Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 5713–5717, August 1986
Medical Sciences

Characterization of the fertilization antigen 1 for the development of a contraceptive vaccine

(high performance liquid chromatography/two-dimensional gel electrophoresis)

R. K. NAZ*†, T. M. PHILLIPS‡, AND B. B. ROSENBLUM§

Departments of *Obstetrics and Gynecology, *Physiology, and ‡Medicine, The George Washington University Medical Center, Washington, DC 20037; and §Department of Pediatrics, The University of Michigan Medical School, Ann Arbor, MI 48109

Communicated by Frederick C. Robbins, April 16, 1986

ABSTRACT **A fertilization antigen, FA-1, was purified from either deoxycholate- or lithium diiodosalicylate-solubilized murine testes by immunoaffinity chromatography using a monoclonal antibody, MA-24, which inhibited fertilization *in vitro*. The FA-1 was recovered at high (11.4) or low (2.8) pH using stepwise elution procedures of the deoxycholate or lithium diiodosalicylate extracts, respectively. Both of these fractions showed a single band of 47 kDa when analyzed by $NaDodSO_4$/PAGE and silver staining. Following removal of the detergent and extensive dialysis at pH 5.8 or treatment with 0.15 M NaCl, even in the presence of detergent, a monomer of 23 kDa was detected. Two-dimensional PAGE of FA-1 showed, four or five polypeptides in the 47-kDa or 23-kDa range. The dialyzed FA-1 contained a major 23-kDa and a minor 48-kDa band when separated on both sucrose and cesium chloride gradients. High performance size-exclusion chromatography showed a major peak at 23 kDa and a minor peak at 50 kDa. Further analysis of the 23-kDa peak by reverse-phase chromatography resolved the antigen into three peaks, which gave similar two-dimensional gel patterns as the native FA-1. Lectin affinity chromatography on a lens culinaris column demonstrated that a part of the antigen was bound to the lectin while the rest was not. The FA-1 revealed a positive reaction with periodic–Schiff reagent and contained glucose and mannose, which together constituted 18.8% of the total antigen mass. Amino acid analysis showed a high percentage of aspartic acid, glutamic acid, serine, and glycine. As a single injection of MA-24 significantly reduced fertilization rates *in vivo*, the purified FA-1 is an attractive candidate for the development of contraceptive vaccine.**

Immunization of male and female animals of various species with extracts of sperm or mature testes results in a significant inhibition of fertility (1, 2). The whole sperm or testes cannot be used for immunoregulation of fertility due to the presence of numerous antigens on the germ-cell surface that could be shared with other somatic tissues (3, 4). The utility of any antigen as a contraceptive vaccine is contingent upon its tissue-specificity, its involvement in fertilization and fertility, and its homogeneity. A few sperm-specific antigens have been purified to homogeneity and characterized, and some of them are relevant to fertility. Immunization with lactate dehydrogenase-X (LDH-C_4) resulted in inhibition of fertility (5, 6), and antiserum to rabbit sperm autoantigen (RSA-1) also showed a reduction in fertility (7).

We reported a monoclonal antibody, MA-24, developed against human spermatozoa that inhibited *in vitro* fertilization of murine oocytes by mouse sperm and zona-free hamster ova penetration by human sperm without agglutinating or immobilizing spermatozoa (8). MA-24 was directed against the 23-kDa antigen of human germ-cell plasma membranes, and the fertilization antigen FA-1 was isolated using immunoaffinity chromatography from human testes and sperm. Interestingly, FA-1 cross-reacted with sera from involuntary immunoinfertile patients indicating, indirectly, its immunogenicity in humans. The monoclonal antibody MA-24, though tissue-specific, showed cross-reactivity with sperm from mouse, rabbit, rhesus monkey, and bull. As the availability of human germ cells is limited for use in the isolation of FA-1, we tried to purify it from murine germ cells. We report here the isolation of FA-1 from murine testes, its characterization, and the effect of the monoclonal antibody MA-24 on the murine fertilization *in vivo*.

