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An isoimmune response to human sperm clathrin in an infertile woman with systemic lupus erythematosus

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ABSTRACT

We have employed a proteomic approach to study the immune response to human sperm in an infertile female patient suffering from systemic lupus erythematosus (SLE). Human sperm antigenic extracts were resolved by means of two-dimensional electrophoresis and electroblotted onto nitrocellulose membranes. The membranes were incubated with serum from the SLE patient. Sperm antigens that were reactive to polyclonal antibodies were next visualized on X-ray film, using the enhanced chemiluminescence (ECL). Three spots corresponding to the positions of sperm immunoreactive antigens on a nitrocellulose membrane were localized in a silver stained gel and subjected to mass spectrometry. A database search of the sequences recognized by the analyzed SLE serum revealed its homology to the clathrin heavy chain (CHC). Further analysis revealed that anti-CHC antibody reacted with multiple sperm antigenic determinants, resolved by either one- or two-dimensional electrophoresis. When studied by immunofluorescence, we demonstrated anti-CHC antibody reactivity with the sperm tail tip (corresponding to the sperm agglutination pattern), also with the principal piece and with cytoplasmic droplets around the sperm midpiece. Live sperm clearly exhibited reactivity with the midpiece. This study demonstrates clathrin heavy chain on human sperm using serum of an infertile individual with a concomitant autoimmune disease.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, mainly afflicting women. Most often it is manifested in the skin, joints, blood and kidneys. In most patients with SLE, antinuclear, anticytoplasmic (ribosomal P proteins) and antiphospholipid antibodies can be found. There is no published data describing a relationship between SLE and isoimmunity to sperm. However, antibodies recognizing antigens present on sperm cells may be primarily initiated by somatic and/or microbial stimuli and then cross react with male gametes (Kurpisz et al., 1989).

Although isoimmunization to sperm in women is quite rare because of the existing mechanisms of tolerance to male gametes in the female genital tract and immunosuppressive factors contained in semen, the reproductive system remains in close contact with exogenous antigens (microorganisms, pathogens, haptens, and allergens) and the immune response might be misled after sustained intercourse, potentially shifting to react with sperm

Abbreviations: ANA, anti-nuclear antibodies; APTT, activated partial thromboplastin time; ASA, antisperm antibody; BSA, bovine serum albumin; CHC, clathrin heavy chain; CLTC, gene coding for clathrin heavy chain; ECL, enhanced chemiluminescence; ELISA, enzyme-linked immunosorbent assay; IDIBT, indirect immunobead binding test; IEF, isoelectric focusing; LE, lupus anticoagulant; MS, mass spectrometry; PVDF, polyvinylidene fluoride; SLE, systemic lupus erythematosus; TDOC, triton/deoxycholate buffer.

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cross-reactive determinants. A previous report showed isoimmunization to sperm in an infertile woman with a high antisperm antibody (ASA) titer in her serum (Isojima et al., 1987). Generation and characterization of heterohybridoma secreting monoclonal antibody with high titers of sperm immobilizing and agglutinating activities against human sperm and seminal plasma antigens were subsequently performed.

We have become interested in the reactivity of antibodies to sperm contained in a serum sample obtained from an infertile SLE patient. We have discovered autoantibodies originally affecting the somatic tissues of this woman, such as antinuclear antibodies (ANA) cross-reactive to sperm antigens. Mass spectrometry (MS) analysis revealed a high homology of one of the antigens to the clathrin heavy chain. To our knowledge, this is the first study to demonstrate the reactivity of an immune-infertile individual to clathrin, which is present on human sperm.

Clathrin is a component of the coat-formed pits and vesicles involved in receptor-mediated endocytosis. Endocytosis plays an important role in different cellular functions ranging from nutrient acquisition to synaptic transmission and antigen presentation (Beattie et al., 2000). The gene coding for the clathrin heavy chain (designated as CLTC) had first been mapped to the chromosome 17 in humans (Dodge et al., 1991). Subsequently, it has been proven that there is a second *locus* coding for the clathrin heavy chain (designated as CLTD) in humans and it is located on chromosome 22 (Sirotkin et al., 1996). The products of these two *loci* share a 93% similarity. CLTD shares an identity of 77% with the *Drosophila* protein.

