INFERTILITY INDUCED IN RATS BY IMMUNIZATION WITH SYNTHETIC PEPTIDE SEGMENTS OF A SPERM PROTEIN

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SUMMARY: Three peptide segments (YAL-198, YAL-201 and YAL-212) corresponding to the extracellular domain of a human sperm protein designated as YWK-II antigen were synthesized as multiple antigen peptide (MAP). Male and female rats were immunized with the YWK-II-MAPS and fertility determined. In a group of 12 female rats immunized with YAL-198, seven animals were infertile and two animals were subfertile. When immunized with YAL-201 and YAL-212, 4 and 2 animals were infertile, respectively. In a group of 15 males immunized with YAL-198, 2 animals were infertile and 6 were subfertile. Two animals immunized with YAL 201 were subfertile. All control male and female rats immunized with bovine serum albumin and adjuvant were fertile. Sera obtained from infertile rats immunized with YAL-198 contained higher titers of antibodies compared to those obtained from fertile animals. The present study shows that immunization with synthetic peptide segments of a sperm protein can effectively reduce fertility.

Infertility of undetermined etiology is a clinical entity of considerable magnitude that requires resolution (1). "Immunologic infertility" is due to the production of anti-gamete antibodies, derangement of B and/or T cell activities in the reproductive tract or alteration in the functional components of the immune network (2-6). There is a consensus that antisperm antibodies with sperm agglutinating and/or immobilizing activities may be a probable cause of infertility; however, direct experimental verification of this thesis is lacking. There are studies supporting this contention. For example, immunization of animals with sperm, sperm extracts or purified sperm proteins will result in a marked reduction of fertility, suggesting that production of antisperm antibodies can reduce fertility or induce infertility (7-14).

To validate that antisperm antibodies with sperm agglutinating activity is a cause of infertility, we have raised monoclonal antibodies (mAb) against specific human sperm proteins with sperm agglutinating activity (15-17). One of the antisperm mAb designated

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as YWK-II induced agglutination of human and rat sperm (18-20) and prevented fertilization of zona-free hamster eggs by human sperm (21). Using an immunocytochemical method, the interacting antigen was located at the equatorial region of the head, midpiece and tail of human sperm (19). The equatorial region of the sperm is purported to be the site of sperm-egg interaction during fertilization (22,23). In the rat, this protein is present on sperm head and produced in all germ cells undergoing spermatogenesis (20).

A 1.8 kb cDNA for the YWK-II protein was isolated from a rat testis λgt11 cDNA expression library and its nucleotide sequence determined (24). The cDNA contained an open reading frame coding for 191 amino acid residues. The deduced polypeptide had high homology to the transmembrane-cytoplasmic domains of the A4 amyloid precursor protein found in brain plaques of Alzheimer’s disease. The extracellular domain of the deduced YWK-II polypeptide, however, was unique. In the present study, peptide segments corresponding to extracellular domain of the YWK-II protein were synthesized as multiple antigen peptide (MAP) (25,26). Data are presented showing that immunization of adult female rats with YWK-II-MAPs induced infertility in a significant number of animals.

**EXPERIMENTAL METHODS**

**Synthesis of YWK-II-MAP**

Three peptide segments corresponding to the extracellular domain of the YWK-II polypeptide were synthesized as MAPs:

YAL-198, residue 21-36, SEEIPPFHPFPFPPSL-Y;
YAL-201, residue 24-47, IPPFHPFPFPPSLSENEDTQPELY; and
YAL-212, residue 5-36, SSISENPVDVRVSSEESEEIPPFHPFPFPPSL-Y.

The synthesis was performed as described by Tam (25). The peptides obtained from the synthesis were dialyzed using a Spectra Por 6 tubing with Mr cut off of 1000 against a solution of 2 M urea, 0.1 M NH₂HCO₃, 0.01 M NH₄HCO₃ and water. The MAPs were lyophilized in water. Amino acid analysis was performed in 5.7 N HCl for 24 h at 110°C. Amino acid analysis showed that the composition corresponds to the theoretical ratio.

**Immunization procedure and fertility assay**

Groups of 15 male and 12 female adult rats were immunized with 100 μg of bovine serum albumin (control) and YWK-II-MAPs with MDP-P-T as adjuvant (27) at 2 week intervals over a 12 week period. Immunized female rats were mated with proven fertile male rats one week after the last immunization. Immunized male rats were mated with fertile female rats. The next day after copulation, the vagina was examined for sperm. On day 14 of expected pregnancy the animals were killed and uterus examined for embryos. The number of embryos in each horn was counted. All control animals were fertile containing 12 to 15 embryos in the uterus. Animals were considered as infertile when no embryo was found and subfertile or reduced fertility when there were 6 to 9 embryos per uterus.