MATERIALS AND METHODS

Virgin mice of the BALB/c or the C57BL/6JXCBA/J strain were used for the study. The testes tissue, homogenized in 0.05 M Tris·HCl, was solubilized with detergent sodium deoxycholate (DOC) or with a milder chaotropic agent, lithium diiodosalicylate (LIS) (9). Briefly, for DOC solubilization, the testes homogenate was extracted with 15 mM DOC in 0.05 M Tris·HCl (pH 8.0) containing 1 mM phenylmethylsulfonyl fluoride, and 5 mM soybean trypsin inhibitor for 1 hr at 4°C by gentle agitation, and the supernatant was removed after centrifugation at 25,000 × *g* for 30 min. For LIS solubilization, the testes homogenate was solubilized in 0.3 M LIS in 0.05 M Tris·HCl (pH 8.0) containing 1 mM phenylmethylsulfonyl fluoride and 5 mM soybean trypsin inhibitor at room temperature for 30 min, then at 4°C for 2 hr and centrifuged at 25,000 × *g* for 30 min. The LIS-solubilized testes supernatant was dialyzed against 0.15 M lithium chloride (LiCl) and then against 0.05 M Tris·HCl (pH 8.0) containing 0.15 M NaCl and 1 mM EDTA.

The development and characterization of the monoclonal antibody MA-24 has been reported (8). The IgG fraction of ascites fluid, prepared either by stepwise precipitation with sodium sulfate (10) or by using a protein A-Sepharose 4B column (Pharmacia), was coupled to CNBr-activated Sepharose 4B (11). The coupled gels were placed in columns and equilibrated with 0.1 M phosphate buffer (pH 8.0). Affinity chromatography was performed at room temperature (approximately 22°C) with either a high pH elution procedure for the DOC-solubilized testes (12) or with a low pH elution procedure for the LIS-solubilized testes. For high pH elution, an immunosorbent column was loaded with 1 ml of DOC-solubilized murine testes (containing 3–5 mg of protein) and incubated for 3 hr at room temperature or overnight at 4°C.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FA-1, fertilization antigen 1; DOC, deoxycholate; LIS, lithium diiodosalicylate; 2D, two dimensional; hCG, human chorionic gonadotropin; 1D, one dimensional; RPLC, reverse-phase liquid chromatography.

†To whom reprint requests should be addressed at: In Vitro Fertilization Laboratory, Department of Obstetrics and Gynecology, The George Washington University Medical Center, 2150 Pennsylvania Avenue, NW, Washington, DC 20037.

Elution was performed in a stepwise manner, first at pH 10 to eliminate loosely bound proteins, and then at pH 11.4 to obtain the antigen. The eluted fractions were neutralized with solid glycine, pooled within each pH group, concentrated, and dialyzed against Tris·HCl (0.05 M, pH 8.0).

For the low pH elution system, 1 ml of the LIS-solubilized murine testes was incubated with the immunoaffinity column as described above and bound proteins were first eluted at pH 4 to eliminate loosely bound proteins and then at pH 2.8 to obtain the tightly bound antigen. The eluted fractions were neutralized with 1 M KH_2PO_4, concentrated, and dialyzed against Tris·HCl buffer.

One-dimensional (1D) $NaDodSO_4$/polyacrylamide gel electrophoresis was performed by the method of Laemmli (13) with 10% gel. Following this procedure, electrophoresis gels were stained with silver nitrate stain (Bio-Rad) or with periodic acid/Schiff reagent (14).

For two-dimensional (2D) gel electrophoresis, samples were diluted (1:1) with solubilization solution containing 9 M urea, 2% (vol/vol) Nonidet P-40, 2% (vol/vol) 2-mercaptoethanol, and 2% (vol/vol) (pH 3.5–10) Ampholines (LKB) and were applied to the first dimension prepared using the Andersons' ISO system (15). Following isoelectric focusing (15 hr at 1200 V and 2 hr at 1500 V), gels were extruded from the tubes and equilibrated with 0.01 M Tris·HCl buffer (pH 6.8) containing 10% (vol/vol) glycerol, 2% (wt/vol) $NaDodSO_4$, 1% dithiothreitol, and 0.01% bromphenol blue and electrophoresed in second-dimension $NaDodSO_4$ gels prepared by using a 10% acrylamide gel. After electrophoresis, the gels were stained with sensitive silver nitrate stain.

The antigen was passed through BioBeads SM-2 and extensively dialysed against 0.1 M Tris·HCl, pH 5.8, in a Model 1200 MA continuous flow microdialysis chamber (Bethesda Research Laboratories) to remove the majority of the detergent used to extract the antigen. Twenty microliters of the dialyzed antigen (10.3 μg of protein) was introduced into a Beckman 340 isocratic HPLC system, equipped with Model 112 pump, a Model 160 UV detector, set at 280 nm, and a 7.6 × 300 mm TSK 3000 size-exclusion column (Beckman). The column was run at room temperature at a flow rate of 1 ml/min with an average run pressure of 1200 psi [1 psi = 6.895×10^3 Pa). The running buffer was 0.1 M Tris·HCl supplemented with 0.1 M sodium acetate, pH 5.8. The chromatogram was developed over 60 min, and the results were recorded on a Shimadzu CRIB automatic peak integrator. Fractions of the isolated peaks were collected in a modified ISCO Cygnet fraction collector.

