The mouse gamete adhesin, SED1, is expressed on the surface of acrosome-intact human sperm

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Objective: To determine whether SED1, a protein secreted by the mouse epididymis that coats sperm and participates in sperm adhesion to the zona pellucida, is present on human sperm and in human epididymal tissue.
Design: SED1 expression was analyzed by immunoblot and indirect immunofluorescence assays.
Setting: Academic clinical and research laboratories.
Patient(s): Human breast milk was donated. Unused semen was donated by men presenting for semen analysis or in vitro fertilization (IVF). Cadaveric epididymal tissue was obtained from the institutional body donor program.
Intervention(s): Human milk fat globule membranes and human seminal plasma proteins were analyzed by immunoblot. Human sperm and epididymis were analyzed by indirect immunofluorescence microscopy. Acrosomal status was determined by staining with fluorescein isothiocyanate-*Pisum sativum* agglutinin.
Main Outcome Measure(s): Immunoblot and indirect immunofluorescence assays.
Result(s): Human SED1 is recognized by two different polyclonal anti-SED1 antisera. SED1 is localized to the plasma membrane of human sperm overlying the intact acrosome. In acrosome-reacted sperm, SED1 is localized to the equatorial segment. SED1 is expressed by the epithelium of the anterior caput epididymis.
Conclusion(s): SED1 is expressed on the surface of acrosome-intact human sperm and in the anterior caput of the human epididymis, similar to that seen in mouse. (Fertil Steril® 2009;92:2014–9. ©2009 by American Society for Reproductive Medicine.)

Key Words: SED1, sperm adhesion, acrosome reaction

SED1 is a sperm-associated protein that facilitates mouse sperm adhesion to the egg zona pellucida (1). SED1 was initially identified as P47, a 47-kDa protein isolated by affinity chromatography of solubilized boar sperm membranes applied to immobilized porcine zona pellucida glycoproteins (2). Sequence analysis demonstrates that P47 is homologous to the short isoform of MFG-E8, also known as lactadherin, a predominant milk fat globule membrane protein found in several species, including pig, cow, and human; P47 is also expressed in pig, cow, mouse, and human testis. The localization and intensity of P47 expression changes during posttesticular maturation and capacitation of porcine sperm (3).

To gain more insight into P47 function, the mouse homologue was cloned and renamed SED1 to indicate a Secreted protein containing two NH_2-terminal notch-like epidermal growth factor (EGF) repeats and two COOH-terminal Discoidin/F5/8 complement domains. Some SED1 is derived from the Golgi complex of spermatogenic cells, but the majority of SED1 is secreted from the initial segment of the epididymis, where it binds to the sperm plasma membrane overlying the acrosome (4). Anti-SED1 antibodies block mouse sperm–egg binding, as does recombinant SED1, which binds to the zona pellucida of unfertilized but not fertilized oocytes. SED1 knockout male mice are subfertile in that they produce smaller litters than control males. Furthermore, sperm from SED1 knockout mice are incapable of binding the egg zona pellucida in vitro, despite apparently normal sperm motility and morphology. Thus, SED1 plays an important role during sperm–egg binding in mouse, as assayed by both in vitro and in vivo models (4). In addition to its role during sperm–egg binding, mouse SED1/MFG-E8 has also been shown to play critical roles during mammary gland development (5), clearance of apoptotic cells during mammary gland involution (6), repair of the intestinal epithelium (7), and phagocytosis of photoreceptor outer segments (8).

Human SED1, also known as human breast carcinoma antigen BA46, has been isolated from the milk of healthy donors as a 50-kDa full-length form and a 30-kDa truncated form containing the COOH-terminal discoidin domains (9). Human and mouse SED1 proteins are highly homologous, except that human SED1 lacks the first EGF-like domain. Although SED1 function in human has not been explored, it has been suggested that SED1/MFG-E8 present in milk may prevent rotavirus infection in breast-fed infants (10, 11). SED1 is also found in the sera of women with breast cancer and has therefore been evaluated for a role in breast cancer diagnosis

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and treatment (12–14). Irregardless, as a first assessment of SED1's potential role in human sperm–egg binding, we determined in this study whether SED1 is expressed on human sperm and in human epididymal tissue.

MATERIALS AND METHODS

Development of Recombinant SED1 and Anti-SED1 Antibody

The cloning of mouse SED1, the production of affinity-purified recombinant SED1, and characterization of rabbit antibodies raised against recombinant SED1 have been described previously (4).

