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Status of Contraceptive Vaccines

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Abstract

Problem—This is a review of antisperm contraceptive vaccines (CV), and synthesis of human scFv antibodies that can be used as immunocontraceptives.

Method of study—Various methods of proteomics and genomics, peptide synthesis, phage display technology, and antibody engineering were used to obtain multi-epitope vaccines and human scFv antibodies from immunoinfertile and vasectomized men. The present review primarily focuses on the effect of multi-epitope vaccines and Izumo on fertility and synthesis and characterization of sperm specific human scFv antibodies.

Results—The immunization with Izumo peptides causes a contraceptive effect in female mice. The efficacy is enhanced by combination vaccination, including peptides based on other sperm antigens. Using phage display technology, we were able to synthesize at least four novel scFv antibodies with unique complimentarity determining regions (CDRs) that reacted with specific fertility-related sperm antigens. These antibodies inhibited human sperm function *in vitro*, and their immunocontraceptive effect *in vivo* is currently being investigated.

Conclusions—The multi-epitope vaccines may provide an efficacious and viable approach to contraception. The human scFv antibodies, if they block fertility *in vivo*, may provide unique and novel immunocontraceptives, the first of its kind for human use. The multi-epitope CV and preformed engineered antibodies of defined specificity may obliterate the concern related to inter-individual variability of the immune response.

Keywords

Contraception; vaccines; infertility; sperm; scFv antibodies

Introduction

The population explosion and unintended pregnancies continue to pose major public health issues worldwide. The world population has exceeded 6.67 billion1. In the first year AD it was 250 million, which increased to 1 billion by 1830. It took the next 100 years for the population to increase by 1 billion, and at the present rate, it is increasing by 1 billion every 12 years. Ninety-five percent of this growth is in developing nations. In the USA alone, half of all pregnancies are unintended, which results in >1 million elective abortions annually 2›3. These women use some type of contraceptive. This calls for a better method of contraception that is acceptable, effective and available both in the developed and developing nations. An ideal contraceptive method should be highly effective, safe, inexpensive, have a prolonged duration of action, be reversible, require infrequent administration, and can be used privately4. Contraceptive vaccines (CV) have been

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proposed as valuable alternatives that can fulfill most, if not all, of the properties of an ideal contraceptive. Since the developed and most of the developing nations have an infrastructure for mass immunization, the development of vaccines for contraception is an exciting proposition. The aim of this article is to review the current status of contraceptive vaccines, with special emphasis on vaccines targeting sperm.

Discussion

Several targets are being investigated in various laboratories for the development of CVs. These can be divided into three main categories: CV targeting gamete production, gamete function and gamete outcome5. The molecules targeting gamete production include luteinizing hormone-releasing hormone (LHRH/GnRH), FSH: gamete function includes sperm antigens and oocyte zona pellucida (ZP), and the gamete outcome targets primarily HCG molecule. CV targeting gamete production have shown varied degrees of efficacy; however, they either affect sex steroids and/or show only a partial effect in inhibiting gametogenesis. However, vaccines based on LHRH/GnRH are being developed by several pharmaceutical companies as substitutes for castration of domestic pets, farm and wild animals, and for therapeutic anticancer purposes such as in prostatic hypertrophy and carcinoma. These vaccines may also find applications in clinical situations that require an inhibition of increased secretions of sex steroids, such as in uterine fibroids, polycystic ovary syndrome, endometriosis and precocious puberty. CVs targeting gamete function such as sperm antigens and ZP proteins are exciting choices. Vaccines based on ZP proteins are quite efficacious in producing contraceptive effects, but may induce oophoritis, affecting sex steroids in many species. They have been successfully tested in controling feral populations of dogs, deer, horses and elephants, and populations of several species of zoo animals. The current research for human applicability is focused on delineating infertility-related epitopes (B-cell epitopes) from oophoritis-inducing epitopes (T-cell epitopes). Vaccines targeting gamete outcome primarily have focused on the HCG molecule. The HCG vaccine is the first vaccine to undergo Phase I and II clinical trials in humans6, and efficacy and lack of toxicity have been reasonably well demonstrated for this vaccine. At the present time, studies are focused on increasing the immunogenicity and efficacy of this birth control vaccine, and examining its clinical applications in various HCG-producing cancers.

