ULTRASTRUCTURE OF THE HAEMOCYTES OF *IXODES SCAPULARIS* (ACARI: IXODIDAE)

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PROHAEMOCYTES
PLASMATOCYTES
GRANULOCYTES
IXODES SCAPULARIS

ABSTRACT: Haemocytes of *Ixodes scapularis* were characterized on the basis of their ultrastructure by transmission electron microscopy of thin sections. Three types of haemocytes were identified: prohaemocytes, plasmatocytes, and granulocytes. Prohaemocytes are undifferentiated cells containing very little cytoplasm (high nucelocytoplasmic ratio). Plasmatocytes are rich in free ribosomes, mitochondria, rough endoplasmic reticulum, and have numerous peripheral vacuoles. Granulocytes are polymorphic cells containing granular inclusions and filopodia on the surface. Granulocytes are divided into two morphological types, type I and II, based on the structure of the granules.

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RÉSUMÉ : Les hémocytes d'*Ixodes scapularis* ont été caractérisés par leur ultrastructure en microscopie électronique. Trois types d'hémocytes ont été identifiés : les prohémocytes, plasmatocytes et granulocytes. Les prohémocytes sont des cellules indifférenciées présentant un rapport nucléo-cytoplasmique élevé. Les plasmatocytes sont caractérisés par de nombreux ribosomes libres, des mitochondries, du réticulum endoplasmique rugueux et des vacuoles périphériques. Les granulocytes sont polymorphes, avec beaucoup de granules et des filopodes Les granulocytes sont subdivisés en deux types morphologiques, types I et II. Cette subdivision est basée sur la structure fine des granules.

INTRODUCTION

Ixodes scapularis Say (formerly *Ixodes dammini* Spielman, Clifford, Piesman & Corwin) (OLIVER *et al.*, 1993) is the principal vector of *Babesia microti* (SPIELMAN *et al.* 1979) and *Borrelia burgdorferi* (BURGDORFER *et al.*, 1982) in the USA. Tick-borne pathogens go through a developmental migration from the digestive tract to the salivary glands. Understanding of the interactions between haemocytes and these pathogens as they move through the haemocel is of major importance.

Successful development of *B. burgdorferi* and *B. microti* in ticks depends, in part, on their ability to evade the tick's immunological defenses. Unfortunately, far less is known about the immune mechanisms of ticks than about those of insects and crustaceans (KHUN & HAUG, 1994).

The spirochetal infection in unfed *I. scapularis*, as well as in unfed *I. ricinus*, is often localized in the midgut (BENACH *et al.*, 1987; RIBEIRO *et al.*, 1987; BURGDORFER *et al.*, 1988; 1989; GERN *et al.*, 1990). This phenomenon could be explained by phagocytosis of *B. burgdorferi* by haemocytes in the hae-

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mocoel. The fact that *B. burgdorferi* is phagocytized by *I. ricinus* haemocytes *in vitro* (KHUN *et al.*, 1994; RITTING *et al.*, 1994) supports this hypothesis. A deficient immunological system in some individual ticks might result in generalized infection with *B. burgdorferi* (ZHIOUA *et al.*, 1994).

EGGENBERGER *et al.* (1990) found that plastic implants (Epon-araldite) were encapsulated by haemocytes in *Dermacentor variabilis*. Encapsulation was biphasic, involving a recognition phase and a capsule-formation phase. Type-II granulocytes, followed by type-I granulocytes, degranulated and initiated recognition of the foreign material. Coagulation of haemolymph involved formation of a coat around the implant with electron-dense granules. Plasmatocytes and type-I granulocytes were responsible for capsule-formation around the implant. *Ixodes ricinus* haemocytes were also involved in the phagocytosis of abiotic and biotic material: Type-I granulocytes phagocytized latex beads, while plasmatocytes and granulocytes phagocytized bacteria (*Micrococcus lysideicticus*) (KHUN & HAUG, 1994).

