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Circulating Haemocytes in Insects: Phylogenic Review of Their Types

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Abstract.- Insect haemocytes belong to different categories that carry out various immune functions in larvae and adults. Survey of their studies in different orders of insects would help and suggest that discrepancy in their nomenclature is based on the individual description of their shapes and metabolic contents and the same would be resolved by putting up their characteristics and features together to name different types of haemocytes consistently. Basically, there are three basic forms, prohaemocytes, plasmatocytes and granular haemocytes forming the bulk of the population. Other three types are spherulocytes or cystocytes, oenocytoids and adipohaemocytes. Plasmatocytes and granular haemocytes are functionally termed as immunocytes.

Key words: Haemocytes, prohaemocytes, plasmatocytes, granular haemocytes, oenocytoides, adipohaemocytes.

INTRODUCTION

Morphologically, haemocytes are distinct variety of cells (Price and Ratcliffe, 1974; Mead *et al*, 1986), comparable to the vertebrate leucocytes (Jones, 1977), which constitute the important and inevitable components of haemolymph in the open circulatory system of insects (hexapods) as well as in other arthropods and invertebrates (Wigglesworth, 1933, 1939,1955, 1956, 1979; Jones, 1962, 1975, 1977; Gupta, 1979a). Haemocytes were first discovered by Swammerdam (1669) according to Jones (1962) and their versatile features in a species and reproduction of similar and compatible shapes amongst different species urged Cuenot (1896) to classify them for the first time into 4 different categories (Millara, 1947). The classification has been revised several times (Yeager, 1945; Wigglesworth, 1956; Jones, 1962; Gupta, 1979; Dean *et al*., 2004; Carlos and Michel, 2006; Qamar and Jamal, 2009; Siddiqui and Al-Khalifa, 2012).

Functionally, haemocytes are the generally accepted cellular defense units in insects and partially are responsible to their immune system, (Ratcliffe and Rowley, 1979). In fact insects and other arthropods present different physico-chemical methods to combat, check or onslaught the challenges exerted by their biological enemies including viruses, bacteria, protozoans, fungi and cestods (Ratcliffe *et al*., 1976; Mead *et al*., 1986). Trespass of these biological agents into haemocoel usually elicits the cellular responses as phagocytosis, nodule formation and encapsulation by the haemocytes (Ratcliffe and Rowley, 1979; Gupta, 1979). The contribution of haemocytes in

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prophenoloxidase system through the involvement of granular and/or plasmatocytes has prompted (Gupta, 1985; Yokoo *et al*., 1995; Tojo *et al*., 2000; Ling and Yu, 2006) to label these two, categories of haemocytes as immunocytes. However, type of immunocytes and their role in insects are debatable and need more information on the subject for clarity (Alfonso and Jones, 2002).

However, to-date, there exist lacunae, pitfalls and controversies on over all knowledge about insect haemocytes thus the scope for fundamental studies on various aspects of insect haemocytes demands more investigations and researches. In view of this, in the present review attempt has been made to unify the haemocytes classification.

TYPES OF HAEMOCYTES IN MAJOR ORDERS OF INSECTS

Haemocytes were reported in a variety of insects belonging to different orders on the basis of staining reactions, shape, and size *etc*. but generally there was no uniform criterion. Following review on the haemocytes of different orders of insects will indicate the diverse nomenclature as well as types. This information is based on the reviews of Wigglesworth (1956), Jones (1962), Gupta (1979a), and Khan (1986) as well as later informations (Alfonso and Jones, 2002; Carlos and Michel, 2006; Wood and Jacinto, 2007).

Thysanura

Francois (1975) reported the microscopic and ultrastructure of the haemocytes in *Thermobia domestics* and categorised these cells as plasmatocytes (PLs), granular haemocytes (GRs) and coagulocytes (COs).

Orthoptera

Akai and Sato (1979) categorised the haemocytes of *Locusta migratoria* as prohaemocytes (PRs), plasmatocytes (PLs) coagulocytes (COs), oenocytoids (OEs), and reticular cells (RTs). However, Sharma and Dutta (1979) recorded the presence of PRs, PLs, GRs, CYs, COs, SPs, ADs, OEs, POs and vermicytes (VEs) in *Chrotogonus trachypterus* Blach and *Acrida exaltata* Walk by using phase contrast microscopy. Eventually, these grasshoppers did have more variety of cells in their haemocytes. Islam and Roy (1982) identified only four types of haemocytes, PRs, PLs, ADs, and SPs in *Schizodactylus monstrosus* Drury. Khan *et al.* (1984) in 5th instar hoppers as well as in adults of *Hieroglyphus nigrorepletus* (rice grasshopper) reported the occurrence of PRs, PLs (spherical, oval and fusiform in shape), GRs and OEs. Later, in the same species Ahmad (1988) and Ahmad and Khan (1986) varified and confirmed these types.

