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Ultrastructure and Populational Ratios of Hemocytes of the Brown Dog Tick, *Rhipicephalus sanguineus* (Latreille) (Ixodoidea, Ixodidae)

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ABSTRACT

Identification of Hemocytes is essential to understand hemocyte-mediated immune responses in invertebrates. The ultrastructure and characteristics of hemocytes of field-collected semiengorged female dog ticks, *Rhipicephalus sanguineus* (Latreille) are herein presented on the basis of hemocyte types, morphological cell features and ultrastructural characteristics of cytoplasmic inclusions. Samples of haemolymph were obtained by cutting the caudal alloscutum and collecting the drop there formed. The samples were fixed and stained with Giemsa solution for light microscopic examination. Ultra-thin sections were prepared, stained and examined by TEM. Light and TE Microscopy observation resulted in the characterization of three basic cellular types: Plasmatocytes (pL), granular hemocytes (Gr) and prohemocytes (ph). Comparison of cell type population ratio showed pL as the most abundant hemocyte (57%), followed by Gr (30%). ph accounted for only 12% of the hemocyte population.

Key words: Ixodidae, *Rhipicephalus sanguineus*, hemocyte class, ultrastructure

INTRODUCTION

Invertebrates defend themselves from infection with an innate immune system without antibodies and memory cells. Hemocytes play a major role in this immune response through phagocytosis, nodule formation and encapsulation of invading foreign materials (Gotz and Boman, 1985; Gotz, 1986). Hemocytes are identified on the basis of their morphology, ultrastructure and physiological function (Jones, 1962; Lackie, 1988; Brehelin and Zachary, 1989). The ultrastructure of hemocytes in some tick species is known (Brinton and Burgdrofer, 1971; Amosova, 1983; El Shoura, 1989; Kuhn and Haug, 1994; Carneiro and Daemon, 1996; Zhioua *et al*., 1996; Ayaad *et al*., 2000; Kadota *et al*., 2003). Three basic types of hemocytes have been described in both hard and soft ticks (Kuhn and Haug, 1994; Inoue *et al*., 2001). At least two types of phagocytic cells in ticks, the plasmatocytes and granulocytes have been reported (Fujisaki *et al*., 1975; Kuhn and Haug, 1994; Inoue *et al*., 2001; Borovickova and Hypsa, 2005; De Silva *et al*., 2006). The fact of the haemolymph serve as a carrier of pathogens (Viruses, bacteria and protozoa) (Dubska *et al*., 2012; Hamel *et al*., 2013) makes the hemocytes of ixodida and argasida subject to intensive studies concerning research of pathogens. This paper describes the fine structure of hemocytes of the dog tick *Rhipicephalus sanguineus* on the basis of their morphological and ultrastructural properties.

MATERIALS AND METHODS

Tick collection: Semiengorged female adults of *R. sanguineus* were manually collected from dogs at the experimental station farm (Abies), Faculty of Agriculture, Alexandria University.

Hemocyte preparation for light microscopy: Ticks were surface sterilized with 70% alcohol and precooled on Ice for 2 min to slow down haemolymph coagulation and bled by cutting the caudal alloscutum. Haemolymph drops (1-2 μL) were collected as described by Arnold (1974) for bright field microscopic examination. Hemocyte identification was under taken using Giemsa-stained solution.

Haemolymph processing for transmission electron microscopy: Haemolymph was fixed in 2% glutraldhyde in 0.1 M Na-Cacodylate buffer, pH 7.2 and subjected to a vacuum for 1-4 min every 15 min for 2 h on ice. Prior to vacuum treatment, floating samples were poked under the buffer surface with pointed metal pokers. Rinsing took place in 0.1 M Na-Cacodylate buffer, pH 7.2, for 45 min, with buffer changes at 15 and 30 min. Further fixation in 1% Osmium Tetraoxide in Na-Cacodylate buffer, under intermittent vacuum and poking, took place for 1.5 h. Samples were then rinsed again in the Na-Cacodylate buffer. Samples were dehydrated through an Ethanol series in buffer: 35-50-70-80-95-100-100% for 60 min each. Then Infiltrate with Resin:

