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MINIREVIEW

Origins and functions of annelide immune cells: the concise survey

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Abstract

Immune cells and structures of *Annelida* represent a complex subject that was studied for over 120 years. An overwhelming mountain of data have been accumulated during these studies, most of them being the subject of many excellent reviews and books. In the this paper we focused on a brief survay of old studies and some reflection on the findings during recent years.

Key Words: *Annelida;* cells; immunocytes; defense

Introduction

Annelids are considered to be the ancestral key assemblage in which important evolutionary novelties have originated. These animals represent virtually a new, highly progressive monophyletic group in which the emergence of new structures brought about better dynamics of locomotion and more effective functional and immune capabilities (Clark, 1964). This allowed these animals an extensive adaptive radiation into all biocenoses of the Earth with exception of the air. Subsequently, evolutionary pressures within these different environments resulted in high diversification leading to approximately 9,000 estimated species which are ordinarily divided in two superclasses-the *Aclitellata* with major group of *Polychaeta,* and the *Clitellata* consisting of *Oligochaeta* and *Hirudinea* (see for details Sima, 1994a).

Compared to their predecesors, the annelids exhibit all three major advanced structural features, such as the metameric body arrangement, the secondary body cavity (the coelom) filled by coelomic fluid constituting the hydrostatic skeleton, and the blood-vascular system (in some taxa already closed). This means that all necessary requirements for more advanced eucoelomate basic body plan were finally present from space for development of more complex and hierarchized organs, to increased regional differentiation, and finally, to the ability to change duplicated parts in ways that might be advantageous for survival. All these hallmarks enabled a more active way of life and a higher adaptive plasticity which are not found in any earlier bilateralian acoelomate animals.

Both the coelom and the closed vascular system are important for emergence of a new type and more effective immune strategy. This also contributed toward their successful survival from an evolutionary point of view. A celomic cavity is composed of paired chambers lying within the third germinal layer the mesoderm which represents a general source of all types of immunocompetent cells found in all eucelomate taxa including *Deuterostomia*. Morphological separation of celom from the vascular system allowed cytogenesis of mutually independent cell lineages and their functional specialization and higher degree of functional cooperation. Consequently, a high number of free and sessile cell types can be found both in the celomic and vascular fluids. Cell types differ in morphology among various annelide species. To this date, no clean-cut classification has been formed and widely accepted. Thus, morphofunctional criteria still remain basic for distinguishing various annelide immunocyte lineages. Roughly speaking, these cells are usually characterized as amoebocytes, eleocytes, erythrocytes and hemocytes (*Polychaeta*), celomocytes, amoebocytes, vascular lymphocytes, eleocytes and macrophages (*Oligochaeta*) and amoebocytes and chloragocytes in *Hirudinea*.

Current literature on annelide colemocytes is vast and far beyond the scope of the review. Therefore, we will focus on a survey of the main immunocyte categories in three annelide subclasses: polychaetes, oligocheates, and hirundineans.

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Table 1 Main categories of polychaete amoebocytes (From Dhainaut and Porchet-Henneré, 1988)

granulocyte	morphology	responding to	presence in families
Type I	fusiform, spindleshape, granules/microfilaments in cytoplasm adherent	hyaline fusiform leukocyte (1)	*Arenicolodae,* *Ophelidae,* *Glyceridae,* *Nephtyidae,* *Nereidae*
Type II*	spindleshape, granular, cytoplasm with vacuoles and without microfilaments adherent	hyaline leukocyte (1)	*Arenicolodae,* *Nereidae,* *Terebellidae,* *Capitellidae*
Type III	small, few granules, large nucleus	small lymphocyte (1)	precursor cell? in many families
Type IV	typical macrophage	common macrophage	found in immunized animals
Type V	large cytoplasmic spherules, stacks of rough endoplasmic reticulum	eosinophil granulocyte (1)	many families

*similar cells also in sipunculids, arthropods and molluscs
(1): Romieu (1923)

Polychaeta

The primary and most numerous defense cells are the amoebocytes that represent a rather heterogeneous population of cells. Amoebocyes are present in almost all the polychete species studied. The most common nomenclature used for amoebocyte is granulocyte, convenient term proposed by Baskin (1974). According Dhainaut and Porchet-Henneré (1988), polychete granulocytes are divided into five types (Table 1).

It is important to note that not all the these call types are present in every *Polychaeta*. Despite decades of research, the relationship among the individual types is still not established. Still unknown is whether they represent different stages of a single cell lineage or discrete series, or if ameobocytes have a common origin with other cell types, e.g., the eleocytes. Similarly, we are not certain of their origin. In some species, such as *Aphrodite*, the distinct “lymph glands” have been described (Fordham, 1925), whereas in *Sabelliade*, the long-shaped extension of nephridia has been suggested as a possible source. Discret tissues derived from celomic peritoneum in *Glycera* might play similar role. In other polychaete species, the mesenchymal tissue agglomerations around the vessels and nephridia might also serve for hemopoiesis (Dehorne, 1922).

