Leukophages in Canine Semen

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Introduction

A pure breed male champion dog was referred with complaints of being unable to sire offspring. There had been no obvious signs of a disease process, other than unilateral cryptorchism, to explain the apparent infertility. Spermatological examination revealed oligozo- and teratozoospermia, accompanied by a striking incidence of non-spermatocytic cells, namely leukocytes and large mononuclear cells. The latter phagocytized neutrophilic granulocytes, but were also attacked by them. The properties and possible origin of the large cells acting as leukophages are the subject of this report.

Materials and Methods

A 2 1/2 year old Cavalier King Charles Spaniel was given a thorough clinical examination, including a complete blood count, blood chemistry and urine analysis, including parasitologic and microbiologic culture. Catheterized urine and prostatic lavage were used for cell analysis and culture for mycoplasma.

Semen examination: The semen samples were obtained by manual stimulation. Sperm count and motility were recorded using standard methods. Spermatocytes and non-spermatocytic cells were examined in wet mounts of fresh and intact ejaculates, using phase contrast microscopy, and on stained preparations (supravital eosin-anilin blue and Feulgen methods).

Cytochemical and electron microscopical examination: Cell samples, collected by prostatic lavage, 2, 4 and 90 days after castration, were used for cytochemistry and electron microscopy. The cytochemical reaction for non-specific esterase activity was done on smears of unfixed cells according to Pearse (1972). Cell samples for EM were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 1% OsO₄ in 0.1 M cacodylate buffer, dehydrated and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate, examined and photographed under a HU-12 Hitachi electron microscope.

Histological examination: Testicular and epididymal tissue samples from the castration were processed and 5 μ thick paraffine sections stained with hematoxylin and PAS.

Results

On clinical examination only the right testis was palpable, being about normal size and consistency. The very small left testis was recovered from the inguinal canal during castration. The prostate appeared normal and there was no abdominal pain. The values of the laboratory tests were within normal range. Urine analysis of catheterized samples showed

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occasional epithelial cells and leukocytes. Microbiological tests recorded very few colonies of staph. aureus, considered to be contaminants. Mycoplasma and B. canis cultures were negative.

Spermiological findings: The volume of ejaculates varied between 1 to 1.5 ml. Sperm density was low and motility was less than 10%. The vast majority of sperm had primary morphological changes (Fig. 1). Sperm cells were broken at the neck and decapitated, and tails were coiled and bent. Often defective sperm occurred in bundles, embedded in a cytoplasmic mass. Primary spermatocytes were observed occasionally. Cellular debris and degenerating spermatids were common. More prominent were lymphocytes, neutrophilic granulocytes and a population of much larger mononuclear cells. Neutrophils actively phagocytized sperm, and, often, confronted and, eventually, destroyed large mononuclear cells (Figs. 2 and 7 to 10), some of which contained one or more neutrophils (Figs. 6 and 7).

Large mononuclear cells: These cells, examined and photographed alive in original seminal plasm, ranged from 25 to 50 μ. They had a large euchromatic nucleus and their cytoplasm contained variable number of vacuoles and granules (Figs. 1 and 2). The cell surface had many thin processes, resembling a microvillous border (Figs. 4 and 5), or very long filamemtous processes resembling hairy cells (Fig. 3). The microvillous border seemed to swell and transform into a hyaline ectoplasm, which in turn could expand in blebs, pseudopodia or undulating membranes (Figs. 4 and 5). Sometimes the large cells occurred in aggregates resembling epithelial formations (Fig. 6). The cells varied in size, shape, inner structure and cytochemical activity of non-specific esterases, which may indicate that the cells did not represent a single cellular species. Some of these cells were seen to engulf cell debris and neutrophils (Figs. 1 and 5), while others contained one or several neutrophils (Figs. 6 and 7 to 10), but, surprisingly, no sperm were engulfed by these cells, hence they were referred to as leukophages.

Ejaculates, obtained 2 and 4 days after castration, were practically free of sperm and their precursors, but still contained neutrophils, lymphocytes and large mononuclear cells. Similar cell populations were obtained by prostatic lavages, taken at day 2, 4 and 90 after castration.

Electron microscopy of cells from the prostatic lavage revealed, in addition to lymphocytes and neutrophils, three types of mononuclear cells, i.e., monocytes, macrophages (Fig. 11) and epithelial cells (Fig. 12). The epithelial cells were identified as urotheliocytes. The largest urotheliocytes seemed to be derived from the superficial layer, while the medium sized ones, were more pleomorphic, pale and dark and came from the middle layer of urothelium. They showed all ultrastructural features distinctive of urothelium, vizs., numerous delicate processes, fusiform vesicles, telolysosomes, tonofilaments and fibrogranular bodies in the nuclei (Tannenbaum - 1979).

No histological evidence of inflammatory changes were found in testes and epididymides. The epididymal lumen contained many defective sperm, exfoliated spermiogenic cells and cellular debris, but no leukocytes or macrophages.

Discussion

That neutrophils can phagocytize sperm has already been reported by Stiasny (1944), Riedel and Schirren (1978) and Riedel (1980) in human semen. The spermiophagy also occurs in semen of domestic species, and is believed to be associated with increased incidence of defective sperm. In this dog neutrophils were present in ejaculates before and after castration and were found in prostatic lavages on day 2, 4 and 90 after castration. They engulfed intact and defective spermatozoa with equal frequency. Histological ex-
Figures 1 to 10 are phase contrast micrographs of cells from fresh intact ejaculate observed in wet mount at room temperature within 60 minutes of ejaculation. Magnification from X 1,000 to X 1,200.