Phenoloxidase activity, an important mode of humoral immunity in insects, is apparently absent in ticks such as *I. scapularis*, *D. variabilis*, and *Amblyomma americanum* (ZHIOUA *et al.*, in preparation). This observation is compatible with the reported absence of melanin granules at the surface of implants in *D. variabilis* (EGGENBERGER *et al.*, 1990).

These results suggest that haemocytes play a dominant role in the tick immune response. In this paper, we describe the ultrastructure of the haemocytes in the haemolymph of *I. scapularis* as observed by transmission electron microscopy of thin sections.

MATERIALS AND METHODS

Ticks (*I. scapularis*) were collected from the Tower forest (Kingston, Rhode Island). Due to the small amount of haemolymph in immature and male ticks, only females were used in this study. Females were fed on a New Zealand white rabbit (*Oryctolagus cuniculus*) and hand-removed after four days engorgement.

Twenty ticks were bled by amputating one or more legs at the coxa level into 0.5 ml of ice-cold anticoagulant (10 mM EDTA, 100 mM glucose, 145 mM NaCl, 30 mM trisodium citrate, 26 mM citric acid, pH 4.6: 370 mOsM) (DURRANT *et al.*, 1993) and fixed by the addition of 2 % glutaraldehyde. The sample was then centrifuged at 6,000 g for 20 min and the cells in the pellet were embedded in molten 1.5 % purified agar at 44° C which was then cooled to solidify. Small pieces of agar containing cells were fixed overnight at 4° C in 2 % glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4, with 2 % sucrose. The specimens were then washed three times with cacodylate buffer and postfixed in 2 % osmium tetroxide in cacodylate buffer for 3 h at 4° C, dehydrated in a graded ethanol series, and embedded in SPURR's epoxy resin (SPURR, 1969). Thin sections were cut on a Dupont/Sorval ultramicrotome with a diamond knife, stained with uranyl acetate followed by lead citrate, and examined with a JOEL 1200EX transmission electron microscope.

OBSERVATIONS

Three types of haemocytes were identified in the haemolymph fluid of *I. scapularis* females.

Prohaemocytes

These undifferentiated cells were rare in the study preparations. Prohaemocytes are small and round (3.8 × 6.8 µm diam.) with a prominent nucleus. A small rim of cytoplasm surrounds the nucleus. The nucleo-cytoplasmic ratio is very high. The cytoplasm appears to contain free ribosomes, mitochondria, and small amount of rough endoplasmic reticulum (RER) (Fig. 1). Inclusion bodies, or granules were not observed.

Plasmatocytes

These cells are ovoid to spindle-shaped and larger than prohaemocytes (8.1 × 15.6 µm diam.) (Fig. 2). Numerous mitochondria surround the nucleus. The rough endoplasmic reticulum is well-developed and

