Antisperm antibody : a monkey wrench in conception / magic bullet of contraception?

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Abstract : Antisperm antibodies can cause infertility by interacting with spermatozoa through immunoglobulin binding protein thereby blocking their penetrance of cervical mucus and / or by interfering with sperm-egg interaction. However, these antibodies appear not to be cytotoxic to embryos since a high implantation rate and consequently high pregnancy rate were achieved by IVF-ET treatment of women with antisperm antibodies. Also the finding that these antibodies do not appear to cause any deleterious clinical symptoms and have yet be associated with infertility suggested that sperm antigens are promising candidates in the development of immunocontraceptives. Some synthetic peptides corresponding to segments of human sperm antigens have effectively induced infertility in female rats when administered as an immunogen. Different peptides, adjuvants and routes of administration should be studied to determine the optimum conditions for inducing high antisperm antibody titers in the host. Moreover, identification of various steps and factors that are involved in regulating the production of antisperm antibodies such as immunoglobulin binding factor may open new paths in the treatment of immunological infertility and at the same time lead to a more effective immunocontraceptive. J. Med. Invest. 46 : 19-28, 1999

Key words **:** *antisperm antibody, infertility, contraception, immunoglobulin binding factor, sperm antigen*

INTRODUCTION

Nonspecific and specific immune reactions against gamete or embryo appear to be physiologically important for the maintenance of homeostasis in reproduction (1). For example, in the male and female reproductive tracts, activation of the complement system by immature and aged spermatozoa can enhance their destruction and clearance during the selection of active motile sperm. This mechanism of sperm selection is physiologically important, in increasing the opportunity of healthy sperm to fertilize the ova (2). Furthermore, we have demonstrated that the occurrence of cellular and/or humoral immune reaction against sperm antigens expressed on the surface of embryos appears to augment their receptivity by the uterus (3).

On the other hand, aberration of the immune homeostasis may give rise to" immunological infertility ". As a matter of fact, the majority of subjects with asthenozoospermia and/or oligozoospermia have a higher incidence of low complement-inhibiting activity and a reduced level of complement regulatory proteins, such as membrane cofactor protein (MCP, CD46) and decay accelerating factor (DAF, CD55) in their seminal plasma, such that nonspecific activation of the alternative complement pathway may occur, inflicting injury to sperm (4).

An immune reaction acting adversely against gametes is a mechanism that contributes to the development of " immunological infertility ". Autoantibodies against zona pellucida may cause infer-

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tility by blocking sperm-zona pellucida interaction (5, 6). These antibodies are found in approximately 6% of infertile women with indeterminate etiology (6) ; whereas the incidence of infertility with associated antisperm antibodies is higher (approximately 18%).

The presence of antibodies in blood, follicular and uterine fluids and cervical mucus can impair reproductive processes that are crucial for a successful pregnancy. Since these antibodies appear not to cause any deleterious clinical symptoms and have yet be associated with infertility, sperm antigens are promising candidates in the development of an immunocontraceptive. Here, we review the significance of antisperm antibodies as a causative factor of infertility and their potential as immunogens in the development of a contraceptive vaccine.

ANTIFERTILITY EFFECT OF ANTISPERM ANTIBODY

Spermatozoa can elicit an immune response in women as an alloantigen and in men as an autoantigen. Antisperm antibodies may cause infertility by 1) impairing sperm motility ; 2) blocking penetrance of cervical mucus by spermatozoa ; 3) interfering with sperm-egg interaction ; 4) interfering with the implantation of blastocysts ; or 5) inhibiting growth and development of embryos.

Impairment of sperm motility

Antibodies capable of agglutinating or immobilizing spermatozoa may impair their motility in the female reproductive tract, thereby preventing fertilization. However, to be effective biologically the titer of the agglutinating antibodies should be sufficiently high. In the case of immobilizing antibodies, high complement activity is required.