Fig. 1: Spermatozoa, most of them with head and tail defects. A large mononuclear cell in apparent contact with cellular debris and expanding hyalinic ectoplasm.

Fig. 2: A large mononuclear cell “under attack” by more than half a dozen neutrophils. Note that some of the neutrophils seem to exert pressure and cause a depression in the ectoplasm.
Figures 1 to 10 are phase contrast micrographs of cells from fresh intact ejaculate observed in wet mount at room temperature within 60 minutes of ejaculation. Magnification from X 1,000 to X 1,200.

Fig. 3: A large mononuclear cell showing an elaborate system of long filamentous processes on its surface, some exceeding the diameter of the cell (upper right corner).

Fig. 4: A large mononuclear cell with microvilli, many in the process of bulbous expansion or swelling, which most likely leads to hyaline ectoplasm.

Fig. 5: A large cell with microvillous border and periodic hemispherical bulges (arrowheads). It has two large pseudopodial expansions, which seem to embrace a degenerated cell (neutrophil?).

Fig. 6: Two large cells (leukophages) in an epithelioid arrangement. The lower cell contains two neutrophils (asterisks), the upper cell a large vacuole.
Figures 1 to 10 are phase contrast micrographs of cells from fresh intact ejaculate observed in wet mount at room temperature within 60 minutes of ejaculation. Magnification from X 1,000 to X 1,200.

Figs. 7 to 10: A series of micrographs taken at room temperature in a sequence of about 15 minute intervals. It depicts a leukophaghe (neutrophil is labelled by asterisk), with an initially narrow and smooth ectoplasm and its reaction and ultimate fate (degeneration) in a concerted attack by neutrophils. One of the neutrophils contains engulfed sperm (arrowhead).

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Fig. 11: Electronograph of a macrophage containing an ingested unidentified cell. X 11,050.

Fig. 12: Electronograph of an urotheliocyte, distinctive by its fine cytoplasmic processes, vesicles, telolysosomes (arrows). There is an abundance of filaments in the cytoplasm and fibrogranular bodies in the nucleus (arrowhead). X 10,100.

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amination of the testes and epididymides indicated that neutrophils and other leukocytes had to enter the seminal duct distal to cauda epididymidis, most likely in pelvic urethra. This site is inferred from simultaneous occurrence of urotheliocytes. If so, sperm would be exposed to neutrophils at the time of or after ejaculation only. In fact, the spermiophagy was observed in progress, and spermiophages were more frequent as the time interval after ejaculation increased. Therefore we conclude that neutrophils and other leukocytes derive from a chronic inflammatory lesion in the urethra or prostate and had no causal relationship with defective or normal sperm. The teratozoospermia and the inflammatory lesion may be coincidental.

It was difficult to distinguish between urotheliocytes and macrophages in wet mounts, but not so in smears where macrophages were identified by their marker enzyme, i.e., nonspecific esterase. While it would seem logical to identify leukophages as macrophages, the possibility that they might be urotheliocytes can not be ignored, especially in light of several reports that epithelial cells of seminal ducts are capable of phagocytosis (Roussel et al. - 1967; Dym - 1976; Cooper and Hamilton - 1977; Riva et al. - 1981). There have been no reports of interaction between urotheliocytes, leukocytes or sperm, but macrophages, mono- or multinuclear, have been repeatedly associated with spermiophagy in seminal ducts (Gdovin et al. - 1956; Phadke and Phadke - 1961; Phadke - 1975; Holstein - 1978; Allen - 1981; Hughes et al. - 1981). What remains to be established is whether they enter the lumen through an intact or broken epithelial barrier.

Riedel (1978) was the first to observe leukophages in human semen. Riedel's leukophages, believed to derive from monocytes, phagocytized leukocytes and spermatozoa. The leukophages, described in this paper, are related to transformed macrophages. Their larger than standard size, variability in surface modification and, occasional epithelioid appearance, are explained by a transformation, which is known to take place in chronic inflammatory reactions (Bloom and Fawcett - 1975). Furthermore macrophages in tissue culture conditions are known to phagodytize effete or dying granulocytes (Bloom and Fawcett - 1975). Our observations indicate that neutrophils may counterattack.

Summary

Spermiophages and leukophages were found in teratozoospermic canine semen. Spermiophagy was the role of neutrophilic granulocytes. Leukophages are believed to be oversize macrophages transformed by and released from a chronic inflammatory lesion in the pelvic urethra or the prostate. The term leukophages was adopted to indicate that the cells phagocytize leukocytes, specifically neutrophilic granulocytes. Direct observations on living cells revealed a surprising interaction between these two cell types. It is inferred that neither of the phagocytic cells are related to the disturbed spermiogenesis nor the teratozoospermia.

Leukophagen im Hundesperma

Zusammenfassung

Im Hundesperma wurden bei Teratozoospermie Spermatophagen und Leukophagen nachgewiesen. Die Rolle der Spermatophagen wurde dabei von neutrophilen Granulozyten übernommen. Leukophagen sind wahrscheinlich übergröße Makrophagen, die im Rahmen einer chronischen Entzündung der im Becken verlaufenden Urethra oder der Prostata transformiert und ins Lumen abgegeben werden. Der Ausdruck Leukophage wurde ge-
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wählt, um zu zeigen, daß diese Zellen Leukozyten phagozytieren, vor allem neutrophile Granulozyten. Eine direkte Beobachtung lebender Zellen enthüllt ein überraschendes Wechselspiel zwischen diesen beiden Zellformen. Es wird festgestellt, daß keine der phagozytierenden Zellen zur gestörten Spermatogenese oder zur Teratozoospermie in Beziehung stehen.

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References


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