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Location, morphology and function of nephrocytes in termites

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ABSTRACT

Insect nephrocytes are cells bathed in hemolymph and considered to have an excretory function. These cells have ambiguous nomenclature and are understudied in termites. This study is the first report on the occurrence, morphology and function of nephrocytes in different termite castes. Cytological characteristics in specific developmental stages and castes enable physiological functions to be inferred. Perforate diaphragms indicate a role in filtration, while the extensive peripheral invaginations of the cell membrane suggest active endocytosis. A sequence of morphologies in putative digestive vacuoles infers a lysosomal system and the occurrence of phosphatases suggests a function involving detoxification of substances sequestered from hemolymph. Pericardical nephrocytes took up the dye trypan blue injected in live termites, suggesting their activity connected to the filtration of the hemolymph. Additionally, histochemical tests showed the existence of stored proteins in their cytoplasm. These cells presented a well-developed Golgi apparatus and abundant rough endoplasmic reticulum, consistent with protein synthesis. This study highlights the importance of nephrocytes in Isoptera and opens perspectives for further research of these cells.

1. Introduction

Various insects have specialized cells with excretory functions belonging to the nephrocyte or athrocyte family (Crossley, 1985; Locke and Russel, 1998). These cells are important for metabolizing a wide range of substances and appear in groups bathed in the hemolymph (Chapman, 1998). Their morphology under light microscopy is characterized by the abundance of cytoplasmic granules and their ultrastructure by labyrinth channels, lysosomes and presence of coated vesicles.

Locke and Russel (1998) criticized the use of the term nephrocyte for insects and preferred to use the term athrocyte as these authors regarded these cells as not being involved in hemolymph filtration. However Weavers et al. (2009) demonstrated that nephrocytes of *Drosophila melanogaster* are podocyte-like cells with a slit diaphragm, implying an uptake function. The most common insect nephrocytes are pericardial cells that are present along the dorsal vessel (Chapman, 1998).

Adult nephrocytes occur laterally to the insect heart (pericardial cells), as well as in the dorsal diaphragm and on the alary muscles (Crossley, 1985; Chapman, 1998; Locke and Russel, 1998). Three types of nephrocytes (athrocytes) were reported for *Bombyx* spp.: pericardial, subesophageal and peritracheal cells (Owa et al., 2006). In the louse *Pediculus*, the nephrocytes form a group on either side of the esophagus (Chapman, 1998), and in Diptera, they are disposed in a dorsal ring, known as Garland cells in *Drosophila* (Crossley, 1972). In larvae of Odonata, these cells are scattered throughout the fat body (Chapman, 1998), while in larvae of bees, they are apparently absent (Cruz-Landim, 2008).

In insects, some nephrocyte roles are well defined, such as the ability of uptake dyes and other large molecules from hemolymph, including toxic substances. These cells are selective in their sequestration of hemolymph materials (Locke and Russel, 1998; Weavers et al., 2009). They sequester proteins from the hemolymph and produce lysozymes with antimicrobial properties (Tobe and Loughton, 1969; Hernándes-Martínez et al., 2013). Additionally, these cells are associated with amino acid metabolism in the roach *Periplaneta americana* (Davey, 1962). The high amount of lysosomes in the cytoplasm suggests that they are involved in protein degradation (Owa et al., 2008).

This study is the first report on the occurrence and morphology of nephrocytes in different developmental stages and castes of termites. Histochemical and ultrastructural analyses were used to address specific roles of nephrocytes in the metabolism of these

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insects. Nephrocyte roles may differ according to the termite species, caste or developmental stage. Moreover, the ambiguous terminology of these cells is discussed.

2. Material and methods

2.1. Insects

Termite specimens of *Cryptotermes brevis* (Kalotermitidae), *Serritermes serrifer* (Serritermitidae), *Coptotermes gestroi*, *Heterotermes tenuis* (Rhinotermitidae), *Cornitermes cumulans*, *Syntermes nanus*, *Neocapritermes opacus* and *Velocitermes heteropterus* (Termitidae) were collected in different locations of Brazil and used in the histological and histochemical studies. The samples were composed of the two termite lines: the apterous line, including larvae, workers and soldiers, and the imaginal line, which included nymphs, alates (future queens and kings) and functional reproductives (queens and kings). Termite nymphs are immature instars with wingbuds that precede alate reproductives.

2.2. In vivo preparation

Twelve individuals of *C. brevis*, being three queens, three kings and six alates, were anesthetized on ice and injected with 1 μL trypan blue dye (Vetec, Rio de Janeiro, Brazil) solution of 10 mg/mL in ultra pure water using a Hamilton syringe (Hamilton Company, Reno, Nevada). Injections were performed within the pleurites in the middle of the abdomen. After 24 h, individuals were dissected in saline solution through a ventral incision. The organs and fat body were removed and the remaining material was examined under a Zeiss stereomicroscope (Stemi SV 11). Then, the material was disposed in slides and observed under a light microscope (Leica DM500).