Of all potential targets, sperm have drawn considerable attention, and a sperm vaccine represents an exciting proposition for contraception. The rationale and feasibility for the development of a sperm vaccine is strong. Sperm have both auto- and isoantigenic potentials and can therefore form antibodies in both men and women. Antisperm antibodies (ASA) affect fertilization and fertility both *in vitro* and *in vivo* by several mechanisms, including inhibition of sperm capacitation, acrosome reaction and sperm-zona interaction and penetration. Up to 70% of vasectomised men produce ASA, 7 and 2-30% of cases of infertility may be associated with the presence of ASA in the male and/or female partner of an infertile couple. 8 Deliberate immunization of male or female animals of various species, 9‚10‚11 including humans (both women and men),12‚13 with sperm antigens causes development of ASA, leading to infertility. Baskin12, injected 20 fertile women, who had at least one prior pregnancy, with their husband's semen and these women developed antibodies 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 number 2103240). Thus, spermatozoa can generate an immune response that is capable of inducing a contraceptive state. However, the whole spermatozoon *per se* cannot be used for development of a vaccine because of the presence of several antigens that are likely to be shared with various somatic cells. 14 Only those antigens that are sperm specific can be employed for CV development. The application of a sperm antigen in CV is contingent upon its sperm specificity, surface expression, involvement in fertility, and ability to raise high titer antibodies to be capable of

intercepting fertility. If an antigen is also involved in human immunoinfertility, it is an especially attractive candidate. The immunoinfertile patients who have ASA are healthy individuals without any disease concomitant with infertility. The sperm-ZP binding site constitutes the most attractive target for immunocontraception.

Several technologies of genomics and proteomics have been employed to delineate sperm antigens that play a role in fertilization/fertility and can be used for the CV development. While 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 animal models. Notable among these are FA-115, $YLP_{12}16$, P10G17, A9D18, and SP5619. Most of these active immunization studies were carried out in the mouse model. The findings of these studies are summarized in Table I. No study has achieved 100% reduction after immunization with any of the antigens. The maximum reduction in fertility after immunization with any antigen is up to ˜75%. It remains to be seen whether this 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 typically 1 egg every cycle. Therefore, there are differences between the mouse and human. It is possible that ˜75% reduction in fertility in the mouse model translates to a 100% block in humans. This may be due to an inherent nature of the mouse model that it is challenging to make mice completely infertile. However, after active immunization or deleting a single gene, one does find a few mice that are totally infertile.

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_4$20. However, a study by another group found no effect on fertility in female monkeys after vaccination with LDH-C_4.21. The reason for this discrepancy is unclear. In another study, male monkeys were immunized with an epididymal protein designated as epididymal protein inhibitor, (Eppin) 22. After immunization, 78% of male monkeys who developed high anti-Eppin antibody titers became infertile, and 71% of those monkeys recovered fertility after immunization ceased. To maintain high antibody titers, booster injections with Freund's adjuvant have to be administered every 3 weeks for almost an entire duration (691 days) of the study. The potential immunopathological effects of immunization were not investigated. This study indicates that anti-sperm CV can also be developed for men.

Recently, using gene knockout technology, >100 novel testis/sperm genes/proteins have been identified that have a vital role in various aspects of fertility23, 24. 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 others in fertilization. The majority of these knockouts also showed an effect on nonreproductive organs concomitant with an effect on fertility. We performed an extensive database analysis to examine how many of these genes/proteins have the characteristics required for the CV development. The findings revealed that only a few of these genes/proteins are expressed on the surface, thus are amenable to antibody binding. Although the genes/proteins that are not expressed on the surface can provide ideal targets for pharmacological inhibition for contraception, they are not suitable for CV development. Very few, if any, knockouts of a single gene have made mice totally infertile.