Human Milk Preparation

SED1 was isolated from donated human milk using a modification of a previously published method (9). Briefly, the cream was isolated from fresh human milk by centrifugation at 4,000 × *g* for 20 minutes and washed with 10 mM Na_2PO_4, phosphate-buffered saline (PBS), pH 7.2. After centrifugation at 4,000 × *g* for 20 minutes, the isolated washed cream was resuspended in five volumes of PBS and sonicated to disrupt the milk fat globule membranes (MFGMs). The resultant MFGMs were collected by centrifugation at 40,000 × *g* for 1 hour. The membrane pellet was resuspended in PBS with 2.5% Triton (vol/vol), sonicated, and mixed overnight at 4°C. Phase separation was achieved by heating the solution in a 37°C water bath for 1 hour, followed by centrifugation at 4,000 × *g* for 20 minutes. The lower detergent phase was collected and the protein precipitated overnight in 10 volumes of acetone at −20°C. The resultant SED1-containing fraction was collected by centrifugation at 4,000 × *g* for 20 minutes. The acetone was removed and the pellet stored at −20°C.

Human Sperm Acquisition

After informed consent, excess semen from 105 men aged 18–50 years presenting for semen analysis or in vitro fertilization (IVF) was donated to the study. Sperm used in this study were from men with normal standard semen analysis parameters, according to World Health Organization 1999 criteria for count and motility (15) and Kruger strict criteria for morphology >4% (16).

Participants were instructed to abstain from ejaculation for 2–5 days before sample collection. Semen samples were collected by masturbation and allowed to liquefy for 30–60 minutes before being centrifuged over an 80% single-layer density gradient (PureCeption; SAGE In-Vitro Fertilization, Inc., a CooperSurgical Company, Trumbull, CT) for 20 minutes. The resultant pellet was suspended in synthetic human tubular fluid (HTF) (Quinn's; SAGE In-Vitro Fertilization, Inc., a CooperSurgical Company) with 6% serum substitute supplement (SSS) (Irvine Scientific, Santa Ana, CA) and centrifuged for 5 minutes. The supernatant was discarded; fresh HTF with 6% SSS was applied over the pellet and incubated at 5% CO_2, 37°C for 30 minutes for capacitation and "swim-up" separation. Sperm from the top portion of the fluid column, the "swim-up" sperm, were used for immunohistochemical analysis.

Western Blot of Human Milk and Seminal Plasma

The MFGM isolated from human milk and the soluble proteins from human seminal plasma were resolved by both one- and two-dimensional electrophoresis. Proteins were transferred to polyvinylidene fluoride, which was subsequently blocked overnight in 5% normal goat serum (NGS) at 4°C. The polyvinylidene fluoride membrane was incubated in rabbit anti-SED1 antiserum at a concentration of 1:2,000 in 5% NGS phosphate buffered saline plus 1% Tween-20 (PBST) for 1 hour. The membrane was washed with PBST three times before incubation with 1:30,000 horseradish peroxidase–conjugated goat antirabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA) in 5% NGS PBST for 1 hour. The membrane was washed three times in PBST before application of electrochemical luminescence Western detection reagent, film exposure, and development. Preimmune antiserum was used at the same concentration to determine background levels of immunoreactivity. Two-dimensional electrophoresis provided the best protein separation to compare SED1 profiles between samples and species. Both sources of anti-SED1 antisera (anti-SED1, anti-MFG-E8) produced similar results.

Induction of the Sperm Acrosome Reaction

A portion of the swim-up sperm was incubated with the calcium ionophore A23187 (10 μM) in 5% CO_2 at 37°C for 30 minutes to induce the acrosome reaction. The sperm were then washed in HTF with 6% SSS for 5 minutes before being dried on slides.

Immunofluorescence of Human Sperm

Sperm were evaluated for SED1 expression using indirect immunofluorescence noting presence or absence of staining on the sperm membrane. A portion of the swim-up sperm was dried on slides, which were subsequently blocked in 5% NGS for 1 hour. Rabbit anti-SED1 IgG (60 μg/mL) was applied to slides for 1 hour. After three washes in PBS, slides were incubated in biotinylated goat antirabbit IgG (1:1,000) (Vector Laboratories, Burlingame, CA) for 1 hour. After three washes in PBS, fluorescent streptavidin (1:1,000) was applied for 1 hour. Slides were rinsed in PBS and water before mounting. Preimmune IgG at the same concentration was used to determine background levels of immunoreactivity. Parallel assays using anti-SED1 antiserum produced similar results as anti-SED1 IgG. To confirm that SED1 was expressed on the intact sperm plasma membrane, live sperm were blocked and exposed to primary antibodies as above before being dried on slides and exposed to secondary antibodies.

Determination of Acrosomal Status

Acrosomal status was determined by staining with fluorescein isothiocyanate–*Pisum sativum* agglutinin (FITC-PSA)