The major defense functions of these cells consist of phagocytosis, waste removal and pathogen clearing. They are involved in antibacterial defense and encapsulation, which was a detailed study in *Nereis* (Porchet-Henneré *et al.,* 1987). The high degree of the cooperation by individual subtypes of granulocytes during encapsulation reaction was documented via means of monoclonal antibodies (Porchet-Henneré, 1990).

A second population of free cells within a polychaete celom is represented by the eleocytes (Rosa, 1896), the relatively large cells (appr. about 40 μm) contained in their cytoplasm nutrition reserves (mainly lipids and glycogen). They have often been described as agranular lymphocytes, trephocytes, or adipo-spherular cells. Eleocytes were found in a row of families belonging to both polychaete subclasses (the *Errantia* and the *Sedentaria*) but not in *Arenicolidae*, *Glyceridae*, *Nephyidae*, and *Syllidae*. Celomic lining epithelia covering blood vessels and somatic peritoneal epithelium were documented as a source of eleocytes in *Nereidae* (Eckelbarger, 1976) and *Terebellidae* (Dhainaut, 1970). Conversely, other authors supposed that they develop from phagocytic amoebocytes (Romieu, 1923; Dales and Dixon, 1981). Their role is mainly trophic, even if their active but non-defensive pinocytosis used for removing of damaged tissues has been documented (Dhainaut and Porchet-Henneré, 1988).

Third cell type found in polychetes constitutes hemoglobin-containing-cells termed erythrocytes (rarely hemocytes, red celomocytes or blood cells). They are found in *Capitellidae, Cirratulidae, Glyceridae,* and *Opheliidae,* exclusively in their celomic cavity, (Goodrich, 1898). According to Dhainaut and Porchet-Henneré (1988) erythrocytes form two lineages differing in morphology and functional specialization.

In general, eleocytes and erythrocytes are primarily involved in regeneration and respiration. As with other immunocytes in this phylum, the origin of these cells is unclear and probably differs among species. While the role of eleocytes in defense is most probably limited, hemoglobin-containing erythrocytes are actively phagocytosing (Goodrich, 1898).

A more questionable population of cells represent hemocytes (blood cells) closed in the vascular systems of various representatives of polychete families such as *Terebellidae*, *Sabellidae, Arenicolidae*, and *Nereidae*. The hemocytes resemble celomocytes in morphology but they are relatively small, around 3-10 μm. With the exception of *Magelona papilliocornis* (Boilly, 1974), they never contain hemoglobin. Their origin is unresolved and, for decades, they were considered to be an immigrant cell population. Only later, electron microscopic studies (Dhainaut and Porchet-Henneré, 1988) provided clearer knowledge and allowed classification into three categories (Types I to III, from which the Type III comprises sessile or attached cells and may be morphologically analogized to blood folicle cells of oligochaetes). Their role in defense reactions remains unknown.

Oligochaeta

From a practical perspective, the *Oligochaeta*, and the earthworms (of the family *Lumbricidae*) in particular, have by far been the most studied annelids and have become the object of interest in various scientific fields such as evolutionary biology and invertebrate immunology as well as a variety of environmental diciplines (agriculture, pedology, environmental pollution, epidemiology, etc.). Extensive studies accomplished during last 50 years have made them one of the most popular models of invertebrate immunity (Bilej, 1994; Cooper and Roch 1994, 2003).

Due to the intense study of the earthworm species, our knowledge of cell population composition in this category is superior. Current classification of free cells is based on old research (Kukenthal, 1885; Rosa, 1896). For detailed information on origin, classification and formation, the reader is asked to refer to the detailed reviews written by Sima (1994b) and Cooper and Roch (1994). Again, the primary source of most information were the *Lumbricus terrestris* and *Eisenia foetida* models and, on rare occasions, from limited representatives of other lumbricid species. Therefore, they might not be universally valid for all oligochaete taxa.

The rather complicated classification can be simplified into two basic categories: the celomocytes (amoebocytes and eleocytes) and the hemocytes (blood cells). Amoebocytes can be, according their morphology, further subdivided into granulocytes (acidophils Type I and Type II) and non-granular hyalocytes (basophils, amoebocytes forming a 70 % majority of free cells and a monolite population of neutrophils). Granular amoebocytes are present in all representatives of lumbricids. Some nomenclatures subdivide them further based on size or type of granules.