Dictyoptera

Jones (1962) studied the microscopic structure of the haemocytes in *Periplaneta americana* and described only two categories of these cells which were named as PLs and CYs. However, later Boerwald (1975) added three more categories of haemocytes *viz*,, PRs, GRs, and SPs in the same species. Hagopian (1971) in *Leucophaea maderae* L. reported PRs, PLs, GRs, SPs, and COs CYs. Arnold (1972) reported, PRs, PLs, GRs and Cystocytes in 16 species of cockroaches but in none of these species COs were observed, although these cells were reported in *P. americana* and *L. maderae*. Scharrer (1972) found similar types of haemocytes in other dictyopterous insects, *viz.*, *Periplaneta australai* F. *Blaberus giganteus* L., *B. discoidalis*, *B. cranifer*, *Blatta germanica* L., *Diploptera punctata* Esch, *Gromphadorhina portentosa, Brysotria fumigata*, *Pycnocellus surinamensis* and *Sphodromantis bioculata*. Amirante and Mazzali (1978) in *Leucophaea maderae* did not find PRs but PLs, GRs, and SPs, were present. Kochetova (1978) added two more types of haemocytes in *Nauphoeta cineria* Oliver, unlike other cockroaches, and labelled them as OEs and ADs. Akai and Sato (1979) in *Panesthia anqustipennis* reported the presence of PRs, PLs, GRs and SPs but coagulocytes (COs) were not seen even under electron microscope. Gupta (1985) labelled PLs and GRs as immunocytes. Gupta and Han (1988) as well as Han and Gupta (1989) followed the term immunocytes in cockroaches, *Periplaneta americana* and *Blattella germanica*.

Phasmida

In *Clitumnus extradentatus*, PRs, PLs, GRs, SPs, and CYs were the main cells (Gupta, 1979a).

Dermaptera

Arvy and Lhoste (1944) investigated the haemocytes in *Forficula auricularia* L. and named them leucoblast, small rounded basophil leucocytes, with dusty cytoplasm and leucocytes with crystalloids. In addition to these, they noticed large cells in the larval stages which were named as megacytes.

Mallophaga

In this group of insects, fewer types of haemocytes have been reported. According to Saxena and Agarwal (1979) in *Lipeurus lawrensis tropicalis* identified three categories of haemocytes *i.e.* PRs, GRs and OEs but universally occurring PLs were not found.

Hemiptera

Phenolic inclusion were the haemocytes in this species. Later, several insect haematologists studied the haemocytes in this order, in *Rhodnius prolixus* Wigglesworth (1956) gave different nomenclature to various haemocytes and labelled them as proleucocytes; phagocytic leuocytes of various shapes such as PLs, POs, SPs, OEs and adipocytes. However, Ballard and Jones (1959) reported these cells as PRs,PLs,GRs,ADs,OEs, and CYs or COs and later Laifook (1970) confirmed these types described by Jones and Bellard using supravital staining and phase optics in *R. prolixus*. But under electron microscope he could identify only PRs, PLs, OEs, and granulocytophagous haemocytes. Le Blanc (1971) also in *R. prolixus* categorised the haemocytes into PRs, PLs, GRs and OEs. Zaidi and Khan (1974) followed the criteria of Jones (1962) and classified the haemocytes of *Dysdercus cingulatus* as PRs, PLs, ADs, GRs and OEs. However, in the same species Qamar (1990) could not observe granular haemocytes (GRs). Boiteau and Perron (1976) in *Macrosiphum euphorbidae*(Thomas) described six types of haemocytes *viz.*, PRs, PLs, GRs, SPs, OEs and wax cells. The haemocytes of *Leptocorisa varicornis* (Siddiqui and Khan, 1979), *Nepa cineria* (Siddiqui and Khan, 1979) and *Nezara veridula* L. (Mall and Gupta, 1978) *D. cingulatus* (Zaidi and Khan, 1974) were studied extensively and six types of haemocytes, PRs, PLs, GRs, SPs, OEs and adipohaemocytes (ADs) were described in general but in *L. varicornis* and *N. cineria,* the term CYs was used in place of SPs whilst Behura and Dash (1978) reported only four types of haemocytes, PRs, PLs, POs and CYs in *Bhopalosiphum maidis* Fitch.