**Assay of antibody titers**

MAPs were radioiodinated with ¹²⁵I using modified chloramine T method by Midgley (28). The circulating antibody titer was expressed as percent binding of ¹²⁵I-MAPs with individual serum diluted to 1:50, determined by radioimmunoassay.
RESULTS

Groups of 12 female rats were immunized with YAL-198, YAL-201 and YAL-242 and their fertility determined (Table I). On immunization with YAL-198, 7 animals were infertile and 2 were subfertile. The uteri of the infertile animals were devoid of embryos (Fig. 1). Immunization with YAL-201 and YAL-212 induced infertility in 4 and 2 female rats, respectively. In male rats, immunization with YAL-198 resulted in 2 infertile and 5 subfertile animals out of a total of 15 animals (Table II). Two male rats immunized with YAL-201 were subfertile and with YAL-212 none were affected (Table II). Of the YWK-II-MAPs tested, YAL-198 was the most effective in inducing infertility in female and male rats.

The antibody titers in sera obtained from YWK-II-MAPs-treated male and female rats were determined by radioimmunoassay. The titers are expressed as percent binding at 1:25 and 1:50 dilution of male and female sera, respectively. Sera obtained from the seven infertile, two subfertile and three fertile females immunized with YAL-198 (Table I) showed binding titers ranging from 41 to 52%, 11 and 22%, and 3 to 18%, respectively. Infertile female rats immunized with YAL-201 and YAL-212 showed antibody binding titers of 30-40% and 25-30%, respectively, and fertile females, 5-20%. Sera (1:25 dilution) obtained from the two infertile males immunized with YAL-198 (Table II) showed antibody titers of 40 and 48% binding; five subfertile males, 15 to 30%; and eight fertile males, 4 to 20%. The binding titers of sera obtained from the two subfertile male rats immunized with YAL-201 (Table II) were 22 and 25%. Those obtained from the fertile male rats immunized with YAL-201 and YAL-212 were 5-20%. The present results suggest that infertility may be related to circulating antibody titers.

DISCUSSION

The present study is the first demonstration that immunization with synthetic peptide segments of a sperm protein can cause infertility. This finding suggests that peptide

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Table I. Fertility of adult female rats immunized with YWK-II-MAP

<table>
<thead>
<tr>
<th>Status of fertility</th>
<th>YAL-198</th>
<th>YAL-201</th>
<th>YAL-212</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertile*</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Subfertile+</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal Pregnancy</td>
<td>3</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Control female rats were immunized with bovine serum albumin and adjuvant; all rats were pregnant with 15 to 20 embryos/animal.

*Infertile, no embryos, non-pregnant.

+Subfertile, reduced no. of embryos: 6 to 9 embryos/animal.
segments corresponding to epitopes or domain of specific sperm protein can be used as antifertility immunogens. The whole intact protein is not required. It should be pointed out that although YAL-198 was the most efficacious immunogen tested, the longer segment, YAL-212 containing the same peptide sequence as YAL 198 was less effective. This finding that the antifertility potency was less with the longer segment is not clear. A possible reason is that YAL-198 contains the critical region for sperm-egg interaction,
whereas in the longer peptide, YAL-212, the extra peptide segment may reduce the antigenicity by structural hindrance that the raised antibodies are less effective in preventing sperm-egg interaction.

Polyclonal antibodies were raised against YAL-198 in rabbits. The antisera induced tail-to-tail agglutination of rat and human sperm and stained a 60-kD human sperm protein by immunoblot (unpublished data). In a previous study, it was demonstrated that YWK-II mAb interacted with a 60-kD human sperm protein and induced agglutination of human sperm (17). Thus the above finding indicates that the YAL-198 correspond to a segment present in the 60-kD sperm protein.

There are several reports showing that fertility is reduced by immunization with sperm proteins. Immunization with a sperm protein designated as PH-20 induced 100% infertility in guinea pigs (14). A sperm specific glycoprotein designated as FA-1 from human and mouse germ cell plasma membranes causes reduction of fertility in actively immunized female rabbits (13). Immunization of female rats with a 24 kD protein isolated from rat testicular cytosol induced infertility in over 80% of treated animals (29). Another soluble antigen, LDH-C4, is effective as antifertility immunogen (30). In these reported studies purified proteins were used; whereas in the present study synthetic peptide segments of known sequence were utilized as immunogens and were effective in reducing fertility.

In summary, active immunization with synthetic peptide segments of a specific sperm protein can induce infertility and/or reduced fertility in male and female rats. The YWK-II MAP designated as YAL-198 should be further evaluated as a potential antifertility vaccine.

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REFERENCES