Blocking sperm penetrance of cervical mucus

Blockage of sperm penetrance of cervical mucus is a common cause of infertility. Fjallbrant (7) showed that the ability of antibody-bound sperm to penetrate normal cervical mucus and the capacity of active motile sperm to traverse cervical mucus containing antisperm antibody are markedly reduced or completely abolished. Kremer and Jager (8) described the" shaking phenomenon "of antibody-bound spermatozoa entrapped in cervical mucus. They hypothesized that the Fc portion of the antibodies interact nonspecifically with the glycoprotein micelles of the cervical mucus (9). A similar observation was made earlier by Isojima et al. (10). The shaking phenomenon observed with spermatozoa found in cervical mucus is induced only by intact complete antibodies ; whereas Fab fragments are inactive. These findings suggest that cervical mucus contains an Fc receptor-like component.

In analyzing cervical mucus for antisperm factors, we have isolated and identified a 15 kDa protein that binds IgA, IgM and all subclasses of human IgG, determined by Western blot (11). The amino acid sequence of the N-terminus was found to be identical to secretory leukocyte protease inhibitor (SLPI). The capacity of SLPI to bind IgG was validated by Western blot using two recombinant SLPI preparations. SLPI has been considered to be an important factor in the defense against inflammatory stimuli by the mucosal epithelial cells (12, 13). Thus SLPI has an important physiological role in the local cellular defense system. Moreover, it is also involved in the pathogenesis of immunological infertility by entrapping sperm in cervical mucus.

Blocking of sperm-zona interaction

Naturally occurring antisperm antibodies, especially sperm immobilizing antibodies can block in vitro sperm-zona interaction (Table 1). We were the first to demonstrate that sperm immobilizing antibodies can block penetration of the sperm through the human zona pellucida (14). This observation has been confirmed subsequently by many investigators (15) and further substantiated by our clinical data (16). Although the definitive mechanism, whereby antisperm antibodies block sperm-zona interaction has not been elucidated, the capacity of antisperm antibodies to inhibit the acrosome reaction of sperm, an indispensable process for successful fertilization, is well established (15, 17)

It is noteworthy that the inhibition is reversible as shown in Fig. 1. Spermatozoa preincubated with antisperm antibody recover their ability to penetrate the zona pellucida upon subsequent washing in antibody-free medium (1). Recovery from the blocking effect of antisperm antibodies on the acrosome reaction is also observed after incubation of the antibody-bound sperm in antibody-free medium (17).

Benoff *et al.* (18) reported that antisperm antibodies inhibit the acrosome reaction and prevent capacitation by blocking the release of cholesterol from sperm membrane ; thereby lowering the membrane fluidity. Finally, antibody-bound spermatozoa

Table 1. Effect of antisperm antibodies on human sperm-zona pellucida interaction and in vitro fertilization (IVF).

	Sperm penetration test		IVF[1)]	
	Number of sperm bound	Number of sperm penetrating	Autoserum	Donor serum
Control serum (n=8)	13.3±14.1	8.4±9.1		
Test serum (n=10) [2)]	0.3±0.9*	0.1±0.3*		
IVF patient				
1 (∞)[3)]	0	0	0/2	3/3
2 (25)	-	-	2/3	2/2
3 (3)	-	-	2/3	1/1
4 (∞)	0	0	-	4/4

1) Serum was admixed as a supplement to the incubation medium. Data show fertilized ova/mature ova.
2) Positive for sperm immobilizing antibodies.
3) Sperm immobilizing value calculated by the formula : $\frac{\%\text{ of motile sperm in control serum}}{\%\text{ of motile sperm in test serum}}$
*p<0.02