The molecules involved in sperm-oocyte membrane fusion are interesting and are being actively examined. Various candidates have been proposed, including DE, cluster of differentiation (CD)4626, equatorin Sperad, sperm acrosomal membrane-associated protein

(SAMP)$_{32}$25, a disintegrin and Metalloprotease (ADAM$_1$), and disintegrin domains (ADAM$_2$ and ADAM $_3$)27, and CD928. Recently, knockout of a gene, designated as *Izumo*, was reported that is very interesting29. *Izumo* is named after a Japanese shrine dedicated to marriage. *Izumo* gene knockout rendered the mice almost totally infertile29. 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 because it is not exposed until the sperm cell undergoes acrosome reaction, and the antibodies have to be present at the particular time and space for binding to Izumo antigen.

In order to examine whether or not: 1) proteins involved in sperm-oocyte membrane fusion can be used for CV development, and 2) immunization with multiple antigens can enhance the contraceptive efficacy, we recently conducted a trial by immunizing female mice with various peptides based upon several sperm antigens (Izumo, FA-1, YLP$_{12}$, and SP56)30. The synthetic peptides were conjugated to four carrier proteins namely keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), chicken λ-globulin, and chicken ovalbumin. Female mice were immunized with various peptide vaccines and each booster injection was given with the peptide conjugated to a different carrier protein30. Two different fertility trials with different doses of the peptide vaccines were conducted to examine the contraceptive effect. Injection of 150 μg of each peptide caused a significantly higher immune response in serum as well as in the vaginal tract causing enhanced contraceptive effect than those observed after injection with 75 μg of the peptides. Immunization with the Izumo-based peptides, which are involved in sperm-egg plasma membrane fusion, caused a significant reduction in fertility (Table II). The contraceptive effect was enhanced by including peptides based upon other sperm antigens (FA-1, YLP$_{12}$, and SP56), resulting in an overall 73.33% reduction in fertility. When the antibodies against all the peptides disappeared after >9-10 months from circulation and genital tract, all the animals regained fertility (Table II). These findings indicated for the first time that the immunization with Izumo and other sperm peptides namely FA-1, YLP$_{12}$, and SP56 induced antibodies in serum and genital tract that cause a reversible long-term contraceptive effect in female mice. The data further indicates that the proteins involved in sperm-egg fusion can also be used for contraceptive vaccine development. The contraceptive effects are enhanced by immunizing with multi-peptide vaccines. Similar effects on enhancement of contraceptive effect after combination vaccination was observed using sperm DNA vaccines31,32. However, even after using multi-epitope vaccines, there was up to 73.3% reduction in fertility, rather than a complete block. This may be due to variability of the immune response among the immunized animals.

The progress in CV development has been delayed due to variability of the immune response after vaccination 5. It is envisaged that this concern may be obliterated by the passive immunization approach using the preformed antibodies. The antibody therapies have been tried and proven to be successful against various infectious diseases, both in animals and humans. Several of these antibodies have become treatment modalities in the clinics 33-35. Phage display technology has been widely used to obtain a variety of engineered antibodies, including single chain variable fragments (scFv) antibodies against several antigens 36-40. ScFv is an antibody fragment that plays a major role in the antigen-binding activity, and is composed of variable heavy (VH) and variable light (VL) chains connected by a peptide linker. The most widely used peptide linker is a repeat of a 15-residue sequence of glycine and serine (Gly$_4$Ser)3. The affinity and stability of the scFv antibodies produced in bacteria are comparable with those of the native antibodies and are maintained by a strong disulfide bond. ScFv antibodies can be produced on a large scale using specially modified bacterial hosts and have an advantage over the whole immunoglobulin (Ig) molecule. ScFv