- **Propylene:** Resin (no accelerator) 2:1 — 1 h
- **Propylene:** Resin (no accelerator) 1:1 — 1 h
- **Propylene:** Resin (no accelerator) 1:2 — 1 h
- **Pure resin:** (no accelerator) — 1 h
- **Pure resin:** (no accelerator) — Overnight
- **Pure resin+accelerator:** — 1-2 h
- **Embed samples into molds:** — 60°C oven

Semi thin sections were prepared on glass slides through cutting at 1 um using the ultramicrotome. Sections were stained with Toluidine blue for 5 min examined by light microscope model M-200 M. Ultra-thin sections were cut using ultramicrotome Leica model EM-UC6 at thickness 90 nm, mounted on copper grids (400 mish).

Sections were stained with double stain (Uranyl acetate 2% for 10 min followed by Lead citrate for 5 min) and examined by transmission electron microscope JEOL (JEM-1400) at the candidate magnification. Images were captured by CCD camera model AMT, optronics camera with 1632×1632 pixel format as side mount configuration. This camera uses a 1394 fire wire boared for acquision (The work was done in TEM lab FARP. Faculty of Agriculture Research Park-Cairo University).

Hemocyte classification and population: With the aid of light and TEM, hemocytes were classified based on the morphology and structure of cells observed in comparison with other cellular phone types described for Ixodida, Argasida and other groups of arthropods. The total hemocyte was counted according to the form that suggested by Jones (1962):

$$\text{Total hemocyte} = \frac{\text{Hemocyte-1 mm}^2 \times \text{dilution} \times \text{depth of chamber}}{\text{No. of 1 mm counted square}}$$

The differential hemocyte count percentage (DHC%) was determined per fixed number (200/slide) of hemocytes counted following the technique of Abu El-Magd (1992).

RESULTS

Hemocyte classification: Three types of hemocytes were observed in the haemolymph of semiengorged adult females of *R. sanguineus*.

Plasmatocytes: Comparison of cell type population ratio showed plasmatocytes (pL) as the most abundant hemocyte (57%). They are polymorphic, varying from broadly oval to irregularly fusiform. Often numerous and at times branched filopodia extend in various directions above the cell surface. Many mitochondria surround the ovoid nucleus. A well-developed rough endoplasmic reticulum (RER) is distended. pL's have numerous organelles such as vacuoles and lysosomes (Fig. 1a-c).

