



Case Report: Dendritic Cells and Macrophages Capture Sperm in Chronically Inflamed Human Epididymis

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Chronic inflammation of the male genital tract is thought to be a primary etiological factor of male infertility. The abundance and activation of macrophages and dendritic cells in patients with chronic inflammation of genital tract were closely associated with oligozoospermia and asthenospermia. Chronic epididymitis appears to be more important than seminal vesiculitis or prostatitis due to the direct interaction between spermatozoa and epididymal inflammatory cells. In this study, we present a case report of a 41-year-old male with oligoasthenospermia and chronic epididymitis. Hematoxylin-eosin staining and immunofluorescence analyses showed that antigen presenting cells including macrophages and dendritic cells were found capturing spermatozoa in the lumen of cauda epididymis. To our knowledge, this is the first case report that directly observed dendritic cells capturing spermatozoa in the lumen of an inflamed epididymis. This finding directly explains chronic epididymitis as the possible cause of oligospermia in patients.

Keywords: chronic inflammation of genital tract (CIGT), oligozoospermia and asthenospermia, chronic epididymitis, macrophages, dendritic cells

INTRODUCTION

Infertility affects approximately 15% of couples, and male factor infertility account for approximately 20~50% of all infertile patients (1, 2). Teratozoospermia, azoospermia, oligozoospermia, and asthenospermia are the main causes of male infertility, accounting for 20~25% of male cases (3). Inflammation of the male genital tract is thought to be a primary etiological factor of oligozoospermia and asthenospermia (4–6). Chronic epididymitis appears to be more important than seminal vesiculitis or prostatitis due to the direct interaction between spermatozoa and epididymal inflammatory cells (7–9).

Normal epididymis environment contains an intricate network of antigen presenting cells including residual dendritic cells and macrophages (10). The identification and removal of abnormal spermatozoa or the possible presence of sperm “mass control” mechanisms is one of the most interesting field of research for epididymal physiology (11, 12). Some earlier studies have presented evidence that the obstruction of genital tract was associated with spermatophagy in human

and monkey epididymis (13). Although the “spermatophagy” phenomenon has been previously reported (13), there had been no convictive evidence presented, until recently, that dendritic cells and macrophages play an important role in the identification and removal of defective sperms in normal epididymis. Recent studies showed that the abundance and activation of dendritic cells in patients with chronic inflamed epididymitis were closely associated with oligozoospermia and asthenospermia (14, 15). However, evidence of dendritic cells and macrophages participation in sperm damage in epididymitis has not been presented in chronically inflamed human epididymis.

CASE PRESENTATION

Here we present a case report of a 41-year-old male with oligoasthenospermia and chronic epididymitis. This male patient is a driver and visited the outpatient department presenting with the complaints of bilateral testicular burning pain for 9 months. Scrotal color Doppler ultrasound showed chronic bilateral inflammatory changes of bilateral epididymis and he was primary diagnosed with chronic bilateral epididymitis. In addition, the patient was diagnosed with oligozoospermia and asthenospermia based on semen samples according to the WHO criteria (WHO laboratory manual for the examination and processing of human semen—5th edition). The parameters of semen were evaluated by a computer-aided sperm analysis (CASA) (NatureGene Corp, Sperm Tracker, USA) and was presented in **Table 1**. This man did not report any disorders with penile erections, orgasm, or ejaculation and he was not taking any medication for any systemic diseases including diabetes mellitus, hypertension, etc. Physical examination showed bilateral testes were in normal position and tender upon palpation. The rest of the physical examination was normal. The patient was referred to many hospitals for conservative treatment including general treatment, medication, physical therapy, and Traditional Chinese Medicine treatment, and the symptoms of pain was not effectively relieved and in fact worsened, which strongly affected his sleep, diet, mental state, etc. The patient already has two children and are willing to receive epididymectomy. Therefore, bilateral epididymectomy was performed at the Urology Center of our hospital on September 8, 2016. Excised epididymal tissue samples were immediately fixed in Bouin’s solution and embedded in paraffin for Hematoxylin-