antibodies lack the Fc portion that eliminates unwanted secondary effects associated with Fc, and due to its small size can be easily absorbed into tissues and gene manipulated41. The mouse monoclonal antibody can elicit strong anti-mouse antibody reaction, chimeric antibody can cause anti-chimeric response, and xenogenic complementarity-determining regions (CDRs) of humanized antibodies can also evoke an anti-idiotypic response, when injected into humans42-44. Antibodies must be of human origin if to be used in humans. The potential poor immunogenicity and toxicity of an antigen, and ethical issues, limit immunizing humans to obtain human antibodies. However, the phage display technology can be used to obtain these antibodies against target antigens if they exist involuntarily in humans, such as ASA in immunoinfertile men and women, and vasectomized men.

We recently did a study to obtain fertility-related scFv human antibodies that can be used for CV immunoinfertility. Peripheral blood leukocytes (PBL) were obtained from antisperm antibody-positive immunoinfertile and vasectomized men, activated with human sperm antigens *in vitro*, and cDNA was prepared from their RNA and PCR-amplified using several primers based on all the available variable regions of VH and VL chains45. The amplified VH and VL chains were ligated and the scFv repertoire was cloned into pCANTAB5E vector to create a human scFv antibody library. Panning of the library against specific antigens yielded several clones, and the four strongest reactive (designated as AFA-1, FAB-7, YLP20, and AS16) were selected for further analysis. These clones were shown to have novel sequences with unique completmentarity determining regions (CDRs) when a search was performed in the immunogenetic database (Table III). ScFv antibodies were expressed, purified, and analyzed for human sperm reactivity and effects on human sperm function. AFA-1 and FAB-7 scFv antibodies, having IgG3 heavy and IgK3 light chains, recognized a specific single sperm protein of 50 ± 4 kD and reacted with the purified and well characterized human sperm FA-1 antigen, which is involved in human sperm function and fertilization (Table IV). These antibodies bound to post-acrosomal, mid-piece, and tail regions of human sperm and inhibited sperm capacitation/acrosome reaction. Although both of these antibodies reacted with FA-1 antigen, they were directed against different epitopes of the molecule. AFA-1 was directed against an antigenic determinant $FA\text{-}1_a$ (human $FA\text{-}1_{200\text{-}219\ aa}$/mouse $FA\text{-}1_{117\text{-}136\ aa}$) and FAB-7 was directed against the determinant $FA\text{-}1_b$ (human $FA\text{-}1_{87\text{-}97\ aa}$/ mouse $FA01_{2\text{-}19\ aa}$) of the FA-1 antigen. The third, YLP20 scFv antibody, reacted with a sperm protein of 48 ± 5 kD, which contains the dodecamer sequence, YLPVGGLRIGG. This sequence is present on acrosomal, mid-piece and tail regions of human sperm and is involved in human sperm function and fertilization. The fourth antibody, AS16, reacted with a 18 kD sperm protein (major band) and was found to be a human homolog of the mouse monoclonal recombinant antisperm antibody (RASA)46. RASA is directed against a sperm agglutination antigen-1 (SAGA-1), that causes agglutination of human sperm47. These antibodies inhibited human sperm capacitation/ acrosome reaction in a concentration-dependent manner (Table IV). This is the first study to report the use of phage display technology to obtain human antisperm scFv antibodies of defined antigen specificities from immunoinfertile/vasectomized men. These antibodies will find clinical applications in the development of novel immunocontraceptives, and specific diagnostics for immunoinfertility in humans. The contraceptive effect of these antibodies *in vivo* is currently being investigated.