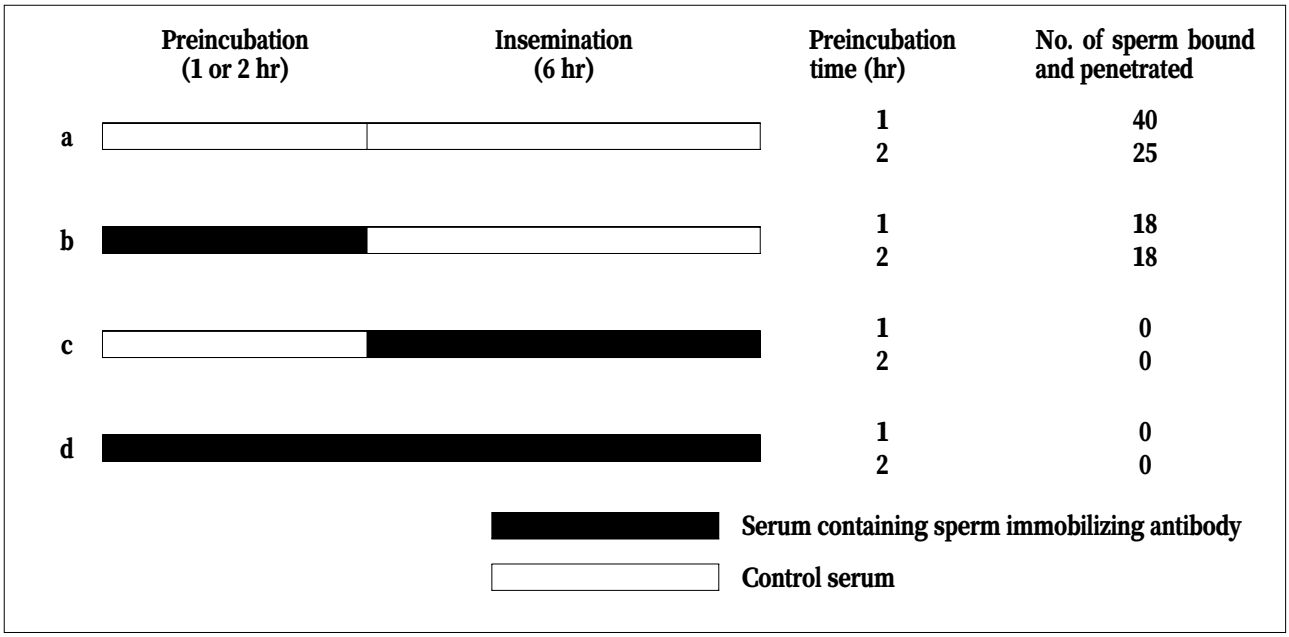

Fig.1. Time course results of zona penetration test using sperm exposed to serum containing sperm immobilizing antibody (cited from reference 1).

failed to express mannose-binding sites, essential for sperm-zona interaction (19, 20).

Inhibition of implantation of blastocyst and embryo growth

Since embryos are capable of expressing genes encoding some sperm antigens, specific antisperm antibodies can be embryotoxic and inhibit their growth (21, 22). We have previously identified the human sperm membrane component, YWK-II, as the target antigen of a monoclonal antibody (mAb) that agglutinates human sperm *in vitro* (23, 24). Passive immunization of pregnant female mice with anti-YWK-II mAb impeded the growth of the embryos (24). Furthermore, the antibody raised against the synthetic peptide, YAL-198, residue 21-36 of the YWK-II polypeptide interfered with the growth of the mouse zygotes beyond the 2-cell stage (Takikawa, in preparation).

To date, reports on the cytotoxic effects of antisperm antibodies on embryonic growth have been restricted to studies with experimental animals. On the other hand, naturally occurring antisperm antibodies found in the sera of immunologically infertile women do not appear to inhibit embryo growth or implantation of blastocysts. The clinical data clearly show that high implantation rates and subsequently high pregnancy rates are achieved

by *in vitro* fertilization and embryo transfer treatment of infertile women possessing sperm immobilizing antibodies in their circulation (13, 16).

MECHANISM OF ANTIBODY PRODUCTION AGAINST SPERM IN THE REPRODUCTIVE TRACT

Immunoglobulin binding factor family

Antisperm antibodies are rarely found in the sera of sexually active women who are constantly exposed to allogeneic sperm or in normal men who are in contact with autoantigenic sperm. This lack of antisperm antibodies production in fertile women and men suggests a possible suppression of immunoreactivity against sperm and the existence of some factors that prevent an immunological response against sperm. Components of seminal plasma may play a role in the immunomodulating network of the female and male reproductive tracts (25).