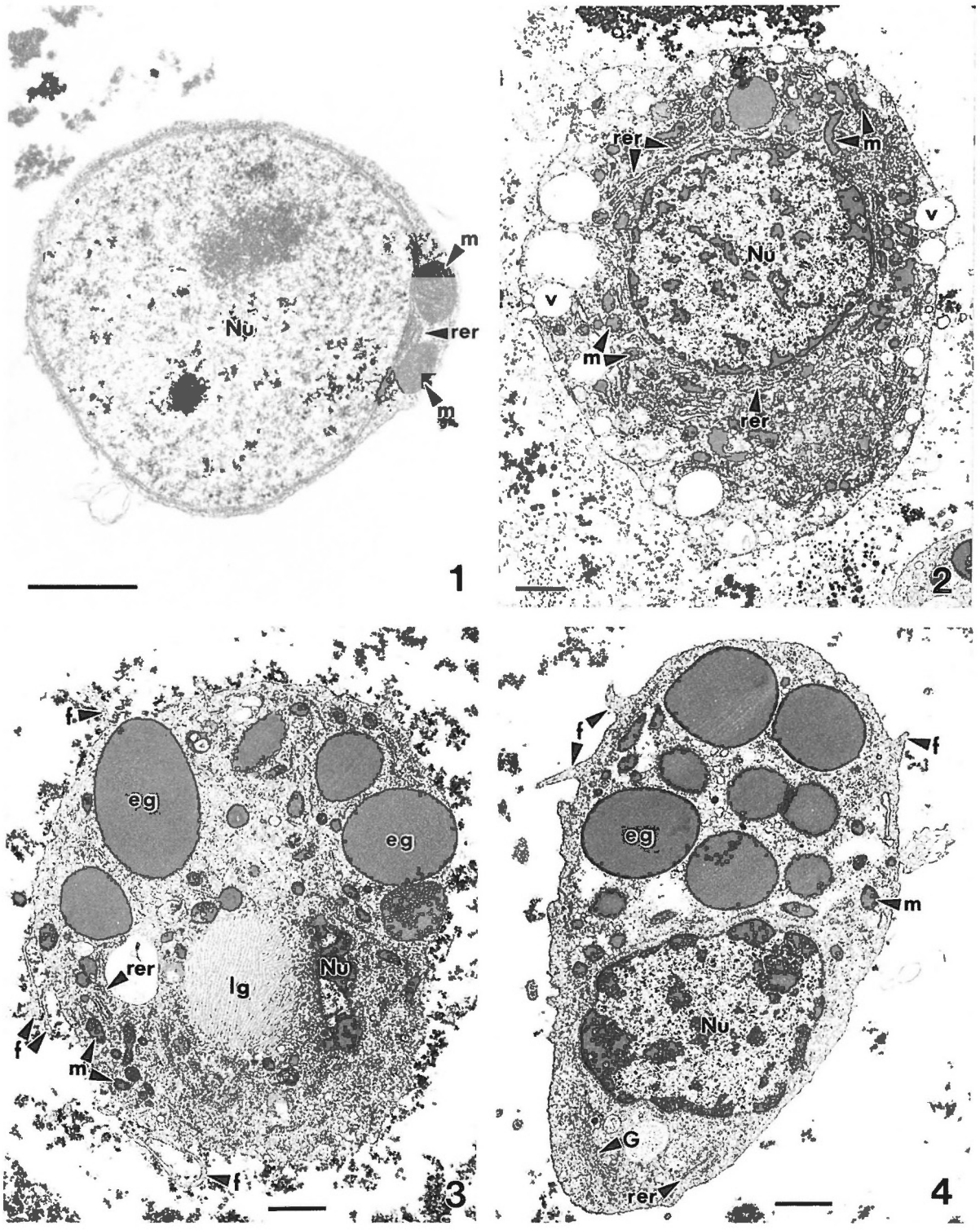

FIG. 1: Prohaemocytes with large nucleus (Nu) occupying most of the cell volume, mitochondria (m) and rough endoplasmic reticulum (rer). × 18,600. Scale bars = 1.0 μm. FIG. 2: Plasmatocyte with central nucleus (Nu), mitochondria (m), rough endoplasmic reticulum (rer) and vacuoles (v). × 8300. Scale bars = 1.0 μm. FIG. 3: Type-I granulocyte with two types of granular inclusions: electron-dense granule (eg), lamellate granule (lg), filopodia (f), rough endoplasmic reticulum (rer). × 9900. Scale bars = 1.0 μm. FIG. 4: Type-II granulocyte with one type of granular inclusion: electron-dense granule (eg), filopodia (f), rough endoplasmic reticulum (rer), Golgi apparatus (G). × 9700. Scale bars = 1.0 μm.

abundant. The cytoplasm is rich in free ribosomes, and lysosomes are prominent. No granular inclusions or filopodia were observed. Peripheral membrane-bounded vacuoles were numerous.