The antigen isolated from the TSK 3000 size-exclusion column was subjected to further analysis by reverse-phase liquid chromatography (RPLC) on a Rainin C_{18} analytical column (16). Twenty microliters of the antigen, recovered from the major peak eluted from the TSK 3000 column, was injected into the same Beckman HPLC system that had been readjusted to an initial organic phase buffer containing 20% (vol/vol) acetonitrile/80% (vol/vol) water, to which 0.1% trifluoroacetic acid had been added. The chromatogram was developed on a 4.6 × 250 mm C_{18} column (Rainin, Woburn, MA) with a linear gradient (actual) running from 20–75% (vol/vol) acetonitrile in water. Trifluoroacetic acid (0.1%) was a constant constituent of the running buffer. The gradient was developed on an Autochrom Model OPG III, low-pressure gradient controller and pumped into the HPLC system via the Model 112 pump. The run was developed at 70°C, and the resulting elution profile monitored at 214 nm. The chromatogram was recorded and analyzed on the CRIB automatic peak integrator. The peaks from the RPLC run were concentrated individually, dialyzed against the Tris·HCl (pH 8), and analyzed on a 2D/PAGE.

The molecular weight of the antigen was further checked by ultracentrifugation using both sucrose and cesium chloride gradients. Four-milliliter gradients with a density range of 10–40% (wt/vol) were constructed for each medium with a continuous gradient former. The gradients were allowed to "age" for 1 hr at 4°C, and then 100 μl of a 1:4 dilution of the dialyzed antigen was carefully layered onto each gradient. The gradient tubes were placed into a Beckman SW 50.1 swinging bucket rotor and centrifuged at 48,000 × g for 24 hr in a Beckman L50 analytical ultracentrifuge. The molecular weights of the isolated bands were calculated from log c versus r^2 plots (17).

The presence of carbohydrate was checked by anthrone/sulfuric acid method (18) and periodic acid/Schiff's staining (14). To confirm that FA-1 is a glycoprotein, ≈25 μg of FA-1 was applied to a lectin (lens culinaris) column equilibrated with 0.05 M Tris·HCl (pH 8.0) containing 0.05% DOC and 0.15 M NaCl. The bound fraction was eluted with 10% (wt/vol) methyl-D-mannoside in the equilibration buffer. After concentration, the fractions were examined by 1D/ and 2D/PAGE. For monosaccharide analysis, FA-1 (10.3 μg) was digested with trypsin (Sigma, type XI, 100:1 molar ratio) for 2 hr at 25°C. The enzymatic digest of the antigen was passed through a Bio-Gel P-30 column to separate the carbohydrate-enriched peptide fraction, monitored by refractive index at ×16 gain. The carbohydrate-enriched peptide fraction was digested with 1 M HCl (110°C for 10 min) to prepare monosaccharides. Monosaccharide analysis was performed isocratically on the Beckman 340 HPLC system using a cation exchange column in the calcium form (Bio-Rad). The chromatogram was developed in double glass-distilled water at 85°C with a flow rate of 0.8 ml/min (19). The eluted carbohydrates were detected using a Pharmacia Refractive Index monitor set at ×32 gain. The carbohydrate elution profile was compared to known carbohydrate standards (Bio-Rad) run under identical conditions.

Amino acid composition was performed with a reversed-phase HPLC (20). The cysteine was analyzed via cysteic acid using a separate analysis. A duplicate sample was oxidized with performic acid before hydrolysis. The amount of cysteic acid detected was normalized to the untreated sample using residues that are generally not affected by the oxidation procedure. That amino-terminal acid residue was identified by the method of Tarr (20) using HPLC.

To investigate the effect of monoclonal antibodies on the murine fertilization *in vivo*, the ascites fluids were heat inactivated (56°C, 30 min) and were checked for their cytotoxicity to the murine epididymal sperm. They were also investigated for their immunoblot reaction with DOC-solubilized murine testes. The immunoblot procedure (21) and the immunodetection of antigen bands followed the procedure described before (22). The ascites, which were positive in the immunoblot and did not show direct cytotoxicity to the sperm, were then used in *in vivo* fertilization experiments.

