding activity of antisperm antibodies present in sera of infertile women.

These results demonstrate that sera from fertile women who are sexually exposed to sperm have anti-idiotypic antibodies that are capable of neutralizing the antisperm antibody activity. These anti-idiotypic antibodies carried an internal image of FA-1 antigen and thus were of the ab-2 ß type. These data suggest that, normally in fertile women, exposure to semen elicits a systemic immune response with antibodies against sperm, as complementary anti-idiotypic antibodies were detected by several methods. These results support the Jerne's network theory (55) of immune modulation regulated through idiotype-anti-idiotype interaction. Based on these findings, one can speculate that the immune response to sperm is generated after exposure to sperm in both fertile as well as infertile women. However, in fertile women, the antisperm antibody (ab-1) generated after exposure to sperm elicits the production of anti-diotypic antibodies (ab-2), which neutralize the ab-1 response. In contrast, in infertile women, the ab-2 production is either weak or absent and thus its insufficiency to neutralize the ab-1 response, results in infertility. It can be hypothesized that female immune infertility may be a consequence of derangement of the idiotype-anti-idiotype network. It will be interesting to investigate whether or not similar idiotype-anti-idiotype network, that is involved in immunoregulation of autoantigenicity of sperm cells also exists in men. Derangement of network, if present, could lead to autoantibody production as seen after vasectomy and in many infertile men. These findings will have application in the development of antisperm contraceptive vaccine(s). Production and enhancement of anti-idiotypic antibodies to antisperm antibodies (to a fertility-related candidate antigen) may be able to modulate the immunogenicity and efficacy of antisperm contraceptive vaccine(s) in both women and men.

4. CONCLUSIONS

Development of contraceptive/antifertility vaccines based on antigens derived from spermatozoa, is a viable and extremely promising approach for regulation of fertility. Advances made in the last five years have made prospects for the development of such a vaccine a reality. Contraceptive vaccines based on sperm antigens, require antibodies probably throughout the reproductive tract at concentrations sufficient to cause a complete inhibition of fertilization. Both male and female genital tracts in humans are immunologically dynamic systems having components of mucosal immunity as well as systemic immunoglobulins in different combinations (9). The infertility in some men and women with antisperm antibodies, can be used as a model in the development of a contraceptive vaccine. Using this type of infertility as a model, it appears desirable to induce immunity not only at the mucosal surfaces of the genital tract but also systemically. An oral vaccine either alone or in combination with a systemic vaccine appears to be a feasible approach for development of a contraceptive vaccine for women.

A multivalent vaccine having most appropriate (specific and effective) epitopes from sperm, and probably zona pellucida and hCG in a single formulation with enhanced immunogenicity and efficacy covering various steps of fertilization process and preimplantation embryogenesis may provide an ideal vaccine for birth control. The sequence of cDNAs encoding for fertility-related sperm antigens showed a minimal sequence homology and no common epitope in these sequences seems to be involved in sperm-zona pellucida binding (56). These findings suggest the involvement of more than one protein (epitope) in sperm-zona pellucida binding (41). The specific and functional epitopes of these proteins (receptors) in a single vaccine formulation could provide potent immunocontraception.

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