LIGHT MICROSCOPY INVESTIGATION OF THE IMMUNE SYSTEM CELLS OF *HIRUDO MEDICINALIS* LINNAEUS, 1758

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ABSTRACT: The present study was carried out to determine hemocyte types of medical leech, *Hirudo medicinalis*. Four hemocytes types were identified; prohemocytes, plasmatocytes, granulocytes and eleocytes. They were characterized by light microscopy according to size, presence or absence of granules and nucleus/cytoplasm ratio. The prohemocytes were the smallest cells with large nuclei in the hemolymph. Plasmatocytes were polymorphic, varied from ovoid to spindle-shaped cells. Plasmatocytes were the most abundant hemocyte type in the hemolymph of *H. medicinalis*. Granulocytes were elliptical in shape and characterized by the presence of cytoplasmic granules. Eleocytes were spherical cells with homogeneous and slightly granular cytoplasm. The aim of this study was to characterise the hemocytes in *H. medicinalis* and to determine whether any differences from other invertebrates in terms of hemocyte types due to use in medical targets.

KEY WORDS: Hemocyte; innate immunity; leech; *Hirudo medicinalis*

INTRODUCTION

The immune system is a host defense system which is distinguishing the cells, tissues and organs that are parts of the host body from foreign things called nonself[4]. After distinguishing between self and nonself, the immune system performs an immune response to eliminate nonself invaders[9]. The immune system is typically divided into two categories, innate and adaptive immunity. All animals possess innate or natural immune mechanism which is believed to be most ancient form of immunity. Another component of innate immunity is complement system which is composed of more than 30 proteins in blood[4]. Recent researches have indicated that invertebrates do not have complement system and adaptive immunity[1]. However several phyla of invertebrates including various insects, crabs and worms have analogue system to complement system called prophenoloxidase system. Like the complement system prophenoloxidase is activated by a series of enzymes[4].

The innate immune systems of invertebrates include humoral and cellular defenses. Effector proteins as phenoloxidase and lysozyme carry out humoral responses[1,4,5,26]. Cellular defense mechanisms contain hemocyte or coelomocyte-mediated responses which are phagocytosis, nodule formation, encapsulation and the production of reactive oxygen intermediates[1,18,27]. Invertebrate hemocytes perform not only host defense but also they perform essential physiological functions such as nutrient transport, digestion, wound healing, shell mineralization and excretion[1,7,13,24].

The first recorded use of medical leeches was known from ancient Egypt. Leech is derived from the Anglo-Saxon word "laece" which means physician. The Romans were also accustomed to leeches, they named as "Hirudo". Later Linnaeus used the term *Hirudo medicinalis* to define the application of leeches to medicine[23,36]. *H. medicinalis* is categorized as a member of phylum Annelida, class Clitellata, order Arhynchobdella, family Hirudinidae and genus Hirudo[38].

Currently, leeches are used in hirudotherapy, microvascular replantation, reconstructive surgery and the treatment of venous congestion[2,31]. Although more than nearly 650 leech species are known, just a small number of them are used as a therapeutic purpose[14]. The aim of this study was to characterise the hemocytes in *H. medicinalis* and to determine whether any differences from other invertebrates in terms of hemocyte types due to use in medical targets.

MATERIALS AND METHODS

The hemocyte monolayers were prepared on clean glass slides. The cell were fixed in methanol. After the fixative dried, the hemocytes were stained with Giemsa stain for 10-15 min, and then the slides were rapidly washed with phosphate-buffered saline. The slides were dehydrated and mounted with Entellan. Observations were carried out under Zeiss Axioscope light microscope that was equipped with AxioCam Erc 5S digital camera.

RESULTS AND DISCUSSION

In this study, four main hemocyte types were identified based on size, presence or absence of granules and nucleus/cytoplasm ratio. The four hemocyte types of the hemolymph in *H. medicinalis* were prohemocytes, plasmatocytes, granulocytes and eleocytes. The hemocytes of insects were categorized into seven main types; prohemocytes, plasmatocytes, granulocytes, spherulocytes, adipohemocytes, oenocytoids and coagulocytes[16]. However, in the silkworm five types of hemocytes were characterized; prohemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids[25,35]. Feitosa et al.[12] identified five types of hemocytes (prohemocytes, plasmatocytes, granulocytes, spherulocytes and adipohemocytes) in an arachnid species, *Rhipicephalus sanguineus*.