2.3. Histology and histochemistry

A minimum of three individuals of each different termite instar and caste were fixed in FAA fixative (ethanol, acetic acid and formaldehyde, 3:1:1) and in 4% paraformaldehyde, embedded in JB4 resin, sectioned at 6 μm thickness and stained with hematoxylin-eosin (HE) and toluidine blue. The material was stained with bromophenol blue and xylidine Ponceau for protein detection, Lison for peroxidases and Gomory for acid phosphatases (Mello and Vidal, 1980; Pearse, 1985; Tolosa et al., 2003).

2.4. Ultrastructure

Electron microscopy was carried out using standard techniques. Abdomens of *C. brevis* queens were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide preceded by dehydration in a graded acetone series. The samples were previously stained with uranyl acetate, embedded in Epon Araldite (Araldite 501/PolyBed 812 kit, Polysciences, Germany) and sectioned with a Reichert Leica ultramicrotome. Thin sections were contrasted with 2% uranyl acetate for 45 min and lead citrate for 10 min and examined under a CM 100 Philips transmission electron microscope.

3. Results

Termite nephrocytes were found in the dorsal region of the abdomen, along the dorsal vessel, in the thorax surrounding the aorta and in the ventral region under the esophagus (Figs. 1 and 2). The cells were rounded and occurred in groups in all castes, except in small white immatures known as larvae, in which they were not identified. Some termite nephrocytes presented two nuclei per cell. The pericardial nephrocytes were anatomically observed in reproductives of *C. brevis* after *in vivo* preparations using trypan blue coloring method (Fig. 3). (See supporting video S1). Nephrocytes sequestered the dye, evidencing their activity in filtration of the hemolymph.

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.asd.2015.03.006.

In workers of *C. cumulans*, the morphology of pericardial and subesophageal nephrocytes was similar because these cells did not exhibit granulations and they presented deep plasma membrane invaginations, evidenced by internal channel spaces (Fig. 4A–B). Workers and soldiers of *H. tenuis* displayed nephrocytes with many cytoplasmic inclusions, including granules and vesicles (Fig. 4C–D). In *H. tenuis*, the number of these inclusions was higher in soldiers than in workers, which have predominantly round vesicles. However, workers and soldiers of *S. serrifer* displayed an extensive storage of different sized granules in the nephrocyte cytoplasm (Fig. 4E–F). The largest diameter of nephrocytes varied from 18 to 36 μm in workers of *C. cumulans*, from 15 to 23 in *H. tenuis* and from 25 to 36 μm in *S. serrifer*.

In all castes, the pericardial nephrocytes were large and conspicuous cells. Mature queens had cytoplasmic granules of different sizes in pericardial nephrocytes, and these granules contained

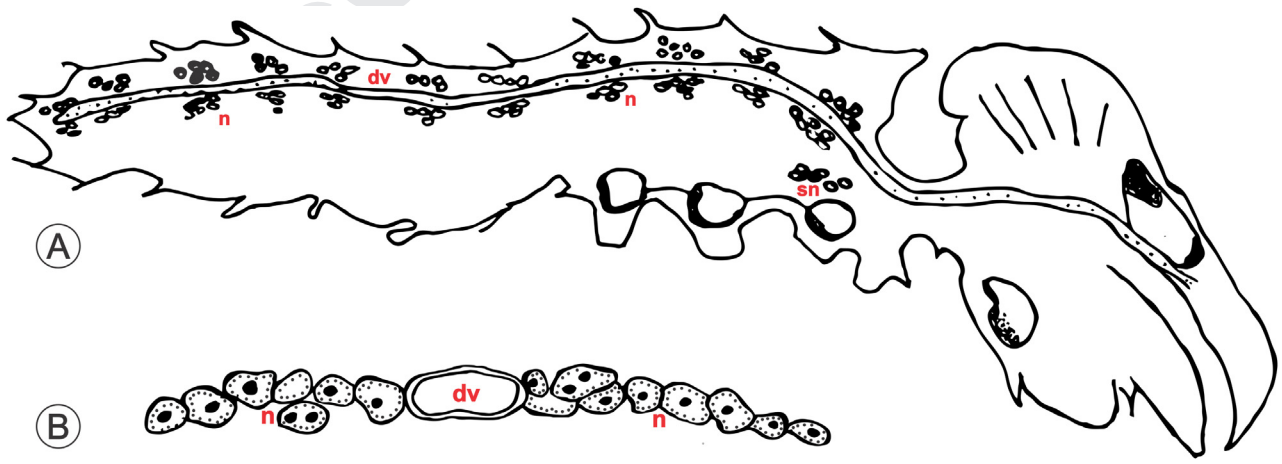

Fig. 1. Schematic representation of the nephrocytes in termites. **A**. Longitudinal section of a worker of *Heterotermes tenuis* showing the location of nephrocytes. **B**. Transversal section of abdominal nephrocytes disposed along the dorsal vessel (dv) in a worker of *Neocapritermes opacus*. n = nephrocytes, sn = subesophageal nephrocytes.

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