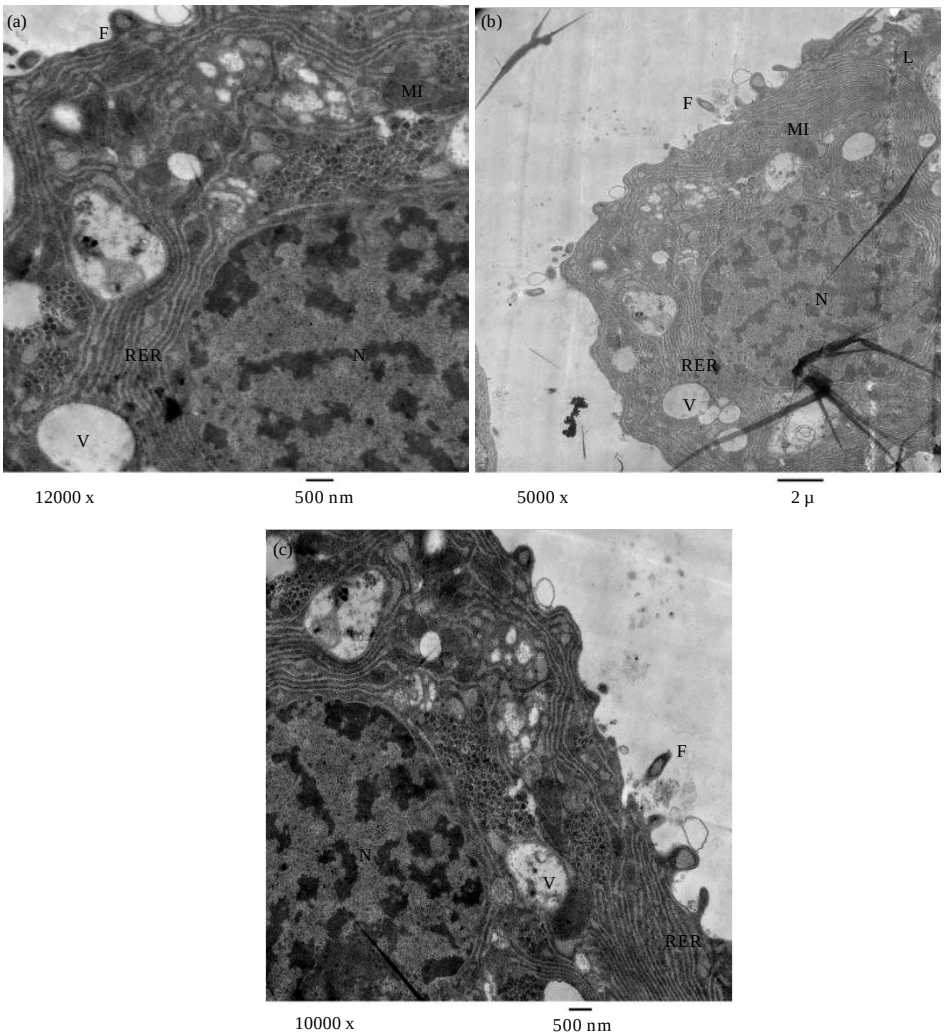

Fig. 1(a-c): (a-c) Ultrastructure of Plasmatocyte show N: Large nucleus, F: Filopodia, V: Vacuoles, MI: Mitochondria, RER: Extensive Rough Endoplasmic Reticular and L: Lysosomes

Granulocytes: Granulocytes (Gr) comprise a considerable proportion, about 30% of the hemocyte population. These hemocytes are characterized by their numerous processes. They contain large amounts of endoplasmic reticulum which is often extensively dilated. Lysosomes and vesicles are well developed. Golgi complex and mitochondria are abundant. Many granular inclusions with varying shapes and sizes (Spindle, Triangular and Spherical) are well represented in the cytoplasm (Fig. 2a-c).

Prohemocytes: (Ph) accounted for only 12% of the hemocyte population. They appeared as small cells, vary from round to broadly ovoid with high nucleo-cytopasmoc ratio. Narrow band of