Prohemocytes were usually small hemocytes in the hemolymph and the cells had a large, centrally located nucleus that occupied much of the cytoplasm of the cell (Fig. 1A). The prohemocytes were oval or spherical cells and measured ~9.8 µm in diameter. Prohemocytes do not perform defensive response such as phagocytosis or encapsulation, and they have low enzymatic activities[8,19]. Kavanagh and Reeves[20] reported that prohemocytes divided and appeared to differentiate into other cell types. Yamashita and Iwabuchi[37] proved approximately 43% of prohemocytes differentiate into plasmatocytes, granulocytes and spherulocytes in hemolymph of *Bombyx mori*.

Plasmatocytes were polymorphic, varied from round to spindle-shaped cells (Fig. 1B, C). The plasmatocytes were small to large with variable size (4.7-8.5 µm wide and 10.2-12.6 µm long). The nucleus was round or elongated and generally centrally located. Plasmatocytes are similar to monocytes of vertebrates[10]. They are generally known as phagocytic cells involved in the removal of apoptotic cells during development as well as in the ingestion or encapsulation of pathogens[17]. Plasmatocytes contain lysosomal enzymes and are the most abundant cell type[29]. Our results indicated that plasmatocytes were the most abundant hemocytes in the hemolymph of *H. medicinalis*.

Granulocytes were elliptical in shape and measured ~6.5 µm wide and ~12.2 µm long with a centralized nucleus or an acentric nucleus type. The granulocytes were characterized by the presence of granules with variation in size (Fig. 1E). Granular hemocytes are similar to neutrophil, basophil and eosinophil of vertebrates. Granulocytes perform immune functions such as wound healing, blood clotting, phagocytosis and encapsulation of pathogens. Additionally, granulocytes are involved in developmental and metabolic functions[17].

Kavanagh and Reeves[20] reported that plasmatocytes and granulocytes were the predominant phagocytic cells. Phagocytosis is one of the prominent innate immune mechanism in both vertebrate and invertebrate animals[3]. Plasmatocytes and granulocytes participate in nodule formation and encapsulation as well as phagocytosis and they are functionally termed as immunocytes[30,34]. The innate immune defenses of invertebrates are not specific and consist of cellular and humoral responses. Hemocyte-mediated responses include phagocytosis, cellular aggregation, encapsulation and production of antimicrobial proteins[6,11,21,32,33]. Cellular aggregation is considered as an immunological response for host defence. Aggregation of hemocytes contribute to prevent the accidental blood loss and resist pathogenic microorganisms[28]. Aggregation of plasmatocytes of *H. medicinalis* was observed (Fig. 1D). The hemolymph/coelom compartments of many taxa contain another