Ovulation was induced in female mice by injecting 7 units of pregnant mare serum administered intraperitoneally, followed 48 hr later by 7 units of human chlorionic gonadotropin (hCG) injected intraperitoneally. These mice were injected intraperitoneally with 0.2 ml of MA-24 ascites fluid containing approximately 2.1 mg of antibodies, before hCG injection. Control mice received equivalent amounts of normal mouse serum or MA-8C10.5 ascites fluid. The 8C10.5 is a sperm-specific monoclonal antibody that has been reported to cause reduction in fertility without causing an inhibition of the fertilization process (12, 22). The animals were kept overnight for mating with male mice of proven fertility. The next morning, females were checked for vaginal plug formation. Some of the mated animals were killed 15–24 hr after hCG injection, and the oviducts were flushed with Hams's F-10 medium supplemented with 0.5% bovine serum albumin to retrieve fertilized zygotes. The fertilization rates were determined by removing the culumus mass with hyaluronidase

(300 units/ml) and counting the ratio of fertilized ova to total ova recovered. Ova were considered fertilized if they had two pronuclei in the cytoplasm and/or had the second polar body. To further confirm that they were fertilized, the fertilized zygotes were cultured in supplemented Ham's F-10 for 24 hr to check for their cleavage. The remaining animals were killed 36 hr after hCG injection to count the percentage of cleaved embryos.

RESULTS

Extracts of mouse testes were applied to an affinity column of monoclonal antibody MA-24 coupled-Sepharose 4B. In the DOC-high pH elution procedure, the fraction eluted at pH 10 showed a weak band of 47 kDa as well as a few other protein bands that were loosely bound to the immunoaffinity column. The fraction eluted at pH 11.4 showed only a single protein band of 47 kDa in 1D/PAGE after staining with Coomassie blue as well as with silver stain (Fig. 1, lane b). In the LIS-low pH elution procedure, the fraction eluted at pH 4 showed a weak band at 47 kDa and a few other bands. The fraction eluted at pH 2.8 had only a single band of 47 kDa (Fig. 1, lane c). When the antigen was passed through Bio-Beads SM-2 column to remove the detergent and then extensively dialyzed against 0.1 M Tris·HCl (pH 5.8) in a Model 1200 MA continuous flow microdialysis unit, the same antigen showed a single band of 23 kDa (Fig. 1, lane d). Protein on the SM-2 column was monitored at 280 nm and showed an 85% recovery of total protein applied to the column. This recovery rate does not account for the loss of the 47-kDa protein but rather the dissociation of 23-kDa monomers from the 47-kDa dimer.

On reduction with 2-mercaptoethanol and dithiothreitol, the 47-kDa protein did not migrate as a 23-kDa band. Treatment of the 47-kDa dimer with 0.1% $NaDodSO_4$, 10% (wt/vol) mannose, 10% (vol/vol) glucose, 6 M urea, 8 M guanidine hydrochloride, or 1.5 M sodium thiocyanate (100°C for 3 min) did not result in the formation of the 23-kDa monomer. However, when the 47-kDa dimer was treated with 0.15 M NaCl, a 23-kDa band was formed indicating that ionic forces were involved in the polymerization of the antigenic molecule. The monoclonal antibody MA-24 reacted with the 47-kDa dimer on the immunoblot containing DOC- or LIS-solubilized murine testes (Fig. 1, lane e). On the immunoblots containing DOC- or LIS-solubilized murine testes, extensively dialyzed as described above, MA-24 recognized the monomeric 23-kDa form.

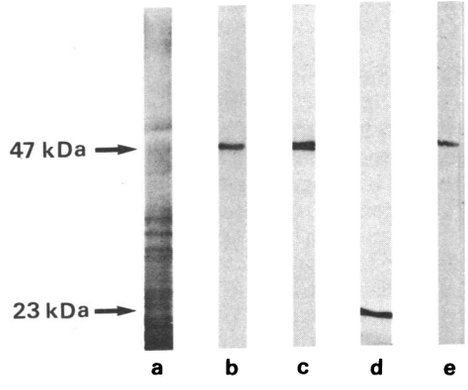

FIG. 1. 1D/$NaDodSO_4$/PAGE of the fertilization antigen FA-1 purified from DOC-solubilized testes (lane b) and LIS-solubilized testes (lane c). When the same antigen solution was passed through Bio-Beads SM-2 column to remove detergent and dialyzed or treated with 0.15 M NaCl, it showed a molecular size of 23 kDa (lane d). The MA-24 recognized a single band of 47 kDa on the immunoblot of DOC-solubilized murine testes (lane e). The whole DOC-solubilized murine testes preparation has been included for comparison (lane a).

