# Dendritic cells in semen of infertile men: association with sperm quality and inflammatory status of the epididymis

Yong-Gang Duan, M.D.,[a,b] Qiongli Zhang, M.D.,[b] Yuchen Liu, M.D.,[b] Lisha Mou, Ph.D.,[b] Gang Li, Ph.D.,[a] Yaoting Gui, M.D.,[a] and Zhiming Cai, M.D., Ph.D.[a,b]

[a] Guangdong Key Laboratory of Male Reproduction and Genetics, Shenzhen PKU-HKUST Medical Center, Peking University Shenzhen Hospital; and [b] Center of Reproductive Medicine and Andrology, First Affiliated Hospital of Shenzhen University, Shenzhen Second People's Hospital, Shenzhen, People's Republic of China

**Objective:** To determine whether dendritic cells (DCs) are present in semen and whether their abundance and activation correlate with sperm quality and inflammatory status of the male genital tract.
**Design:** In vitro prospective study.
**Setting:** University hospital laboratory.
**Patient(s):** Eighty infertile men and twelve control fertile men were enrolled in this study.
**Intervention(s):** None.
**Main Outcome Measure(s):** The percentages of DCs, intracellular staining of cytokines, and spermatozoa DNA fragmentation index were assessed by flow cytometry. Seminal cytokines, neutral $\alpha$-glucosidase, zinc, and fructose were measured with commercial kits.
**Result(s):** A significant number of $CD11c^{+}$ $HLA\text{-}DR^{+}$ DCs were detected in ejaculates from patients with chronic inflammation of genital tract, which was negatively correlated with spermatozoa motility, vitality, and DNA integrity. Intracellular staining of cytokines of seminal DCs showed enhanced ability to secrete inflammatory cytokines (interleukin [IL] 23p19, tumor necrosis factor [TNF]–related apoptosis-inducing ligand [TRAIL], and TNF-$\alpha$). Furthermore, a significant correlation was found between DCs and the seminal concentrations of IL-6, IL-17, IL-23, TRAIL, and neutral $\alpha$-glucosidase, the marker of epididymal function, in the inflammatory group but not in the noninflammatory and fertile groups.
**Conclusion(s):** The abundance and activation of seminal DCs of infertile men may be closely associated with poor epididymal function and sperm quality. (Fertil Steril® 2014;101:70–7. ©2014 by American Society for Reproductive Medicine.)
**Key Words:** Dendritic cells, spermatozoa, epididymis, inflammatory cytokines, male infertility

**Discuss:** You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/duanyg-dendritic-cells-semen-male-infertility-sperm/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Infection and inflammation of male genital tract are considered to be significant etiologic factors in male infertility. However, it is difficult to diagnose subacute or chronic inflammatory reaction in the male reproductive tract, owing to its mostly asymptomatic course and unspecific diagnostic criteria (1–3). Recent studies have demonstrated that induction of testicular inflammation is associated with a $T_H17$-cell–mediated autoimmune response, i.e., disruption of the immune privilege and spermatogenesis (4, 5). In this context, it is most likely that antigen-presenting cells (APCs; such as macrophages, dendritic cells) orchestrate $T_H17$-cell immune response in the inflamed testis, because APCs are capable of not only capturing and processing antigens but also inducing antigen-specific immune response (6–8). Moreover, elevated levels of several cytokines in semen

Received June 25, 2013; revised August 18, 2013; accepted September 4, 2013; published online September 29, 2013.
Y.-G.D. has nothing to disclose. Q.Z. has nothing to disclose. Y.L. has nothing to disclose. L.M. has nothing to disclose. G.L. has nothing to disclose. Y.G. has nothing to disclose. Z.C. has nothing to disclose.
Supported by the Emerging Scientist Project of Shenzhen Second People's Hospital (no. 2012004), Science and Technology Project of Shenzhen (no. 201202023), Medical Research Fund of Guangdong Province (no. B2012319), and Scientific Research Foundation for the Returned Overseas Chinese Scholars (no. 2013693).
Reprint requests: Zhiming Cai, M.D., Ph.D., Center of Reproductive Medicine and Andrology, First Affiliated Hospital of Shenzhen University, Shenzhen Second People's Hospital, 518035 Shenzhen, People's Republic of China (E-mail: caizhiming2000@163.com).

Fertility and Sterility® Vol. 101, No. 1, January 2014 0015-0282/$36.00
Copyright ©2014 American Society for Reproductive Medicine, Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.fertnstert.2013.09.006

from men with genital infections suggest that they could be involved in immune defense of male genital tract. On the other hand, inflammatory responses have been reported to impair semen quality and sperm function (9, 10).

Seminal leucocytes represent only a small portion of the total numbers of cells in ejaculates from fertile men, and low numbers of leucocytes may play a beneficial role by removing abnormal and degenerative spermatozoa (11–13). Polymorphonuclear (PMN) granulocytes account for 50%–60% of seminal leucocytes, macrophages for 20%–30%, and T lymphocytes for 5% (14, 15). However, the presence of dendritic cells (DCs) and their immunologic properties in semen remain unknown. DCs are a heterogeneous population of APCs that are key regulators of immune response and immunologic tolerance in numerous organs (16–19). DCs migrate as immature cells that are potent phagocytes capable of capturing and processing antigens from the bone marrow into peripheral tissue. On receiving an activated signal associated with pathogens or inflammation, DCs up-regulate major histocompatibility complex (MHC) class II/human leukocyte antigen–D-related (HLA-DR) molecules and costimulatory molecules. After activation, DCs produce cytokines and inflammatory mediators, such as tumor necrosis factor (TNF) $\alpha$, interleukin (IL) 20, IL-23, and TNF-related apoptosis-inducing ligand (TRAIL) (20, 21). In addition, these inflammatory DCs induce allogeneic T cell proliferation and polarize T cells toward $T_H17$ and $T_H1$,2 types of T lymphocytes implicated in many diseases (4, 19, 22).