We have identified in human seminal plasma a component that binds IgGs of several species and interacts with anti-Leu-11b monoclonal antibody (mAb) raised against FcγRIII/CD16 (26-28). The protein is designated as" Immunoglobulin Binding Factor (IgBF) "This designation is based on the findings that it failed to interact with another mAb raised against FcγRIII/CD16 (3G8), showing that the interaction was not dependent on the presence of a specific antigenic determinant, but rather due to a common structure of immunoglobulins.

Three components having IgBF activity were separated by HPLC (Fig. 2) (29). The amino acid sequence of the major IgBF had structural identity to β-microseminoprotein (β-MSP)/prostatic secretory protein composed of 94 amino acids (PSP94) and β-inhibin. Furthermore, identity in the structure and molecular mass of the major IgBF and β-MSP/PSP94 was confirmed by mass spectrometry. In addition a large IgBF and β-MSP consisting of 93 amino acids were detected. The ability of β-MSP to bind human IgG and anti-Leu-11b antibody were demonstrated by Western blot analysis. Thus, this family of proteins is composed of at least three isoforms with β-MSP/PSP94 as the principal member, and should be designated as the IgBF family (30, 31).

Immunosuppressive activity of immunoglobulin binding factor

The in vitro effects of IgBF purified from human seminal plasma on mitogen-induced lymphocyte blastogenesis are depicted in Fig. 3. IgBF specifically inhibited PWM-stimulated lymphocyte blastogenesis (32), but did not influence PHA-stimulated blastogenesis and had a slightly inhibitory effect on Con A-stimulated blastogenesis. Furthermore, IgBF may not be directly involved with the immunosurveillance system, since it does not influence natural

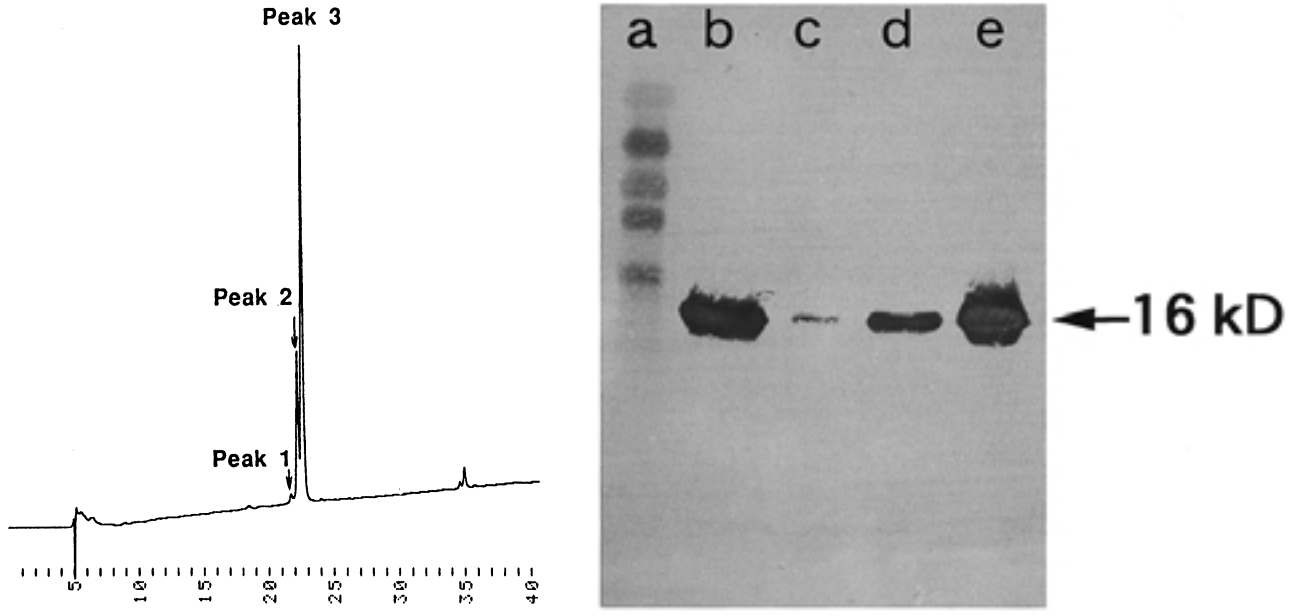

Fig. 2. Reversed phase HPLC (left) and Western blot analysis (right) of immunoglobulin binding factor (IgBF) purified by gel filtration on Sephadex G-100 column. Three peaks (left) possessing Ig binding activity were separated. Lane b : IgBF purified by Sephadex G-100 ; Lanes c-e : correspond to peaks 1-3 separated by HPLC (left). Molecular markers (lane a) : 80, 49.5, 32.5, 27.5, 18.5 kDa ; top to bottom.