The silver-stained 2D gel of the reduced antigen showed only four or five polypeptides in the 47-kDa range (Fig. 2). The antigen treated with 0.15 M NaCl revealed four spots of 23 kDa.

On passing the extensively dialyzed FA-1 through the size exclusion TSK 3000 column, two peaks were resolved. A small peak was 50 kDa, and the major peak had a molecular size spread between 21 and 24 kDa. The tip of this peak was estimated to be 23 kDa (Fig. 3*a*). The 23-kDa peak eluted from the TSK 3000 column was resolved into three peaks on the C_{18} RPLC column (Fig. 3*b*). The first major peak was eluted at 10.45 min at the 50% (vol/vol) acetonitrile point on the linear gradient. The second peak was eluted at 15.69 min at the 63% (vol/vol) acetonitrile point on the linear gradient. This was a small peak. The third peak was eluted at 16.49 min at the 65% (vol/vol) acetonitrile point on the linear gradient. Clearly the first and third peaks exhibited a large difference in their polarity but the difference between the second and the third peak was small, yet large enough to consider these peaks as separate (Fig. 3*b*). The 2D gel analysis of these peaks revealed a polypeptide pattern similar to that of the original FA-1.

In both the sucrose and the cesium chloride gradients, two bands were isolated. The lighter of the two bands was the major band while the second, heavier band appeared as a small diffuse band on the sucrose gradient and a sharper band on the cesium chloride gradient. The log c vs. r^2 plots revealed the lighter band had a molecular size of 23 kDa, while the heavier band had a molecular size range between 48 and 50 kDa on the sucrose gradient and 47 and 48 kDa on the cesium chloride gradient. Both gradient types were carefully searched for the presence of other bands, but none were found.

The antigen was anthrone/sulfuric acid/periodic acid/Schiff's positive. On passing the FA-1 through lectin (lens culinaris) column, the unbound flow through fraction showed a band of 23 kDa, and the eluted fraction also showed a band of 23 kDa. The unbound fraction passed through the lectin column for a second time did not bind indicating that the initial nonbinding was not due to overloading of the column. The 2D gel pattern of these two fractions showed the same four polypeptide spots in the 23-kDa range.

Carbohydrate analysis of the single carbohydrate-enriched fraction isolated from a P-30 column was performed on the ion-exchange column and revealed three (two major and one minor) peaks (Fig. 4). All peaks corresponded to standard

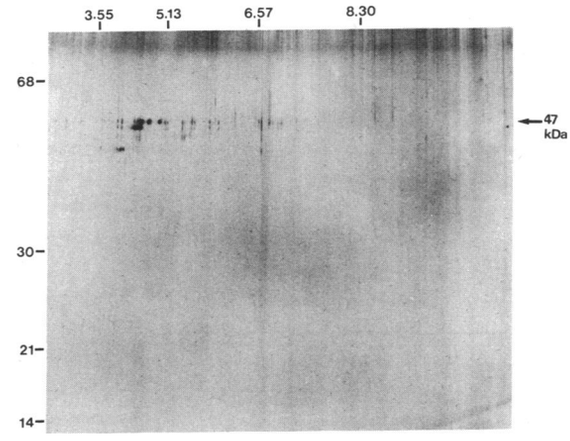

FIG. 2. 2D/PAGE pattern of the reduced FA-1 stained with silver showing four or five polypeptides in the 47-kDa range. Molecular sizes (in kDa) are indicated at the left, and the pH values of the isoelectric points are indicated at the top of the gel.

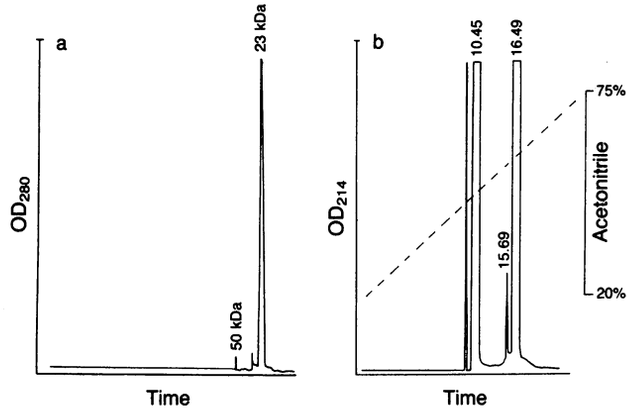

FIG. 3. HPLC analysis of the FA-1. (*a*) TSK 3000 size-exclusion chromatography run at 1 ml/min with the detector set at 280 nm, 0.8 AUF (absorbance unit, full-scale). Two peaks were eluted at 50 kDa and 23 kDa (second peak tip). (*b*) RPLC of the 23-kDa peak. Chromatogram was produced by running the column at a flow rate of 1 ml/min with the detector set at 214 nm, 0.04 AUF. Three peaks were resolved at 10.45, 15.69, and 16.49 min on a 20–75% (vol/vol) acetonitrile/water gradient containing 0.1% trifluoroacetic acid.