Granulocytes

Granulocytes were the largest (11.9 × 25.0 μm diam.) and most common cells in the haemolymph fluid of *I. scapularis*. These cells are extremely polymorphic and are differentiated from the other types of haemocytes by the presence of granules and filopodia. Two types of granulocytes were identified: Type I and Type II. Type I granulocytes contain two morphological types of granules (Fig. 3). One granule has a lamellate structure (Fig. 5) while the other granule has a uniformly electron-dense appearance (Fig. 6). Both types of granules are membrane-bound, and vary in size and shape. In type I granulocytes, the RER is well-developed, often with enlarged cisternae. Numerous Golgi complexes in association with small vesicles and primary lysosomes were also observed. Type II granulocytes contain only the uniformly electron-dense granules (Fig. 4). These electron-dense granules are round to oval-shaped and often fill the cytoplasm. The RER is also well-developed, with narrow elongate cisternae surrounding the nucleus. Golgi complexes are adjacent to long cisternae and are associated with small vesicles. Filopodia were observed in both types of granulocytes.

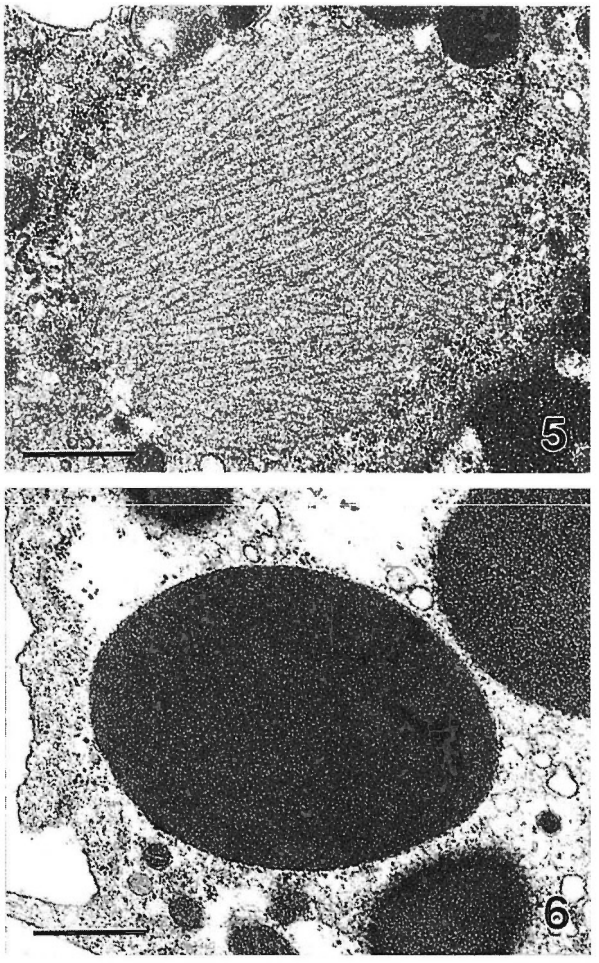

FIG. 5 : Lamellate granule. × 31,400. Scale bar = 0.5 μm.
FIG. 6 : Electron-dense granule. × 31,600. Scale bar = 0.5 μm.

DISCUSSION

The haemocytes of *I. scapularis* females were classified according to the scheme of BREHELIN & ZACHARY (1986). Three types of haemocytes were recognized on the basis of their ultrastructure: prohaemocytes, plasmatocytes, and granulocytes. These types of haemocytes have previously been identified in *I. ricinus* (KHUN and HAUG, 1994), *D. variabilis* (SONENSHINE, 1991), *Hyalomma asiaticum* (AMOSOVA, 1983), *Argas arboreus* (EL SHOURA, 1986) and *Ornithodoros erraticus* (EL SHOURA, 1989). However, BRINTON & BURGDORFER (1971) reported four types of haemocytes in *Dermacentor andersoni;* prohaemocytes, plasmatocytes, spherulocytes and oenocytes. These authors considered granular cells as spherulocytes and they subdivided them into four subtypes. It appears that prohaemocytes, plasmatocytes, and granulocytes are the most common haemocytes in ixodid, as well as in argasid ticks.

