Involvement of the inflammasome in abnormal semen quality of men with spinal cord injury

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Objective: To study the mechanism leading to elevated semen cytokines in men with spinal cord injury (SCI) and to understand if inflammasome pathways are involved in this process. To investigate inflammasome components and end-product cytokines in semen of SCI and control subjects.
Design: Prospective study.
Setting: Major university medical center.
Patient(s): Men with and without SCI (n = 28 per group).
Intervention(s): None.
Main Outcome Measure(s): Seminal plasma concentrations of caspase-1, interleukin (IL) 1β, and IL-18 were quantified by ELISA. Caspase-1 in sperm fractions and apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) in seminal plasma and sperm fractions were identified by Western blot. Localization of proteins in sperm was accomplished by immunocytochemistry.
Result(s): ASC, caspase-1, IL-1β, and IL-18 concentrations were elevated in the seminal plasma of SCI subjects compared with control subjects. ASC and caspase-1 were elevated in sperm cells of SCI subjects. Immunocytochemistry revealed that ASC was located in the acrosome, equatorial segment, and midpiece, and caspase-1 in the midpiece.
Conclusion(s): This study provides the first evidence of ASC in human semen and demonstrates the involvement of inflammasome proteins in semen of men with SCI. These findings suggest an immunologic basis for abnormal semen quality in men with SCI. (Fertil Steril® 2013;99:118–24. ©2013 by American Society for Reproductive Medicine.)
Key Words: Inflammasome, caspase-1, sperm, seminal plasma, spinal cord injury

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Every year in the United States, there are ~12,000 new spinal cord injuries (1) with hundreds of thousands more worldwide. In the United States, the mean age at injury is 33.9 years, with 80.7% of these new injuries occurring in men (2). Parenting is of great concern in this age group (3). The majority of these men are infertile owing to erectile dysfunction, ejaculatory dysfunction, and poor semen quality (4–6). Treatments are available for erectile and ejaculatory dysfunction. No treatments are available for poor semen quality in these men (7–9).

The semen of SCI men is characterized by normal sperm concentrations but abnormally low sperm motility and viability (6). Furthermore, these sperm are fragile, as evidenced by faster loss of motility and viability, and higher DNA damage than sperm from noninjured control subjects (10, 11). Because of poor semen quality, advanced assisted reproductive technologies (ART) are often required to assist conception in couples with SCI male partners (12).

In previous studies, we showed that seminal plasma from men with SCI is

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toxic to sperm (13, 14). We hypothesized that this effect was due to elevated semen concentrations of specific inflammatory cytokines (interleukin [IL] 1β, IL-6, and tumor necrosis factor α), the neutralization of which led to improved sperm motility (15, 16). The mechanism leading to elevated semen cytokines in these men is unknown. It is possible that the inflammasome plays a role in elevating semen cytokines in men with SCI.

The inflammasome is a multiprotein complex responsible for activating the innate immune response (17, 18). The most common inflammasomes consist of a nucleotide oligomerization domain–like receptor (NLR), the adaptor protein apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC), and caspase-1. The activation of the inflammasome results in cleavage of procaspase-1 into active caspase-1, which then cleaves pro–IL-1β and pro–IL-18 into their active forms. IL-1β and IL-18 are potent proinflammatory cytokines that play a major role in innate immunity and the body's response to tissue injury, including lymphocyte activation, recruitment of other inflammatory cells and their products and cytokines, and induction of secondary inflammatory cytokines and other cellular products in T cells and natural killer cells (19, 20). The inflammasome complex itself may be activated by a wide variety of causes, such as bacteria, fungi, yeasts, viruses and many of their products, cell wall components, toxins, nucleic acids, foreign compounds, such as asbestos, particles, such as urates, and other things, such as lipopolysaccharides and beta-amyloid.

As a first step toward determining if the inflammasome contributes to elevated cytokines in semen of men with SCI, the present study investigated whether components of the inflammasome were present in the semen of men with SCI as well as in noninjured healthy men as control subjects.