monosaccharides. The first peak was eluted at 9.63 min and corresponded to α-glucose residues. The second peak was eluted at 12.63 min and corresponded to the mannose standard. A small third peak was eluted at 13.48 min and corresponded to the fructose standard. This peak was very small and appeared as a shoulder on the tail of the second peak. Calculation of the peak heights and areas showed that the glucose residues were 1.75 times more abundant than the mannose residues and that the glucose constituted 12% of the total antigen mass. The mannose constituted 6.8% of the antigen mass. This calculated to a total of 18.8% carbohydrate content of the antigen mass of 23 kDa.

The amino acid analysis is summarized in Table 1 and indicates a relatively high percentage of acidic amino acids (aspartic and glutamic acids) as well as a high percentage of serine and glycine. Preliminary amino-terminal analysis revealed serine and aspartic acid in the first cycle and no amino acid in the second and third cycle.

A single intraperitoneal injection of 0.2 ml of MA-24 ascites

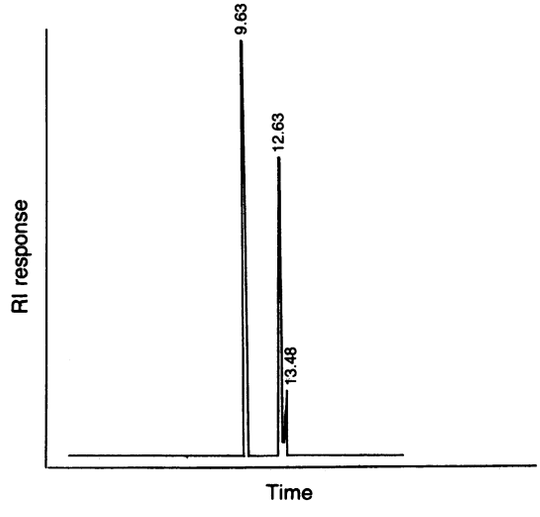

FIG. 4. Carbohydrate analysis of the carbohydrate-enriched polypeptide fraction of FA-1. Chromatogram was developed by running the double-distilled water at 0.8 ml/min with monitoring the refractive index at ×32 gain. Three peaks were resolved at 9.63, 12.63, and 13.48 min that corresponded to glucose, mannose, and fructose, respectively.

Table 1. Percentage of amino acid residues in FA-1

Amino acid	FA-1, %	Insulin A chain,* %
Ala	7.4	4.7
Cya†	1.6	0
Asp	11.4	9.4
Glu	14.2	18.8
Phe	1.8	0
Gly	17.6	5.6
His	1.3	0
Ile	2.3	3.3
Lys	2.9	0
Leu	4.3	9.8
Met	0.8	0
Pro	4.3	0
Arg	2.9	0
Ser	15.3	12.2
Thr	5.5	0
peC‡	0	18.8
Val	4.3	8.5
Try	2.2	9.8
Total residues	243.8	21.3

*Reference peptide.
†Cysteic acid.
‡Permethylated cysteic acid.

fluid per mouse resulted in a significant reduction in percent of fertilized ova recovered (29.6% as compared to 69% in controls) 15–25 hr after the hCG injection (Table 2). The fertilized ova, when cultured *in vitro* for the next 24 hr, cleaved into 2- or 4-cell embryos, further confirming that they were fertilized. There was a significant reduction in percentage of cleaved embryos obtained 36 hr after hCG injection from mice injected with MA-24 ascites fluid (30.3%) as compared to controls injected with normal mouse serum (77.1%). There was no significant reduction of fertilization rates in mice injected with MA-8C10.5 ascites fluid when checked at 15–24 hr (63.4%) or 36 hr (66.6%) after hCG injection. These results indicate that even a single injection of MA-24 monoclonal antibodies causes a significant reduction in fertilization rates *in vivo*.