Lepidoptera

In this order haemocytes were comparatively studied in myriad species. Yeager (1945) gave an extensive description and classification of the haemocytes of *Prodenia eridania* larvae and on the basis of the variation in shape, size and staining reactions he classified the haemocytes into ten classes; proleucocytoids, small chromophil cells, OEs, PLs, POs, vermiform cells, CYs, sheroidocytes, eruptive cells and degenerative cells. These classes were further divided into 32 types. But the free haemocytes of *P. eridania* larvae were labelled as PRs, PLs (polymorphic), POs and SPs by Jones (1959) who rather simplified the classification given by Yeager (1945). Arnold (1952a) identified only four categories of haemocytes in the haemolyph of *Ephestia kuhniella* as PRs, PLs, SPs and OEs. Gupta (1979) investigated the haemocytes of the larvae of *Bombyx mori,* which were only differentiated as PRs, PLs, SPs and OEs. However, they also described two subclasses of PRs as macronucleocytes and micronucleocytes depending on their size. Nittono (1960) also studied the haemocytes in adult *Bombyx mori* and classified them into PRs, PLs, GRs, SPs, imaginal spherule cells and OEs. Jones (1967) in *Galleria mellonella* identified PRs, PLs, GRs, SPs, and OEs. But in the fully grown larvae of *G. mellonella,* Neuwirth (1973) reported only PLs, GRs, SPs and OEs. Rabaglio (1970) made comparative study of the free haemocytes of six Japanese and Italian races of *Bombyx mori* and described micronucleocytes, PRs, GRs, SPs and OEs and dropped the term PLs as used by Nittono (1960). In *Spodoptera littoralis,* he included PLs, PRs and POs whilst GRs represented OEs but ADs had separate identity. Arnold (1975) made a comparative study of the haemocytes of two closely related species, *Euxoa compestris* (Grote) and *E. declarata* but in these species he concentrated on the ratio of Length-width of fusiform PLs and differences were recorded in between to evaluate their taxonomic imporrtance.

Later, Arnold (1982) made an extensive survey of the haemocytes of eighty-four species from the family noctuidae and identified five categories; PRs, PLs, GRs, SPs and OEs but then he could not relate haemocyte shapes to the taxonomic divisions. Nishi (1982) in *Spodoptera litura* described PRs, PLs, POs, GRs, CYs, ADs, SPs, and OEs and Khan *et al.* (1984) further confirmed these cells in the 5th & 6th instar larvae, prepupae and pupae of *S. litura*. Ahmad (1986) extended observations of Nishi (1982) in *S. litura* to its larvae of 3rd and 4th instars and adults of both sexes and further verified that the types of haemocytes were the same in these stages as mentioned by Nishi (1982) in other stages of this species further it was also added that no change in types occurred between various stages of *S. litura*. Ahmad (1988) also described the haemocytes of midd1e aged larvae of 3rd, 4th, 5th and 6th instars, prepupae, pupae and adults of *Spilosoma obligua*. These haemocytes were only characterized as PRs, PLs, POs, OEs and ADs, which were uniformly found in all stages. But in *Heliothis armigera* larvae PRs, PLs and OEs were common with both *S. litura* and *S. obligua*. Whereas, presence of CYs and SPs was like that of *S. litura* (Khan *et al.*, 1990).

Diptera

In *Sarcophaga falculata*, four types of haemocytes were described and were labelled as PRs, phagocytes, GRs and OEs (Dennel, 1947). Rizki (1953) investigated the haemocytes in the haemolyph of *Drosophila willistoni* in its three instars and classified them into PLs, POs, SPs, OEs, nematccytes and crystalloid cells. According to him, there was a sharp decrease in the number of PLs and increase in the population of POs towards the end of third instar. The reason for this change was described as transformation of PLs into POs. Jones (1956) observed the haemocytes of *Sarcophaga bullata* by phase contrast microscopy and reported only the occurrence of PLs, GRs and SPs. However, Whitten (1964) studied haemocytes in *D. melanogaster* and *D. bullata* and found the presence of PLs, GRs, ADs, SPs and OEs in both the species but aside these cells a few multinucleate haemocytes were also reported by him in the haemolyph of adults only. Five types of haemocytes were observed in *D. melanogaster* by electron microscope, which were labelled as PRs, PLs, GRs, crystal cells and OEs by Yu (1976) as compared to the observation of Whitten (1964) in this species. Kaaya and Ratcliffe (1982) studied the haemocytes in *G. morsitans*, *Aedes aegypti*, *Culex guinguefasciatus*, *stomoxys calcitrans*, *Calliphora erythrocephala* and *Lucilia sericata* and described seven types of haemocytes as, PRs, PLs thrombocytoids, GRs, ADs, OEs, and spindle cells. But by electron microscopy they were able to observe only PRs, PLs and SPs.