MATERIALS AND METHODS

Subjects

Semen samples from 28 men with SCI and 28 age-matched control subjects were examined in this study. Control subjects were healthy volunteers who were specifically recruited for this study. All control subjects were noninjured normospermic men with no known history of infertility. The mean age (±SEM) of SCI subjects was 35.0 ± 1.6 years (range 21.0–55.0 years) and 31.0 ± 1.6 years (range 20.0–50.0 years) for the control subjects. All SCI subjects were past the period of spinal shock (i.e., ≥12 months after injury). Their level of injury ranged from C4 to T8. All SCI and control subjects were in good general health and were participants in the Male Fertility Research Program of the Miami Project to Cure Paralysis, University of Miami Miller School of Medicine. The study was approved by the University of Miami Institutional Review Board, and informed consent was obtained from each of the subjects.

Semen Collection

Semen was obtained from SCI subjects using the standard methods of penile vibratory stimulation (PVS) or electroejaculation (EEJ) as previously described (7, 21). Only antegrade semen specimens were used in this study. Non-SCI control subjects collected their semen by masturbation. Semen was analyzed for sperm concentration and sperm motility according to World Health Organization criteria (22).

Preparation of Seminal Plasma

Each semen sample was divided into two portions: one for gradient separation of sperm, and the other for recovering seminal plasma. Seminal plasma was obtained by centrifugation of an aliquot of semen at 1,000*g* for 15 minutes at room temperature. The seminal plasma (i.e., the supernatant) was collected and stored at −80°C.

Purification of Sperm with Discontinuous-Gradient Separation

Semen specimens were processed with the use of a discontinuous gradient (Allgrad; Life Global) as follows: 2 mL Allgrad 90% was placed in a 15-mL centrifuge tube then another 2 mL Allgrad 45% was added. The liquefied semen (0.5–2.0 mL) was carefully added to the top of the 45% layer and centrifuged at 300*g* for 20 minutes. The resulting pellet was recovered, washed, and resuspended in 0.5 mL of Allgrad Wash. This fraction was referred to as the pure sperm fraction (F1). The supernatant from gradient was collected, washed with Allgrad wash, centrifuged at 500*g*, and the resulting pellet resuspended in 0.5 mL Allgrad Wash. This fraction was referred to as the mixed cell fraction (F2). An aliquot of the pure sperm fraction (F1) was stained with ortho-toluidine reagents and examined microscopically for leukocytes (23). Specimens containing traces of leukocytes were labeled $F1_{WBC+}$ and those free of leukocytes were labeled $F1_{WBC-}$. Sperm cells from all three fractions ($F1_{WBC+}$, $F1_{WBC-}$, and F2) were centrifuged to a pellet, washed with phosphate-buffered saline solution (PBS), and resuspended in PTN50 lysis buffer (50 mmol/L sodium phosphate buffer, pH 7.4, containing 50 mmol/L NaCl and 1% Triton X-100, 1 mmol/L phenylmethylsulfonyl fluoride, and 1% protease inhibitor cocktail; Sigma-Aldrich). Specimens were then subjected to freezing and thawing twice in dry ice. Protein was recovered by centrifugation at 5,500*g* for 5 minutes; protein concentration was determined with the use of bicinchoninic acid protein assay reagents (Thermo Scientific).

Western Blot Analysis

Seminal plasma samples were retrieved from storage at −80°C and thawed at room temperature. Seminal plasma (1 μL) from each subject was mixed with loading buffer (to a final concentration of 50 mmol/L Tris-HCl, pH 7.0, containing 2% sodium dodecyl sulfate [SDS], 10% glycerol, 5% β-mercaptoethanol, and 0.002% bromphenol blue), heated at 95°C for 10 minutes, and subjected to SDS-polyacrylamide gel electrophoresis (Mini-protein GTX 4%–20%; Biorad). An ASC-positive sample was included in each gel to serve as a positive standard. The gel was blotted to an immune-blot polyvinylidene difluoride membrane (Biorad). The membrane was blocked with 0.4% I-Block (Applied Biosystem) in PBS (pH 7.4) containing 0.1% Tween-