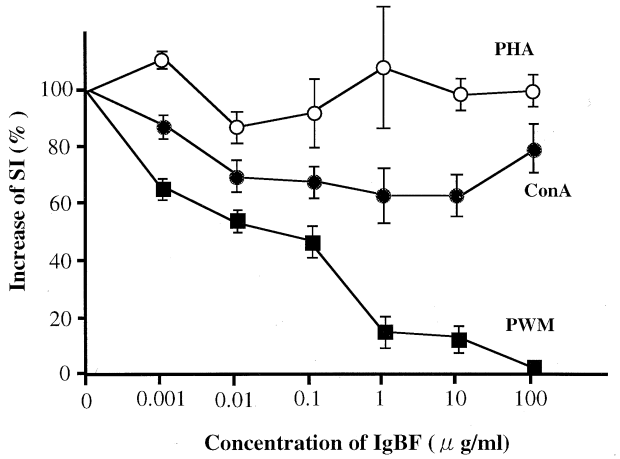

Fig. 3. Effect of purified immunoglobulin binding factor (IgBF) on mitogen-induced lymphocyte blastogenesis. SI : stimulation index ; PHA : phytohemagglutinin ; ConA : concanavalin A ; PWM : pokeweed mitogen (cited from reference 32).

killer cell activity, antibody-dependent cell-mediated cytotoxicity or complement activity (32). These findings support the hypothesis that seminal plasma IgBF suppresses B cell activation directly or represses the function of helper T cells, thereby preventing antibody production against sperm in the reproductive tracts.

Localization of immunoglobulin binding factor in the female reproductive tract

IgBF is a sperm-coating component. This contention is supported by the finding that anti-IgBF antibodies agglutinate and immobilize human sperm and is validated by its localization on the sperm surface by immunofluorescence staining (33).

Production of IgBF by cervical epithelial cells of the uterus was recently demonstrated by reverse transcription-polymerase chain reaction and in situ hybridization (Kamada, unpublished data) and high levels were determined in the cervical mucus by enzyme-linked immunosorbent assay (34).

Activation of immunoglobulin binding factor

IgBF exists in two forms : a 16 kDa monomer under reducing condition and a 27 kDa homodimer under non-reducing condition (28). The two forms can be differentiated in that the 16 kDa form of IgBF binds IgG and anti-Leu-11 b antibody ; whereas the 27 kDa homodimer form does not (28). Furthermore, the binding of IgG by the monomer is lost following carboxymethylation (28). In summary, native IgBF is present as the inactive dimer and is converted to the active monomer with free thiol groups required for the binding of IgG. To validate the above hypothesis that IgBF prevents the production of antisperm antibodies *in vivo*, the presence of an activating system capable of converting the native inactive dimer to the active form under physiological conditions has been demonstrated.

During the process of immunization with IgBF the dimer is transformed to the active monomer. This contention is supported by the finding that when rabbits are immunized with native IgBF, the raised antibodies interact predominantly with the active monomer rather than with the dimer (35, 36).

As shown in Fig. 4, native IgBF treated with reduced glutathione (GSH) migrated as a 16 kDa band in SDS-PAGE under non-reducing conditions and was found to react with anti-Leu-11b antibody and human IgG assayed by Western blotting (Fig. 4, lane e and f, respectively). Fig. 4 also shows that native IgBF treated with protein disulfide isomerase (PDI), a molecular chaperone, is converted to active monomers that bind anti-Leu-11b antibody (lane g) and human IgG (lane h) under non-reducing conditions. The present finding suggests that one of the activating systems of IgBF is PDI, which mediates the rearrangement of intramolecular disulfide bonds of IgBF. Auxiliary activation systems are the cleavage of IgBF by 20S proteasomes and arginylendopeptidase producing active fragments (37).