eosin staining and immunofluorescence analyses (16). Hematoxylin-eosin staining was carried out following standard protocols. Briefly, paraffin-embedded epididymal biopsies (4 μ m sections) were dewaxed (dimethylbenzene), hydrated (100, 90, 80, 75, and 70% alcohol gradient) and stained in hematoxylin solution for 3 min. The slice was then decolorized in acid alcohol solution (0.4% HCL in EtOH) for 10 s in hydrochloric acid ethanol differentiation buffer solution, washed in phosphate-buffered saline (PBS, pH 7.45), and stained with eosin solution for 2 min. After sealing with neutral resin seal, the slides were examined under a digital microscope (Olympus BX 53 microscope, Tokyo, Japan). Interestingly, we observed large numbers of spermatozoa-attached cells in the lumen of inflamed epididymis which indicated that those spermatozoa were directly captured and degraded by infiltrating cells during the process of chronic epididymitis (**Figures 1A–C, Supplementary Figure 1**). In addition, we further analyzed the immune phenotype of infiltrating cells in the lumen of inflamed epididymis by performing immunofluorescence analyses. Immunofluorescence staining was carried out by the following steps. Specifically, the prepared paraffin-embedded biopsies were fully immersed in xylene (I) for 10 min, xylene (II) for 10 min, anhydrous ethanol (I) for 5 min, anhydrous ethanol (II) for 5 min, 95% alcohol for 5 min, 90% alcohol for 5 min, 80% alcohol for 5 min, 70% alcohol for 5 min, and then soaked in distilled water for 1 min. Antigen retrieval was carried out with Tris-EDTA (pH 9.0) buffer solution for 30 min in a temperature control water bath. The sections were placed in 0.3% hydrogen peroxide solution for 15 min to eliminate the influence of endogenous peroxidase. Then 5% goat serum was added and the tissues were incubated at room temperature for 1 h to block non-specific sites. The primary antibody of antigen presenting cells associated markers including CD68 (66231-1-Ig, IgG1; ProteinTech, Wuhan, CHN) and CD11c (60258-1-Ig, IgG2a; ProteinTech, Wuhan, CHN) was added and then stored overnight at 4°C environment. Incubate the tissue for 1 h with the secondary antibody in the dark at room. The tissues were incubated with DAPI for 15 min. The sections were mounted with anti-fluorescence quencher, and were visualized under a confocal microscope (ZEISS LSM 880 confocal microscope, Jena, Germany). Immunofluorescence staining results showed that both the CD11c+ dendritic cells and CD68+ macrophages were found capturing spermatozoa in the lumen of the inflamed epididymis (**Figures 2A, B**). In this case report study, the negative controls were carried out by substituting the primary antibodies with its

TABLE 1 | The parameters of semen.

Volume	PH	Liquefaction time	Color	Sperm concentration	WBC	Round cells	Neutral α -glucosidase
2.0 ml	7.45	50	Gray white	2.5×10^3 /ml	3.0×10^6 /ml	9.0×10^6 /ml	11.94 IU/per ejaculation
Total activity	Fast forward motion	Survival rate (live sperm)	Teratogenesis rate	Fructose	Acid phosphatase	Zn ²⁺	MAR test
0%	0%	0%	100%	6.04 g/per ejaculation	396.6 U/per ejaculation	3.8 mmol/per ejaculation	5%

WBC in semen were identified by toluidine blue oxidase staining in this study. MAR, represent mixed antiglobulin reaction; WBC, represent white blood cell; a, represent fast forward movement; b, represent slow forward motion; c, represent non-forward motion; d, represent not moving sperm.

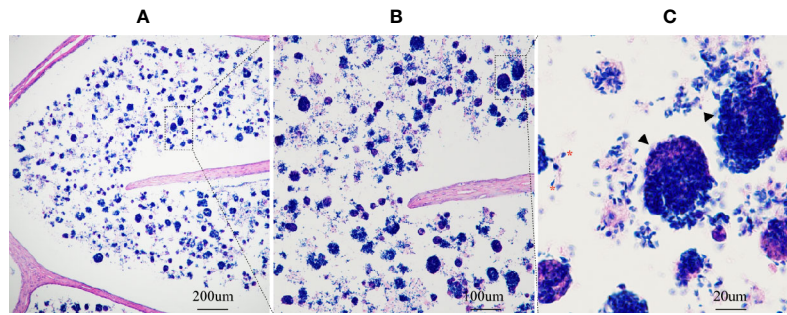


FIGURE 1 | Sperm-attached cells in the lumen of inflamed epididymis. Magnification: $\times 10$ (A), $\times 40$ (B), and $\times 100$ (C); * represent the spermatozoa; Triangle represent the infiltrating cells.

corresponding isotype control including IgG or IgG1, respectively, during the immunofluorescence staining process (data not shown).

DISCUSSION

Current studies on male infertility mostly focuses on the spermatogenic origins, whereas researches on the role of the rest of the male genital tract remains relatively sparse. Epididymis is a male-specific organ where sperms gain the ability to fertilize an oocyte in order to development an embryo (17). In addition, both testis and epididymis are considered as peripheral immune tolerant to the immunologically foreign spermatogenic cells and spermatozoa (18). Importantly, the incidence of immune tolerance shift in the epididymis micro-environment is more prevalent than the testicular micro-environment, as the elaborate blood-epididymis barrier appears to be more vulnerable compared to the blood-testis barrier (19). Under a certain condition, the blood-epididymis barrier and the residual immune cells of human epididymis can cooperate in an intricate network to prevent immunologically antigens from entering the peripheral circulatory system and initiating a pathological immune defense against spermatozoa (20).