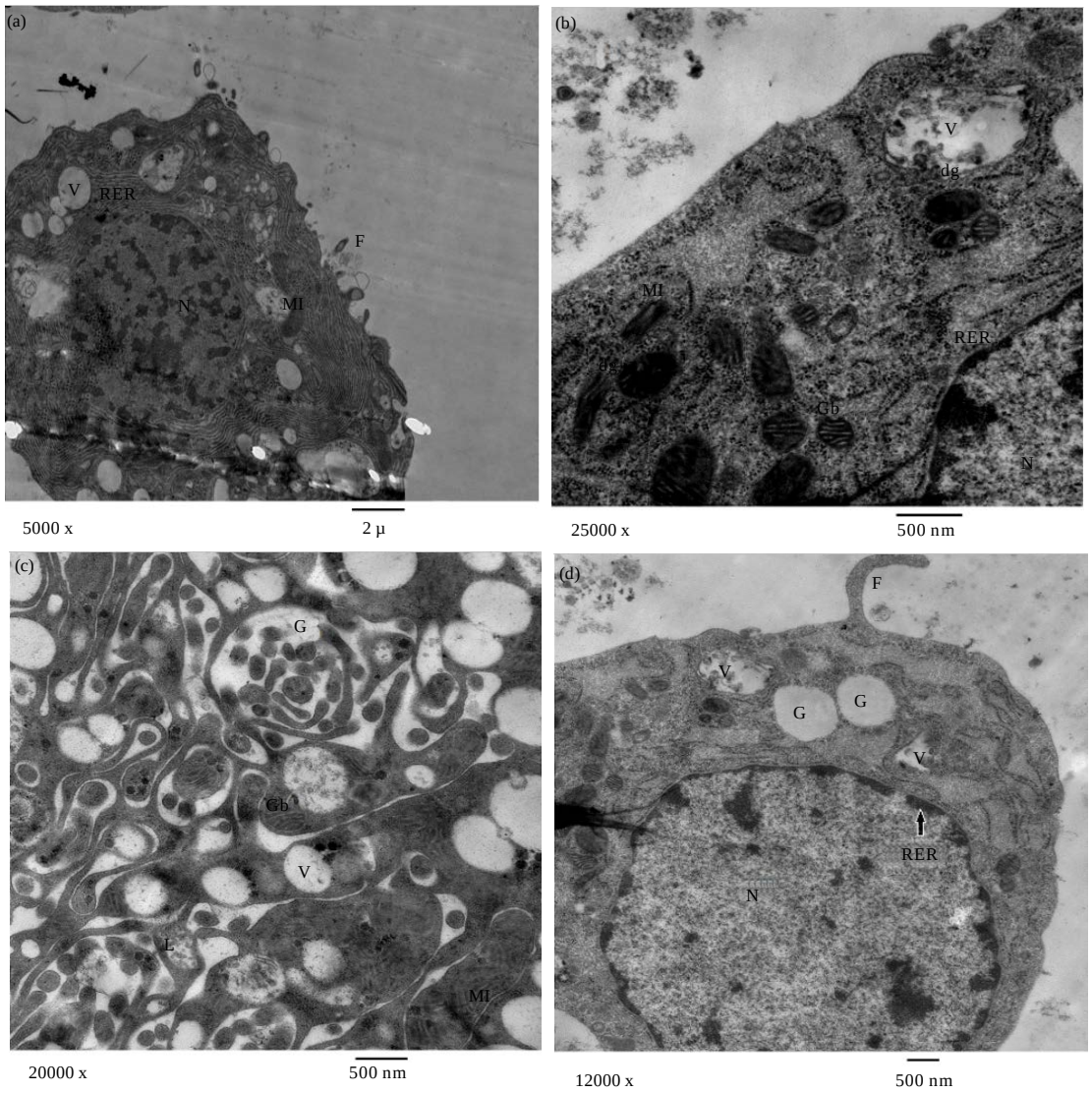

Fig. 2(a-d): Ultrastructure of hemocyte morpho types, (a-c), Gr: Granulocytes with dg: Dense granules, Gb: Golgi bodies, N: Large nucleus, F: Filopodia, V: Vacuoles, MI: Mitochondria, RER: Extensive rough endoplasmic reticular and L: Lysosomes and (d) Ph: Prohemocyte with N: Large nucleus, RER, MI, F, V and G: Granules

cytoplasm around the nucleus was detected with prominent rough endoplasmic reticulum (RER) net work. Round to rod like numerous mitochondria are dispersed throughout the cytoplasm. Small filopodial extensions of the cell surface were noted frequently. The cell cytoplasm is usually homogeneous or occasionally contains few granule-like inclusions (Fig. 2d).

DISCUSSION

The hemocytes of ticks are presumably similar to those of other groups of arthropods and characterization morpho-functional and logic of these have been based on studies carried out in class to being insects, more widely studied. Three basic types of hemocytes have been described in both hard and soft ticks (Kuhn and Haug, 1994; Inoue *et al*., 2001). Several earlier studies on the ultrastructure of hemocytes in ticks have described three types of cells, namely prohemocytes plasmatocytes and granulocytes in *Argas arboreus* and *A. perisicus* (Roberta, 1970; El Shoura, 1986); *Hyalomma anatolicum excavatum* and *H. dromedarii* (Roberta, 1970; Amosova, 1983); *Ixodes ricinus* and *I. scapularis* (Kuhn and Haug, 1994; Zhioua *et al*., 1996); *Boophilus annulalus* (Ayaad *et al*., 2000) and *Rhipicephalus sanguineus* (Carneiro and Daemon, 1996). Table 1 summarizes the highlights of some of these studies. The three types of hymocytes observed in semiengorged *R. sanguineus* females in our study agreed with the classification scheme for insect hemocytes (Brehelin and Zachary, 1989; Chapman, 1998). To obtain reliable ultra structural results, rapid sampling and fixation procedures are important because hemocytes are very reactive cells and preform rapid transformations on contact with foreign bodies (Baerwald, 1979; Jones, 1979; Binnington and Obenchain, 1982). Ultrastructural data revealed that plasmatocytes might contribute to the attachment and Lysosomal degradation of foreign organisms as well as decomposed midgut molecules of foreign origin through their endocytic activity in hard ticks (Kuhn and Haug, 1994; Pereira *et al*., 2001). Subsequently, Kuhn and Haug (1994), Inoue *et al*. (2001), Borovickova and Hypsa (2005) and De Silva *et al*. (2006) showed the involvement of