GSH and proteasome are widely distributed as cellular enzymes in vivo. Also we demonstrated the production of PDI mRNAs in the uterine cervix, endometrium and fallopian tube by reverse transcription-polymerase chain reaction (Fig. 5). It

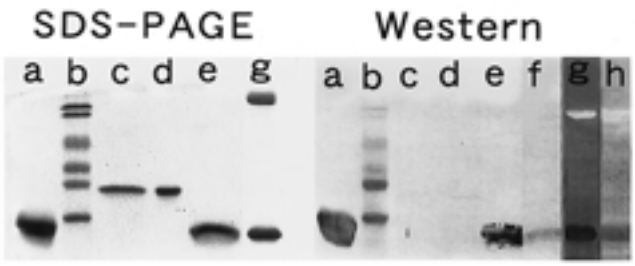

Fig. 4. SDS-PAGE (left) and Western blot (right) analysis of immunoglobulin binding factor (IgBF). Binding activity determined with anti-Leu-11b monoclonal antibody (mAb) raised against FcγRIII or human IgG. Carboxymethylated IgBF migrates as at 25 kDa monomer under reducing (left, lane c) and non-reducing (left, lane d) condition. Both failed to bind Ig (right, lanes c, d). IgBF treated with GSH migrates as a 16 kDa monomer and binds mAb (lane e) and human IgG (right, lane f) under non-reducing condition. Treatment of IgBF with protein disulfide isomerase results in the formation of active monomers that bind mAb (lane g) and human IgG (lane h) under non-reducing condition. Native IgBF under reducing condition interacts with mAb (lane a). Molecular marker (lane b) : 106, 80, 49.5, 32.5, 27.5, 18.5 kDa ; top to bottom. (cited from reference 37)

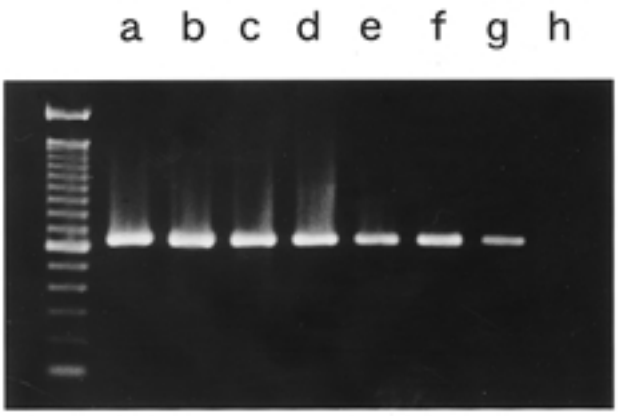

Fig. 5. cDNAs produced by reverse transcription-polymerase chain reaction (RT-PCR) of mRNAs, transcribing protein disulfide isomerase (PDI) as template. Agarose gel showing PCR-amplified PDI cDNA derived from mRNA prepared from human endocervix (lane a), endometrium (lane b), fallopian tube (lane c), ovary (lane d) ; chorion as a positive control (lane e) ; fetal membrane (lane f) and decidua (lane g). RT-PCR performed without mRNA template (lane h, negative control). Left lane, molecular markers. (cited from reference 37)