The role of clathrin in mature human sperm has not been described so far. It has been found, however, that monkey spermatogonia, spermatocytes, spermatids, testicular and epididymal spermatozoa have specific binding sites on the plasma membrane associated with clathrin-like coated pits and vesicles (Gerard et al., 1991). Studies in a *Drosophila* model showed that a mutation in the clathrin heavy chain gene caused sterility in homozygous males (Bazinet et al., 1993).

In this report, we describe the identification and localization of clathrin on human sperm. Its function in sperm and the connection to infertility of the antibodies reactive to clathrin must now be elucidated.

2. Materials and methods

2.1. Case description

A serum sample was obtained from an infertile 34-year-old woman diagnosed with SLE. This serum sample contained a high level of ASA, as determined by means of the indirect immunobead binding test (IDIBT) (Domagala et al., 1998). The ASA were of both the IgG (19%) and IgM class (55%) (Fig. 1). IgM antisperm antibodies recognized antigens mostly localized on the sperm head, as concluded from the topography of immunobead reagent binding and the spontaneous sperm agglutination pattern (head to head) while IgG antibodies in a neat serum sample provoked the tail tip sperm agglutination. This serum sample also contained anti-cardiolipin antibodies of both IgG and IgM class (at 1:64 titer as detected by enzyme-linked immunosorbent assay; ELISA) and anti-nuclear antibodies (ANA-detected on Hep-2 cells using the Colorzyme method at 1:160 titer). Lupus anticoagulant (LE) and activated partial thromboplastin time (APTT) assays were within the normal range. We did not detect ASA in a cervical mucus sample obtained from this woman.

Control ASA-negative sera samples were obtained from healthy, fertile women. These women become pregnant through regular intercourse and conceived healthy children. Sera samples were collected within 1 year of conception.

2.2. Sperm preparation and solubilization of the membrane antigenic fraction

Human semen samples were obtained from healthy, normozoospermic individuals, after 3–4 days of sexual abstinence. Semen analyses were performed according to the WHO manual (World Health Organization, 1999). The University of Virginia Investigation Committee granted permission for written consent to be obtained from all the donors included. The sera samples were negative for HIV.

Preparation of the sperm membrane antigenic fraction was performed by using 2-D Sample Prep for Membrane Proteins (Pierce Biotechnology, Rockford, IL, USA), according to the manufacturer's procedure. The resulting detergent (Triton X-114) phase of sperm membrane proteins was collected and the protein concentration was determined by means of the RCDC Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA).

2.3. Two-dimensional electrophoresis

The procedure of isoelectric focusing (IEF) together with the second dimension has been described in detail elsewhere (Naaby-Hansen et al., 1997). After electrophoresis, gels were either silver stained or transferred to polyvinylidene fluoride (PVDF) membranes for Western blot analysis.

2.4. Cell culture of HeLa cells

HeLa cells were a generous gift from Dr. Danuta Dus (Institute of Immunology and Experimental Therapy, Wrocław, Poland). The cells were grown in Dulbecco's modified Eagle's medium (DMEM, Sigma, Steinheim, Germany), supplemented with 10% (v/v) fetal calf serum (Sigma, St. Louis, MO, USA), 1× concentrated glutamax (Invitrogen Corporation, UK) and 1× concentrated antimycotic–antibiotic solution (Sigma).

2.5. Preparation of sperm and HeLa cells antigenic extracts

Swim-up sperm and HeLa cells (30×10^6/ml) were washed twice in PBS and then resuspended in 1 ml of triton X-100/deoxycholate (TDOC) buffer (Snow and Ball, 1992). Extraction of antigenic fractions was performed at 4 °C for 1 h, with constant agitation. A protease inhibitor cocktail (Roche, Mannheim, Germany) was utilized during