In conclusion, development of CV targeting sperm is an exciting proposition, and may provide a valuable alternative to the presently available methods. As limitation with other vaccines, the progress in CV development has been delayed due to variability of immune response after vaccination. The multi-epitope vaccines may enhance the efficacy and obliterate the concern of the inter-individual variability of the response. Also, this concern may be addressed by the passive immunization approach using preformed human antibodies. Several antibodies are being tried as therapeutic agents. At the present time, >100 antibodies

are in clinical trials and ~20 FDA-approved monoclonal antibodies are available in the market for various clinical conditions, including cancer and infectious diseases. Over 80% of these antibodies are genetically engineered[48,49]. The scFv antibodies that we have synthesized *in vitro* using cDNAs from antisperm antibody-positive immunoinfertile and vasectomized men may provide useful, once-a-month immunocontraceptive. These human antibodies are sperm-specific and inhibit sperm function *in vitro*. Their immunocontraceptive potential *in vivo* is presently being investigated.

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Table I

Active Immunization with Sperm Peptides on Fertility

Peptide	a.a. (n)	Fertility Reduction	Reference
A. Mouse			
rFA-1	Whole molecule	71%	Naz and Zhu15
YLP_{12}	12	70%	Naz and Chauhan16
P10G	10	>70%	O'Rand et al17
A9D	9	50%	Lea et al18
SP56	16	>70%	Hardy and Mobbs19
B. Primates			
LDH-C4-bC5-19	15	62%	O'Hern et al20
LDH-C4-bC5-19	15	0%	Tollner et al21
rEppin	Whole molecule	78%	O'Rand et al22

Table II

Effect of Vaccination with Various Sperm Peptides on Fertility of Female Mice

Group	Peptides	Dose of each peptide/animal	Pups born/animal (% fertility reduction) (mean ± SD)	
			92-102 days[a]	>9-10 months[a]
Group 1	Izumo	150 μg	2.6 ± 2.4 (56.66%)*	6.3 ± 0.8
Group 2	FA-1 and YLP_{12}	150 μg	3.33 ± 2.3 (45%)*	6.1 ± 1.1
Group 3	Izumo, FA-1, and YLP_{12}	150 μg	1.6 ± 1.7 (73.33%)†	6.4 ± 0.9
Control	—	—	6.0 ± 0.4 ‡	6.2 ± 1.0

Values with different superscripts are significantly different, P<0.0001; all others are nonsignificant (P>0.05)

[a]Days after last injection

Table III

Amino Acid Sequence of CDRs for scFv Antibodies

scFv antibody	Light chain			Heavy chain		
	CDR1	**CDR2**	**CDR3**	**CDR1**	**CDR2**	**CDR3**
AFA-1	GYIFTSYD	IFPGEGST	ARGDYYRRYFDLW	ASSSIRY	DTS	QEWSGYPYTF
FAB-7	GYSFTTSS	IIYPGDSE	ARLPESIPHYYGMDV	QSVSSGY	GAS	QQYGSSPLT
YLP20	GFTVSSSA	VVYVDGTT	ARSNWHYVTAMYN	QSVTMNY	AAT	QQYGSSPPGVTF
AS16	GYKFTGYW	IYPNSGDT	DSAVYFCARGDYGCPFVY	QSLLHSDRSTY	EVS	SQSIHVPPT

Table IV

Immunobiological Parameters of scFv Antibodies

scFv antibody	Sperm antigen recognized	Molecular mass	Epitope sequence	Acrosome-reacted sperm
AFA-1	FA-1	~50 kD	Human FA-$1_{200\text{-}219\ aa}$/ Mouse FA-$1_{117\text{-}136\ aa}$	36 ± 7^a
FAB-7	FA-1	~50 kD	Human FA-$1_{82\text{-}97\ aa}$/ Mouse FA-$1_{2\text{-}19\ aa}$	44 ± 6^a
YLP20	YLP_{12}	~48 kD	YLPVGGLRRIGG	42 ± 3^a
AS16	SAGA	18 kD (major) 37, 55, 100 kD (minor)	Unknown	Unknown
CAB-3 control	None	None	None	74 ± 5

^{a}Significantly different compared to control, P<0.01 to P<0.001