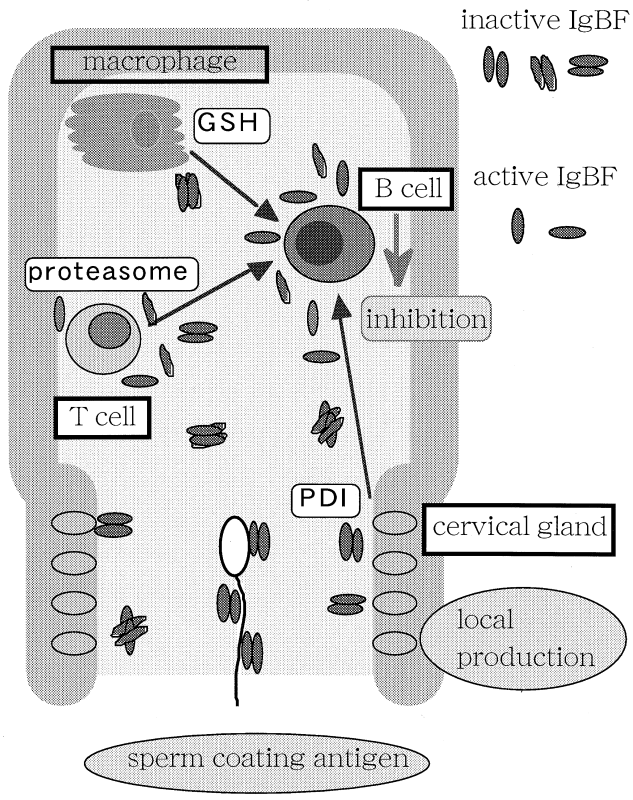

Fig. 6. Schema of a hypothesized mechanism of suppression of antibody production against sperm in female reproductive tract. Immunoglobulin binding factor (IgBF) produced by cervical glands and transported as sperm-coating antigen in their inactive form. Protein disulfide isomerase (PDI) distributed widely in the female reproductive organs can activate IgBF. Furthermore, GSH and proteasome, produced by macrophages and T cells, upon contact with allogeneic sperm can activate IgBF. Activated IgBF inhibits the production of antibody against sperm antigens.

is important to note that all these activating systems are related to the immune network. Proteasome is a threonine protease produced abundantly in activated human T cells (38). It is reasonable to assume that allogeneic sperm in the female reproductive tract attract and activate the T cells to produce considerable amounts of proteasomes. During the activation of macrophages, the intracellular GSH level increases greatly. Also the synthesis of DNA in mitogenically stimulated lymphocytes is accompanied by the generation of acid-soluble thiol as cysteine (39). Interferon-β, which is secreted by fibroblasts and white blood cells, e. g. macrophages, B cells and granulocytes, induces expression of PDI in fibroblasts (40).

Suppression of antibody production against sperm by immunoglobulin binding factor and its breakdown

A schema depicting a hypothesis of antibody suppression by IgBF is presented in Fig. 6. Immunocompetent cells activated by their interaction with allogeneic sperm generate GSH and PDI or induce their production in uterine tissue. Thus, in the female genital tract, intracellular or secreted GSH, PDI and 20S proteasome can activate IgBF, which in turn inhibit proliferation of B cells. The mechanism of the latter process is unclear.

A decrease in the secretion of IgBF by the cervical glands and/or a failure of the activation systems may result in a stimulation of antibody production. Inflammation such as cervicitis may decrease IgBF secretion, thereby promoting antibody formation. As a corollary finding, significantly high levels of antisperm antibodies are found in the sera of prostitutes, the majority of whom are afflicted with a variety of sexually transmitted diseases (41). Additional factors such as cytokines may be involved in regulating this process, and need to be identified.

SPERM ANTIGENS AS AN IMMUNOCONTRACEPTIVE VACCINE rSMP-B and YWK-II

Several laboratories have identified sperm antigens and their encoding genes by using monoclonal antisperm antibodies (42) or naturally occurring antisperm antibodies found in the sera of immunologically infertile women (43). With the use of mAb, we have identified two sperm antigens ; rabbit and human membrane proteins designated as rSMP-B and YWK-II, respectively. rSMP-B is an antigen located on the surface of the tail and midpiece of human sperm (44, 45). It has been tested as an antifertility immunogen

in rats (44). The YWK-II protein was identified as the target antigen of an mAb raised against human sperm (23, 24). These two antigens appear to be involved in the fertilization process since both mAb blocked in vitro fertilization of zona-free hamster eggs by human sperm (45, 46). The cDNAs encoding rSMP and YWK-II proteins were isolated from the rat testis λgt11 expression library and found to contain open reading frames encoding polypeptides composed of 146 and 191 amino acids residues, respectively (23, 47).