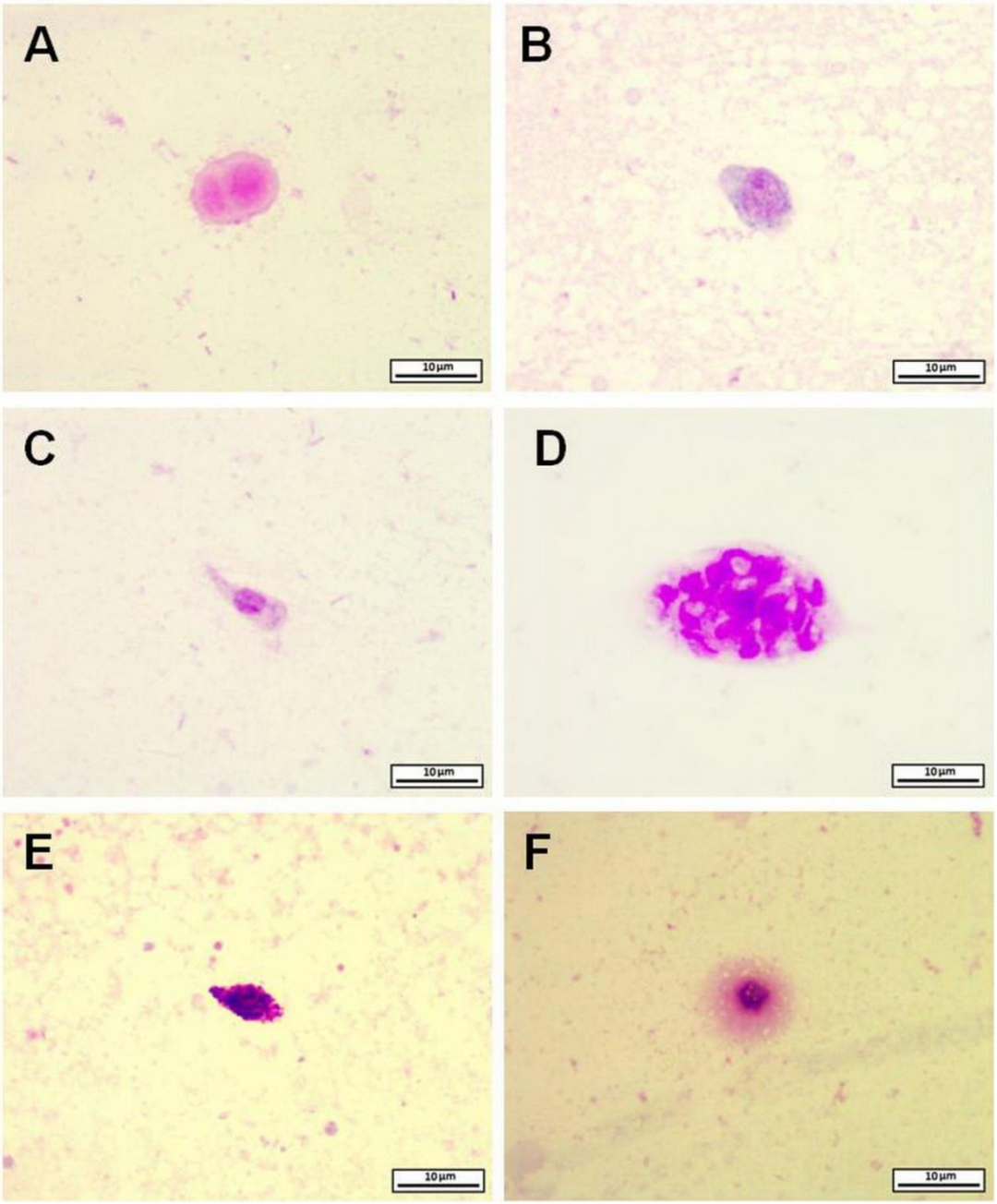

Fig. 1. Light microscopic view of Giemsa-stained hemolymph smears of *H. medicinalis* A: Prohemocyte division B,C: Plasmatocyte. D: Aggregation of plasmatocytes E: Granulocyte F: Eleocyte

cell type that includes lipid or crystalline inclusions.There are many different names in use, among them eleocytes, chloragogen cells,
vacuolated cells, spherulocytes, adipohemocytes and oenocytoids. In *H. medicinalis* spherical cells with homogeneous cytoplasm, eleocytes, were observed and measured ~12.2 µm in diameter. The plasma membrane was without micropapillae or filopodia. The cytoplasm was slightly granular and the nucleus was small and round (Fig. 1F). Oenocytoids are one of the hemolymph cell types filled with lipid or crystalline cytoplasmic granulles and appear to be unique to arthropods[17]. The oenocytoids have phagocytic activity and contain prophenoloxidase[15,20,22]. Hemocyte types and their functions have been assessed for insects, crustaceans and other arthropods taxa. Prohemocytes, plasmatocytes and granulocytes resemble their annelid counterparts form. Other hemocyte types, spherulocytes or oenocytoids, resembling eleocytes in annelids are hemolymph cells with variably sized cytoplasmic inclusions[17].

In conclusion, four distinct hemocyte types (prohemocytes, plasmatocytes, granulocytes and eleocytes) were identified. Plasmatocytes were predominant cells in the hemolymph of *H. medicinalis*. Hemocytes classification of invertebrates is often controversial because there is no universally accepted definition of hemocyte types in invertebrates. Although there are many variations in hemocyte types of invertebrates, the most common hemocytes types from invertebrates are prohemocytes, plasmatocytes, granulocytes. Compared with the studies published to date, our results added information for characterization of hemocytes of *H. medicinalis***.**

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