Coleoptera

According to Arvy and Lhoste (1946) in *Leptinotarsa decemlineata* (Say) the haemocytes were classified into leucoblasts (mother cells), small rounded basophil leucocytes with dusty cytoplasm and leucocytes with crystalloids. They further found megacytes in the larvae of *L. auricularia*. Jones (1950) studied the haemocytes in *Tenebrio molitor* and recognized them as PRs, smooth contour chromophilic cells and oenocyte like cells, PLs, vermiform cells cystocytes (or coarsely granular haemocytes); SPs and degenerating cells. Jones (1954) further studied haemocytes in *T. molitor* by phase contrast microscopy and grouped these cells into PRs, PLs, OEs and CYs. Again in *T. molitor* ultrastructure of the haemocytes was studied by Zachary and Hoffmann (1973) who reported clotting cells, spindle shaped, amoebocytes and phagocytic amoebocytes. Later Devauchelle (1971) described the haemocytes of *Melolontha melolontha* as PRs, PLs, GRs, ADs, SPs and OEs. According to Ahmad (1974) the haemocytes of *Aulacophora fovicollis* were PRs, PLs, SPs, OEs and CYs whereas, in *Mylabris pustulata* the haemocytes were classified into PRs, PLs, CYs, OEs, ADs, and lamellocyte. The haemocytes in *Dermestes vulpinus*, *Dermestes maculates*, *Hybosorus illegari* and *Dineutes aerius* were studied by Al Khalifa and Siddiqui (1985) who reported PRs, PLs, GRs, SPs, OEs and ADs. Al-Khalifa and Siddiqui (1986) described six types of haemocytes, PRs, PLs, GRs, CYs, OEs in *D. vulpinus* and conforming these four coleopterous species, Siddiqui and Al-Khalifa (2012) found similar six types of haemocytes in red palm weevil, *Rhynchophorus ferrugenius*.

Hymenoptera

Muller (1925) studied the haemocytes of honey bee, *Apis mellifera* larvae and described single category prior to caping and observed second category appearing after caping. He could find three types of haemocytes in the haemolymph of honey bee, *Apis mellifera* as leucocytes and pycnonucleocytes and further extended his studies on the haemocytes in the haemolymph of eggs, larvae and pupae of worker honey bee of *Apis mellifera*, in which he found PRs only in the eggs and larvae while neutrophils, eosinophils, basophils, normal leucocytes, pycnonucleocytes, hyaline leucocytes and stem cells were reported in the heamolymph of the pupae. In the haemolymph of *Polistis hebrocus* free haemocytes contained PRs, PLs, GRs and a special type of cell in the females whereas the males of this species had only PRs and PLs (Ahmad, 1974, 1986).

CONCLUSION

Circulating haemocytes have been extensively studied in the different phylogenic orders, although lacunae, discrepancy, pitfall remained destined to this aspects. Terminology need uniformity in adapting the ultratechnique of studies by transmission electron microscope (TEM) and scanning electron microscope (SEM) and at the same time physical condition of insect should be closely monitored before preparing the specimen study as myriad factors are known to effect their shape and size. Hypothesis to reach the agreement on uniform terminology would further open the vistas of information on the type study of haemocytes. A common criterion to classify the haemocytes is based on structure that fits the function. In the open circulatory system, haemocytes play key role in the survival of the insect species of medical and extreme economic importance. However, there is no single method to determine the haemocyte types and different methods must be used (Carlos and Michel, 2006). Functional study of phagocytosis and encapsulation depends on several factors before reaching to conclusion, the size of the particular particle like india ink *i.e.* 1µm thick and silicon beeds, 5µm like haemocytes in *G. mellonella* easily engulf the silicon beed and fail to intake the india ink particles (Tojo *et al*., 2000). Further phagocytosis depends on the formation of vesicles by golgi apparatus, lysosomes. Concept that haemocytes are interchangeable in their forms and structure in the different phases of the life cycle have been established by THC and DHC reports in different species. Ling and Yu (2006) have studied the phagocytosis and reported that biological particles are phagocytosed by granular haemocytes whereas, inert beads are phagocytosed by only palsmatocytes.

Monoclonal antibodies (MAb) should also be tried as a tool for identifying the different categories of circulating haemocytes and such studies are scanty (Gardiner and Strand, 2000). In using the MAb as specific markers of antigens shown to be specific to a haemocytes must be based on studying its signal pathways activated during differentiation (Lebestky *et al*., 2000). Another approach is lectin labeling and it also has the similar limitation as labeling by MAbs.

Concluding remark include the diversified nomenclature of the haemocytes should be resolved by comparing the features of different types and use the commonest terminology for the analogus types and it seems evident that there are far more similarities in the haemocyte picture of the different species than disparities and resolution should be reached by consistant and uniform criteria of characterization.

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