ACTIVE IMMUNIZATION WITH SYNTHETIC PEPTIDES CORRESPONDING TO SEGMENTS OF SPERM ANTIGENS

The use of peptides as a contraceptive vaccine offers distinct advantages since they can be synthesized readily and in sufficient amounts for biological testing. Also a region of the polypeptide specific to germ cells can be selected, thereby avoiding potential unexpected immunological cross-reactivity of induced antibodies with somatic cells of the testis and other tissues. Goldberg and associates (48) successfully tested a synthetic peptide corresponding to the immunodominant B cell epitope of human sperm-specific lactate dehydrogenase (LDH-C4) as a contraceptive vaccine in female baboons. A 75% reduction in fertility was observed compared to the controls. We have attempted to develop an immunocontraceptive by using small synthetic peptides whose sequences correspond to the hydrophilic extracellular domain of the above two sperm antigens (Table 2). Three peptide segments corresponding to the extracellular domain were synthesized as multiple antigen peptides. Female rats were actively immunized with the synthetic peptides with MDP-P-T as an adjuvant (49). Only YAL-198, corresponding to residues 21-36 of the YWK-II polypeptide significantly (9/12) reduced fertility (49). By the same method, active immunization with rSMP-230, residues 1-28 of the rSMP-B protein, administered with complete Freunds 'adjuvant induced infertility in 83% of treated female rats (50). As the next step, before testing in primates, female rats were immunized with the synthetic peptides without adjuvant. The peptides were conjugated to tetanus toxoid as a carrier protein. Fertilization was reduced by 38% with YAL-198 and 45% with rSMP-230 as compared to the controls (51). It is noteworthy that antibody titers against each peptide were higher in the non-pregnant groups than in the pregnant groups.

Another approach is mucosal immunization using recombinant *Salmonella dublin* expressing YAL-198. The microorganism was administered orally or vaginally to female rats. Both routes of immunization induced significant levels of antibody titers in sera and vaginal secretions associated with infertility in 60% (3/5) and 80% (4/5) of treated animals (Shan *et al*., in preparation).

CONCLUSION

Antisperm antibodies can cause infertility by

Table 2. Active immunization of female rats with synthetic peptides corresponding to segments of human sperm components.

	Immunization procedure			
peptide [1]	MAP+adjuvant [2]	TT [3]	Oral [4]	Vaginal [4]
YAL-198	70% (7/10)	38% (5/13)	60% (3/5)	80% (4/5)
YAL-201	33% (4/12)	n.d. [5]	n.d.	n.d.
YAL-212	17% (2/12)	n.d.	n.d.	n.d.
rSMP-230	83% (10/12)	45% (5/11)	n.d.	n.d.
rSMP-229	25% (3/12)	n.d.	n.d.	n.d.
Control		0% (7/7)	0% (5/5)	0% (5/5)

1) YAL-198 (residue 21-36), YAL-201 (residue 24-47) and YAL-212 (residue 5-36) of YWK-II antigen, and rSMP-230 (residue 1-28) and rSMP-229 (residue 29-53) of rSMP-B.
2) Synthesized as multiple antigen peptide (MAP). MDP-P-T and complete Freunds 'adjuvant were used in the immunization as adjuvant for YAL and rSMP, respectively.
3) Synthesized as a single peptide conjugated to tetanus toxoid and administered without adjuvant.
4) Oral and vaginal immunization with recombinant Salmonella vaccine expressing YAL-198.
5) not determined

blocking reproductive processes including sperm penetration of the cervical mucus and the acrosome reaction, which are essential steps for a successful fertilization. Although these antibodies disrupt sperm function, they appear not to be systematically detrimental. These two tenets are the basis for the development of an immunocontraceptive using sperm antigens. Nonetheless, identification of sperm-specific antigens is crucial. Our recent studies show that synthetic peptides or recombinant proteins and the method of mucosal immunization are promising breakthroughs in the strategy of developing an effective antifertility vaccine. Moreover, identification of various steps and factors involved in regulating the production of antisperm antibodies may open new paths in the treatment of immunological infertility and at the same time may lead to a more effective immunocontraceptive.

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