Immunological balance shift in the vas deferens, epididymis, and testis can have adverse effects on spermatozoa and inhibits male reproduction (21). Dysfunction of immune tolerance to sperm in human epididymis leads to the development of sperm-associated autoantibodies and is a considerable problem, resulting in oligozoospermia and asthenospermia which account for 5~10% of male infertility cases (22). Professional antigen-presenting cells including dendritic cells and macrophages are a versatile manager between innate and adaptive immunity system, which promoting initiate specific defense mechanisms by recruiting antigen-specific T lymphocytes (23). An intricate network of dendritic cells and macrophages was detected in mouse epididymis and are especially intensive in the caput region, indicating that these cells are particularly active in the caput of epididymis (24). Our previous study showed that dendritic cells with a potentially tolerogenic phenotype is especially numerous in the caput region of human epididymis (15). It is possible that these dendritic cells and

macrophages in caput epididymis play a role in preventing sperm autoimmunity (25). Overall, the number and structural prominence of dendritic cells and macrophages in epididymis are much greater in the caput region than either in the cauda or corpus, and this needs to be considered in conjunction with the quantitatively different responses of the caput and corpus (or cauda) to immunological challenges such as chronic epididymitis.

An earlier study has showed that the macrophages in the obstruction genital tract was associated with spermatophagy in epididymis (13). Although the “spermatophagy” phenomenon has been previously reported, there had been no convictive evidence presented, until recently, that dendritic cells and macrophages are associate with spermatophagy in chronic epididymitis. In this case report, we directly observed large numbers of spermatozoa-attached cells in the caput region of chronic epididymitis samples which indicated that during the process of chronic epididymitis sperm might be directly captured and degraded by infiltrating immune cells. In addition, our further immunofluorescence analysis showed that both the CD11c+ dendritic cells and CD68+ macrophages were found capturing spermatozoa in the lumen of epididymis which might explain the cause of oligospermia in patients with chronic epididymitis. Chronic inflammation continues to activate dendritic cells and macrophages, producing cytokines that damage the epididymal epithelium, causing dendritic cells and macrophages to infiltrate the epididymal lumen, misrecognizing normal sperm, resulting in sperm capture and immune damage, which could be the possible mechanisms of phagocytosis of sperm cells under inflammatory.

A previous study identified a functional population of mononuclear phagocytes in the epididymal epithelium of mice responsible for maintaining the integrity of the blood-epididymis barrier (26), which has some similarities with our report: 1. It was observed that epididymal immune cells including macrophages and dendritic cells could clear sperms by morphological experiment; 2. There are a certain number of macrophages and dendritic cells in epididymal epithelium, and they have extensive signal communication with surrounding cells. On the other hand, our report is also very different from it: 1. Our research is human-based (chronic epididymitis patients), and theirs are mice-based; 2. Their study emphasizes the macrophages and dendritic cells