DISCUSSION

Using an immunoaffinity column and the DOC-high pH elution procedure, we have purified the fertilization antigen FA-1 from murine testes to a single band (of 47 kDa or 23 kDa) as judged by 1D/PAGE with ultrasensitive silver stain. The same pattern was observed when the antigen was isolated using a milder chaotropic agent (LIS) in a low pH elution procedure. This is important as the antigen isolated with DOC is limited in its utility for biological use. FA-1 in DOC binds weakly to the plates used for the ELISA and the high pH may destroy a few carbohydrate residues. LIS/ELISA has been reported for detecting and titrating anti-sperm antibodies in serum from infertile couples (23). DOC and high pH can interfere in the characterization of the antigen and may increase deamination and hydrolysis of sugars from carbohydrate chains resulting in changes in the immunogenicity of the antigen molecule.

On 2D gel electrophoresis, the FA-1 revealed four or five polypeptides in the 47- or 23-kDa range. The slight difference in charge observed in the polypeptide spots may be due to differences in the carbohydrate content of various polypeptide chains. Preliminary amino-terminal analysis revealed serine and aspartic acid in the first cycle. The high concentration of aspartic acid and glutamic acid residues may also contribute to the charge differences observed in these polypeptide spots. FA-1 was purified from testes where this

Table 2. Effect of a single intraperitoneal injection of monoclonal antibodies on fertilization

Treatment	15–24 Hr after hCG injection			36 Hr after hCG injection		
	Mice, no.	Ova fertilized/ ova recovered, no./no.	Ova fertilized,* %	Mice, no.	Ova cleaved/ ova recovered, no./no.	Ova cleaved,* %
Control (normal mouse serum)	5	29/42	69	13	91/118	77.1
MA-24 ascites	6	16/54	29.6	14	58/191	30.3
MA-8C10.5 ascites	3	26/41	63.4	5	49/69	66.6

*Significance checked by χ^2 analysis: control (15–24 hr) vs. MA-24 ascites (15–24 hr), $P < 0.001$; control (36 hr) vs. MA-24 ascites (36 hr), $P < 0.01$; MA-8C10.5 ascites (15–24 hr) vs. control (15–24 hr), and MA-8C10.5 (15–24 hr) ascites vs. control (36 hr) insignificant.

protein is being synthesized. The antigenic molecule having the same polypeptide composition at various stages of maturation or modifications (glycosylation) or degradation is expected to be present. Mature sperm contain the intact antigen in low quantities, which makes it difficult to isolate enough for this type of analysis.

Reduction of the dimer protein with mercaptoethanol or dithiothreitol did not reduce it to a 23-kDa antigen indicating that polymerization is not due to disulfide linkage. The forces causing polymerization are mainly of an ionic nature and can be broken using 0.15 M NaCl or by changing the pH. After removing the detergent and extensive dialysis of the antigen at pH 5.8, the dimeric form was converted into monomeric form as detected by HPLC and gradient centrifugation. Sometimes, even after extensive dialysis and at low pH, a small quantity of dimer was present as seen after passing the FA-1 through size-exclusion columns and sucrose or cesium gradients. Freezing resulted in autopolymerization of the antigen. The antigen preparation that had a 23-kDa band polymerized to form a polymer of 47 or 69 kDa on freezing. Membrane proteins have a tendency to aggregate, especially in the presence of detergents (24, 25).

FA-1 is a 23-kDa glycoprotein comprised of polypeptide(s) with slightly different charges. Using reverse phase HPLC, the antigen can be split into three peaks of different polarity, but these peaks revealed similar polypeptides spots, indicating that there are 4 or 5 charged species of the same antigenic molecule.

FA-1 is a glycoprotein containing about 18.8% (wt/wt) carbohydrate. Most of the membrane glycoproteins, including immunoglobulins, have a percent of carbohydrates in the same range.

The ascites containing MA-24 monoclonal antibodies showed binding only to the specific protein bands on the immunoblots of testicular membrane extract. A single injection of these specific monoclonal antibodies showed a significant reduction in fertility *in vivo*. Effects may be enhanced by injecting a greater quantity of the antibodies. Preliminary data indicate that FA-1 is immunogenic in mice and rabbits, and active immunization with FA-1 causes a significant reduction in fertility in these species (26). The FA-1 isolated from murine testes shows a cross-reaction with sera from immunoinfertile patients.

The antigen can be isolated in large quantities using the immunoaffinity column. We have used the same immunoaffinity column without loss of activity for more than a year. The FA-1 constitutes approximately 3.7% of the LIS-solubilized and 4.8% of the DOC-solubilized murine testis proteins. Approximately 6.5–15 μg of the FA-1 can be isolated from a single adult murine testis. The concentration of FA-1 is low in mature sperm.