The ultrastructure of the prohaemocytes of *I. scapularis* is similar to that of other tick species (BRINTON & BURGDORFER,1971; KHUN & HAUG, 1994; AMOSOVA, 1983; SONENSHINE, 1991; and EL SHOURA, 1986; & 1989) as well as that of insect prohaemocytes (BREHELIN & ZACHARY, 1986). These latter cells are generally considered stem cells from which other types of haemocytes differentiate

(EL SHOURA, 1986; 1989, SONENSHINE, 1991). However, the role of prohaemocytes in haemocyte production in *I. ricinus* is unclear, and evidence concerning an haemopoietic organ is lacking (KHUN & HAUG, 1994). According to FUJISAKI *et al.* (1975), prohaemocytes might change to plasmatocytes.. Hence, further studies are needed to establish the role of prohaemocytes as stem cells.

Plasmatocyte ultrastructure in *I. scapularis* is similar to that in other tick species, including *I. ricinus* (KHUN & HAUG, 1994), *D. variabilis* (SONENSHINE, 1991), *H. asiaticum* (AMOSOVA, 1983), *O. erraticus* (EL SHOURA, 1989), *A. arboreus* (EL SHOURA, 1986), *D. andersoni* (BRINTON & BURGDORFER, 1971), and in insects (BREHELIN & ZACHARY, 1986). These plasmatocytes are generally characterized as having many peripheral membrane-bound vacuoles and the absence of granular inclusions. DOLP (1970) classified the plasmatocytes as early or advanced, based on the number of vacuoles. The abundant vacuoles and lysosomes in these cells indicate high endocytic activity. The plasmatocytes of *I. ricinus* have been shown to phagocytize *M. lysideicticus* (KHUN & HAUG, 1994), and those from *D. andersoni* phagocytized mitochondria (BRINTON & BURGDORFER, 1971). In argasid ticks, plasmatocytes phagocytized bacteria of the genera *Spirochaeta* and *Brucella* (BINNIGTON & OBENCHAIN, 1971), while in *D. variabilis* they played a role in hemocytic encapsulation (EGGENBERGER *et al.*, 1990).

Two types of granulocytes were distinguished in *I. scapularis* by the ultrastructure of the granular inclusions in these cells. Type I granulocytes are characterized by two types of granular inclusions; electron-dense granules and granules with lamellate elements. These granulocytes are similar to the type IV spherulocytes of *D. andersoni* (BINNINGTON & OBENCHAIN 1971). Type II granulocytes contain only electron-dense granules. These cells are similar to the type I spherulocytes of *D. andersoni* (AMOSOVA, 1983). SONENSHINE (1991) reported two types of granulocytes in *D. variabilis*, type I and type II, and a fourth type of haemocyte (called a spherulocyte), which is a small, round cell containing large granules filled with a matrix of fibrillar material resembling those found in type I granulocytes. In *I. ricinus*, two types of granulocytes were also described; type I and type II, which differ in the shape of the granules. Type I granulocytes are characterized by spindle-shaped granules, sometimes with a lamellate substructure. They are similar to type IV spherulocytes and/or type I granulocytes of *D. andersoni*. Type II granulocytes contain only spherical electron-dense granules (KHUN & HAUG, 1994). In the argasid ticks, *O. erraticus* and *A. arboreus*, only one type of granulocyte was reported, which contained only electron-dense granules (EL SHOURA, 1986; 1989). In insects, four types of granulocytes (I to IV) were recognized by BREHELIN and ZACHARY (1986). Type-I granulocytes are characterized by three morphological types of granular inclusions: membrane-bound granules with a lamellate structure, membrane-bound electron-dense granules, and heterogeneous inclusions with multivesicular bodies, resorptive in nature. Type I and II granulocytes have been shown to be involved in phagocytosis of foreign bodies (KHUN & HAUG, 1994) and in haemocytic encapsulation (EGGENBERGER *et al.*, 1990).

The description of the haemocytes of *I. scapularis* is a step toward understanding the immune response mechanism of this genus and the relationship between *B. burgdorferi* and its vector. Further studies are needed to determine the function of each cell type, their enzymatic activity (e.g., acid phosphatase), and the role of the tick immune system in the epizootiology of Lyme Borreliosis. Similar studies in other acarine taxa are needed to determine the distribution of haemocyte-types among the ticks and related mites.

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