Table 1: Summary of hemocyte characterization in argasid and Ixodid ticks

Hemocyte type	Tick species	Stage	Ratio	Shape	Podia	Granule	Phagocytic activity	Mitochondria	RER	Lysosomes	Vacuole	Golgi body	Free ribosomes	References
Prohemocyte	Aa	Adult		Rou	---	Small		+	+				+	1
	Ba	SF	1	Rou-ovo.	---	Few							+	2
	Ir	Adult	<1	Sphe.	Filo	---		+	F				+	3
	Is	Female	Rare	Rou.	---	---		+	F				+	4
	Da	eF	<1	Rou-ovo.	Filo	---		+	+				+	5
Plasmatocyte	Aa	Adult		Rou-oval		E.d.	+	+	+		+		+	1
	Ba	SF	42	Rou-spin	Filo	---	+	+	+			+		2
	Ir	Adult	20	Poly	Filo	---	+	+		+	+	+		3
	Is	Female		Ovo-spin	---	---		+	+	+	+		+	4
	Da	ef		Poly	Filo	---							+	5
Granulocyte	Aa	Adult	Com		Pseu	E.d.	+	+	+				+	1
1	Ba	SF	37		Filo		+	+	+	+		+		2
2	Ba	SF	20		Filo	T.g.	+	+	+			+		2
1	Ir	Adult	20			Spindle	+		+		S	+		3
2	Ir	Adult	60		Lopo	E.d.,l.e.d.	+		+		S	+		3
	Is	Female	Com	Poly	Filo	E.d.			+		S	+		4

Aa: *Argas arboreus*, Ba: *Boophilus annulatus*, Ir: *Ixodes ricinus*, Is: *Ixodes scapulari*: Da: *Dermacentor andersoni*, SF: Semiengorged female, ef: Engorged female, com: Common, rou: Round, ovo: Ovoid, sphe: Spherical, spin: Spindle, poly: Polmorphic, filo: Filopodia, pseu: Pseudopodia, Lopo: Lopopodia, e.d.: Electron-dense granule, fib: Fibrillar inclusion, spindle: Spindle inclusion, l.e.d.: Less e.d., Lam: Lamellate granule, F: Few, S: Small, 1: El Shoura (1986), 2: Ayaad *et al*. (2000), 3: Kuhn and Haug (1994), 4: Zhioua *et al*. (1996) and 5: Brinton and Burgdrofer (1971)

granular hemocytes as well as plasmatocytes in Phagocytosis in both hard and soft ticks. Also, plasmatocytes and granulocytes might participate in the coagulation of the Haemolymph following recognition of foreign material as indicated by the degranulation process upon contact with glass surface *in vitro*. Eggenberger *et al.* (1990) reported the role of these haemocytes in initiating and coagulation phase of encapsulation in *D. variabilis*. Similarly, Kuhn and Haug (1994) indicated the participation of these cells in the coagulation of the Haemolymph of *I. ricinus*. The data obtained in this study agree with the statement of Carneiro and Daemon (1996), in which prohemocyte cells occur only in small proportions in population of hemocytes in *R. sanguniues*.

Studies have been taken to detect the role of hemocytes in secretion of hemagglutins or lectins, Lysozymes, cystatin and antimicrobial peptides like defensin and Ixodidin and to find out what surface determinants are unique to different types of hemocytes. Understanding the role of hemocytes in pathogen defense is essential to discover a new targets for pesticide research and new approaches to the management of tick-borne diseases.

CONCLUSION

In conclusion, light and electron microscopy study of semiengorged female dog ticks, *Rhipicephalus sanguineus* haemolymph reveals three primary cell types, based on cytoplasmic fine structure and inclusion bodies, plasmatocytes (pL), granular hemocytes (Gr) and prohemocytes (ph). The plasmatocytes constituted the most abundant hemocyte population (57%), followed by Gr and ph at (30%) and (12%), respectively.

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