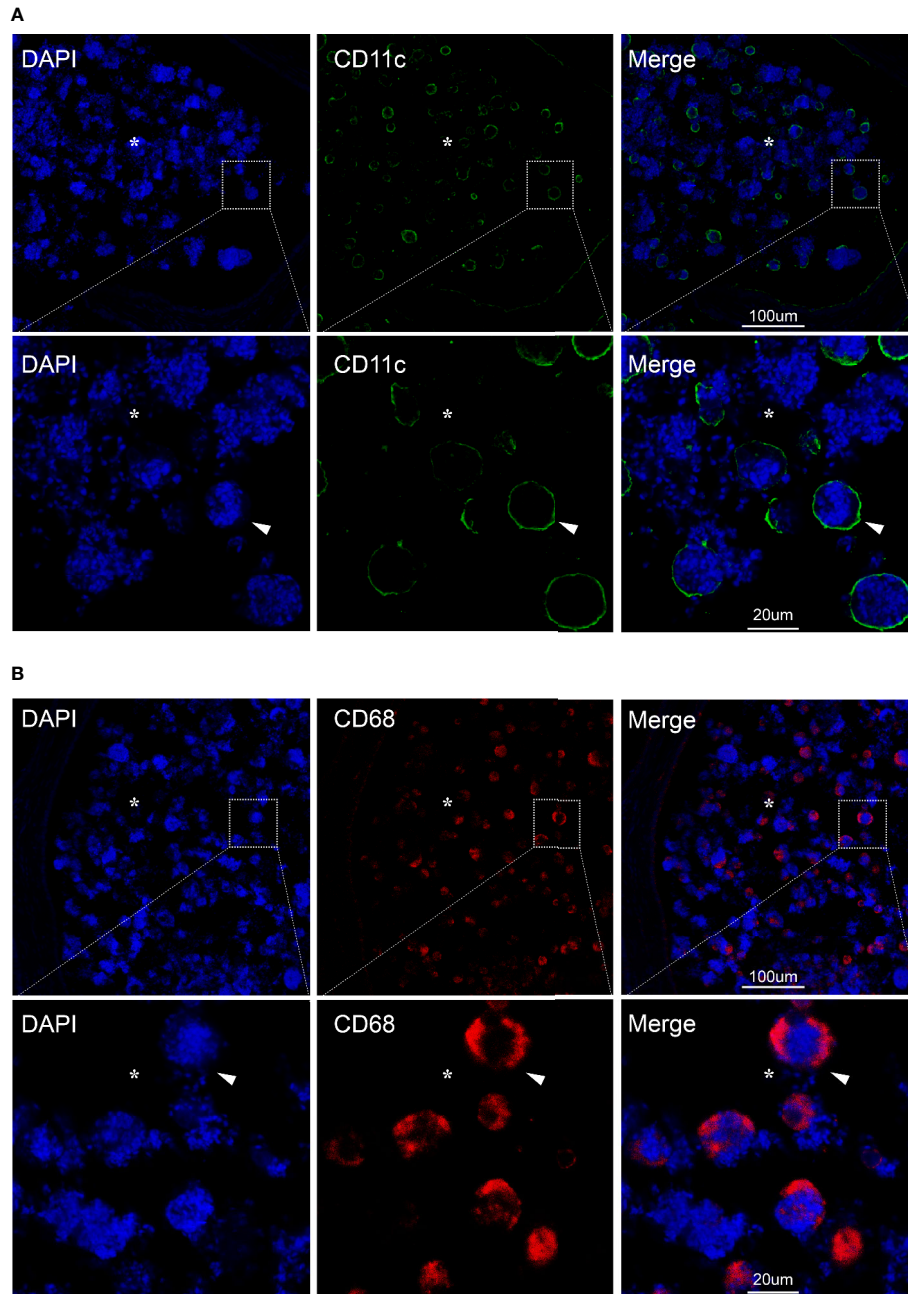


FIGURE 2 | CD11c+ dendritic cells and CD68+ macrophages in the lumen of chronic inflamed epididymis. Immunofluorescence staining of CD11c+ dendritic cells (A) and CD68+ macrophages in the lumen of human epididymis (B); Nuclei were labeled with DAPI; CY3, red fluorescence; FITC, green fluorescence; Arrows represent the positive cells; *represent the lumen of epididymis; Bar = 100 µm (or 20 µm); Original magnification: ×100; further magnification: ×400.

clearance of damaged cells in normal conditions, while our study emphasizes the destruction of normal sperm by macrophages and dendritic cells in under the condition of abnormal activation of inflammation. 3. Their study emphasizes the role of immune cells and peripheral cells in epididymal epithelium of normal mice, while our study emphasizes abnormally activated immune cells penetrating the blood-epididymis barrier and directly causing immune damage to sperm in the lumen of epididymis.

Our report has some limitations. First, to confirm spermatozoa engulfment, electron microscope analysis would represent the gold standard, but our case was unable to fulfill it due to availability reasons. Our study is a retrospective case report study, we are unable to complement the electron microscope specimen, and the patients with chronic epididymitis undergoing epididymectomy are very rare in clinic, the specimen is invaluable. From this report, we observed that a large number of spermatozoa adhered to the

cluster like structure in the epididymal lumen by hematoxylin-eosin staining, which indicated that spermatozoa might be captured by phagocytic immune cells. Immunofluorescence staining can be used to identify the types of immune cells that phagocytize the sperm. In addition, there is a big gap between the size of sperm and immune cells, which also helps us to distinguish them. Second, we could not ascertain inflammation to be the sole cause of oligoasthenospermia, because impairment of spermatogenesis by chronic inflammation, endocrine abnormalities, and genetic variation could also be the cause of oligoasthenospermia.

In summary, we described the first reported case that both dendritic cells and macrophages were found capturing spermatozoa in the lumen of cauda epididymis in an oligoasthenospermia man with chronic epididymitis. However, since impairment of spermatogenesis by chronic inflammation, endocrine abnormalities or genetic variation could not be ruled out, the precise process and molecular mechanisms of macrophage and dendritic cells associated spermatophagy in chronic epididymitis remains to be elucidated in future study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Union Hospital of Fujian Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual

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for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ML drafted and edited the manuscript, and critically discussed the study. WZ drafted and edited the manuscript, and evaluated the immunological data. SZ drafted and edited the manuscript. SJ and ZL performed the hematoxylin-eosin staining and immunofluorescence analyses. XC prepared the epididymal tissue paraffin specimen. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2021.629680/full#supplementary-material>

Supplementary Figure 1 | Immune cells infiltrate in the interstitial of the epididymis.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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