Preliminary observations indicate that the antigen may be crystallizable. The crystallization of FA-1 will delineate the topology of the molecule and thus will help in synthesizing large quantities of biologically active fragments for large scale practical use. Besides lactate dehydrogenase-X (6), rabbit serum autoantigen (RSA-1) (7), and germ-cell antigen (GA-1) (27), FA-1 constitutes the most promising candidate for developing an anti-sperm contraceptive vaccine for humans. Its homogeneity, strong immunogenicity, involvement in involuntary immunoinfertility in humans, and the significant reduction in fertilization and fertility by its antibodies makes it an especially attractive molecule.

The excellent technical assistance of Karen Wayns-Lee and Anne Dullea is gratefully acknowledged. We thank Dr. Nancy Alexander for help in some earlier work on monoclonal antibodies. The editorial assistance by Linda Vaughan is gratefully acknowledged. We thank the University of Michigan Protein Sequencing Facility for performing amino acid composition and amino-terminal analysis. This work was supported by a grant from the Program for Applied Research on Fertility Regulation, Northwestern University, under a Cooperative Agreement with the U.S. Agency for International Development (DPE-0546-A-00-1003-00).

1. Jones, W. R. (1982) in *Immunological Fertility Regulation*, ed. Jones, W. R. (Blackwell-Mosby, St. Louis, MO), pp. 90–146.
2. Talwar, G. P. & Naz, R. K. (1981) *Arch. Androl.* **7**, 177–185.
3. Schachner, M., Wortham, K. A., Carter, L. D. & Chaffe, J. K. (1975) *Dev. Biol.* **44**, 313–325.
4. Mathur, S., Goust, J.-M., Williamson, H. O. & Fudenberg, H. H. (1981) *Am. J. Reprod. Immunol.* **1**, 113–118.
5. Goldberg, E. (1974) in *Immunologic Approaches to Fertility Control*, ed. Diczfalusy, E. (Karolinska Institute, Stockholm), Vol. 9, pp. 202–222.
6. Goldberg, E., Wheat, T. E., Powell, J. E. & Stevens, V. C. (1981) *Fertil. Steril.* **35**, 214–217.
7. O'Rand, M. G. (1981) *Biol. Reprod.* **25**, 621–628.
8. Naz, R. K., Alexander, N. J., Isahakia, M. & Hamilton, M. S. (1984) *Science* **225**, 342–344.
9. Naz, R. K., Bradford, C. R. & Menge, A. C. (1985) *Arch. Androl.* **13**, 279–287.
10. Kekwick, R. A. (1940) *Biochem. J.* **34**, 1248–1257.
11. Cuatrecasas, P. (1970) *J. Biol. Chem.* **245**, 3059–3065.
12. Naz, R. K., Rosenblum, B. B. & Menge, A. C. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 857–861.
13. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
14. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617.
15. Rosenblum, B. B., Hanash, S. M., Yew, N. & Neel, J. V. (1982) *Clin. Chem. (Winston-Salem, NC)* **28**, 925–931.
16. Takagi, T. (1981) *J. Chromatogr.* **219**, 123–127.
17. Creeth, J. M. & Pain, R. H. (1967) *Prog. Biophys. Mol. Biol.* **17**, 217–231.
18. Wilson, C. M. (1959) *Anal. Chem.* **31**, 1199–1201.
19. Schwarzenbach, R. (1979) in *Biological/Biomedical Applications of Liquid Chromatography*, ed. Hawk, G. L. (Dekker, New York) pp. 193–222.
20. Tarr, G. E. (1986) in *Microcharacterization of Polypeptides: A Practical Manual*, ed. Shively, J. E. (Humana Press, Clifton, NJ), pp. 155–194.
21. Towbin, H., Stachlin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
22. Naz, R. K., Saxe, J. M. & Menge, A. C. (1983) *Biol. Reprod.* **28**, 249–254.
23. Alexander, N. J. & Bearwood, D. (1984) *Feril. Steril.* **41**, 270–276.
24. Javaid, J. I. & Winzler, R. J. (1974) *Biochemistry* **13**, 3635–3642.
25. Segrest, J. P., Jackson, R. L., Andrews, E. P. & Marchesi, V. T. (1971) *Biochem. Biophys. Res. Commun.* **44**, 390–395.
26. Naz, R. K. (1986) in *Immunological Approaches to Contraception and Promotion of Fertility*, ed. Talwar, G. P. (Plenum, New York), pp. 183–190.
27. Naz, R. A., Poffenberger, R. J. & Menge, A. C. (1986) *J. Reprod. Immunol.*, in press.