Given the pivotal role of DCs in innate and adaptive immunity and of their maturation in triggering inflammatory process, the aim of the present study was to determine whether DCs are present in semen and whether their abundance and activation correlate with sperm quality and the inflammatory status of the male genital tract.

## MATERIALS AND METHODS

### Subject Recruitment

Infertile patients who had a history of $\geq 1$ year of failed attempts to achieve a pregnancy after regular sexual activity were recruited in this study. Patients showing seminal IL-6 $>30$ pg/mL and/or peroxidase positive cell numbers $>1.0 \times 10^6$/mL in semen were considered to have chronic inflammation of the genital tract (CIGT) (23, 24). Fresh semen samples were collected from the infertile patients presenting to the Center of Reproductive Medicine and Andrology for routine semen analysis ($n = 80$). These patients were divided into two groups: infertile men with CIGT (inflammatory group; $n = 32$) and infertile men without CIGT (noninflammatory group; $n = 48$). Fertile men had past proven fertility with normal semen parameters according to World Health Organization (WHO) criteria were recruited in this study as a fertile group ($n = 12$).

All samples were collected after getting informed consent from the patient. This study received Institutional Review Board approval from Shenzhen PKU-HKUST Medical Center Research Ethics Committee and Shenzhen Second People's Hospital Research Ethics Committee, and the clinical investigation was performed according to the principles expressed in the Declaration of Helsinki. Ejaculate samples were analyzed for standard semen parameters according to the WHO criteria (24). The hypo-osmotic swelling test was used to assess spermatozoa vitality according to WHO criteria, and the lower reference limit for vitality (membrane-intact spermatozoa) is 58% (24).

### Immunofluorescence

The experiment was performed in a standard manner as previously described (4, 19). Rabbit anti–human monoclonal antibody (mAb) CD11c (dilution 1:100, EP1347Y; Lifespan Biosciences) and mouse anti–human mAb HLA-DR (dilution 1:100, LN-3; Lifespan Biosciences) were used as the primary antibodies for immunofluorescence of double staining.

### Detection of DCs in Semen by Flow Cytometry

For flow cytometry, ejaculate was washed and centrifuged in phosphate-buffered saline solution (PBS). The supernate was discarded and the pellet suspended in PBS. Ejaculate measuring 100 $\mu$L was incubated with phycoerythrin (PE)–Cy5–conjugated mouse anti–human HLA-DR (Mouse $IgG_{2b}$; eBioscience) and PE-conjugated mouse anti–human CD11c (Mouse $IgG_1$; eBioscience) for 30 minutes at 4°C, and the leucocytes were gated by HLA-$DR^+$ cells. To detect the phenotype of DCs in semen, costimulatory molecules CD80 (Mouse $IgG_1$; eBioscience) and CD86 (Mouse $IgG_{2b}$; eBiosicence), as well as CCR6 (Mouse $IgG_1$; BD Pharmingen), CCR7 (Mouse $IgG_{2a}$; eBioscience), CD14 (Mouse $IgG_{2a}$; BD Pharmingen), and CD83 (Mouse $IgG_1$; eBioscience), were used in this study. Serving as isotype control samples, 100 $\mu$L ejaculate was incubated with PE-Cy5–conjugated mAb $IgG_1$, $IgG_{2b}$, fluorescein isothiocyanate (FITC)–conjugated mAb IgG2a, and/or PE-conjugated mAb $IgG_1$ (eBioscience) for 30 minutes at 4°C. After the incubation time, samples were washed in PBS and analyzed with the use of a FACScan flow cytometer (Epics XL 4CLR flow cytometer; Beckman Coulter). Acquired data were analyzed with the use of Flowjo software (Tree Star).

### Intracellular Staining of Cytokines of CD11c$^+$ HLA-DR$^+$ DCs in Semen

HLA-DR$^+$ cells were enriched from the semen samples of patients suffering from CIGT by positive selection with the use of mouse anti-human HLA-DR antibody conjugated with magnetic beads according to the manufacture's protocol (130-046-101; Miltenyi Biotech). For intracytoplasmic staining of IL-23p19 (Mouse $IgG_{2b}$; eBiosicence), TRAIL (Mouse $IgG_1$; BD Pharmingen), and TNF-$\alpha$ (Mouse $IgG_1$; BD Pharmingen), enriched HLA-DR$^+$ cells were cultured with brefeldin A (10 $\mu$g/mL; Sigma) to inhibit cytokine release as previously described (19). After 12 hours of culture, cells were harvested, stained for DC marker CD11c PE-Cy5 and TRAIL PE, fixed, and permeabilized with BD Cytofix/Cytosperm Kit (cat. no. 554714; BD Biosciences). Subsequently, the cells were stained with IL-23p19 PE and TNF-$\alpha$ FITC and analyzed with the use of a FACScan flow cytometer.

Download English Version:

https://daneshyari.com/en/article/3938407

Download Persian Version:

https://daneshyari.com/article/3938407

Daneshyari.com