It was generally supposed that, at least in lumbricids, free celomocytes originated from mesodermal peritoneal cells comprising the visceral or parietal epithelial lining of the coelom (splanchnopleure and somatopleure) (Fischer, 1993; Sima and Slipka, 1995; Sima *et al.,* 1995). Other possible sources are epithelial lining of the blood vessels and septa forming specialized poietic structures (Liebmann, 1942; Valembois, 1971). The third eventuality, the origin from specialized hemopoietic organs named blood glands or blood folicles described in several representatives of the *Lumbricullus* sp. and genera *Sparganophilus*, *Maoridrilus*, *Pheretima* and *Pontodrilus*, must be taken into account as well (see for review Sima, 1994b). It was further observed that losts of celomocytes after their depletion induced by irritation (*via* dorsal pores in the body wall) are followed by extensive cell proliferation in coelomic lining in the typhlosole and metanepridial regions (Homa *et al.,* 2008). The mesodermal origin of all subpopulations of lumbricid free cells was recently confirmed using different mammalian antigen-specific monoclonal antibodies reacting with distinct coelomocyte surface markers (Engelmann *et al.,* 2002, 2005).

Both types of amoeboid celomocytes form an extremely important part of the immune system of annelids. As with most invertebrates, they are involved in non-self recognition, transplantation reaction, cytotoxicity, encapsulation, endocytosis and enzymatic digestion of engulfed material. In addition, they actively participate in regenerative processes and wound healing. Phagocytosis alone has been studied in earthworms since Metchnikoff (1891). Due to the availability, this model has been extensively studied and, in the last several decades, a vast amount of data has been obtained. Those seeking detailed information should read these excellent reviews (Cameron, 1932; Davis, 1978; Bilej, 1994; Cooper and Roch, 1994, 2003; Salzet *et al.,* 2006).

Two main different subpopulations among earthworm immunocompetent free celomocytes were identified by flow cytometric analyses (Cossarizza *et al.,* 1996; Engelmann *et al.,* 2002, 2005). A subpopulation of large celomocytes (25-50 μm in diameter) is active in phagocytosis and encapsulation of bacteria but no surface markers were identified using monoclonal antibodies. Small celomocytes (10-25 μm) reacting with anti CD11a, CD45RA, CD45RO, CDw49b, CD54, CD90, and beta 2-microglobulin are cytotoxic. Non-granular coelomocyte subpopulation recognize and neutralize foreign antigens. These cells exprime surface markers Thy-1 (CD90) and beta 2-microglobulin, both molecules belonging to the immunoglobulin superfamily (Shalev *et al.,* 1981; Roch *et al.,* 1983).

Eleocytes are again a heterogenous group of cells, usually divided into ergastoplasmic chloragocytes (which are probably the stem cells of this group), chloragocytes and free eleocytes. Their morphology can vary with oval, round or oblong cell´s shape 10-60 μm in diameter. A majority of eleocytes possess spherical granules (1-3 μm) and chloragosomes within their cytoplasm. Chloragocytes play a role in nutrition, excretion and osmotic balance. The defensive roles of these cells are less studied but they are certainly less pronounced. These cells proved inactive in phagocyting bacteria or other particles (Dales and Kalaç, 1992). In addition, they differ among individual species, e.g., they phagocytose in *Eisenia* but not in *Lumbricus*.

With respect to hemocytes, the information is scarce and the current information has not substantially improved since the last century.

Other Oligochaete families

If someone gained the impression that the situation concerning the cells and cellular structures in earthworms is confusing, an even more complicated

Table 2 Main categories of coelomic cells of the *Theromyzon tessulatum* (*Hirudinea*) (From Lefebvre *et al.,* 2008)

coelomocyte	morphology	responding to	function
large coelomic cell (chloragocyte)	100-150 μm free or attached to mesothelia granules in cytoplasm	oligochaete chloragocyte	non-phagocytic presence of recognition surface molecules encapsulation? vitellin production?
granular amoebocyte	30-70 μm granules in cytoplasm numerous pseudopodia	oligochaete granular amoebocyte	chemotactic phagocyting G+ and G- bacteria
small coelomic cell	7-12 μm without cytoplasmic granules hyaline cytoplasm rich endoplasmic reticulum	oligochaete hyaline cell	non-phagocyting

situation can be found in other *Oligochaeta* families. Even when these members commonly possess the same basic cell types, additional, more-or-less specialized or distinguished cell types have been described. *Pontodrilus bermudensis* serve as an example (Wamper and Jamieson, 1986). On the other hand, genera such as *Achaeta, Analycus*, or *Grania*, have only one type of celomocytes. *Buchholzia* possesses two types (Brinkhurst and Jamieson, 1971).

Hirudinea

General body anatomy responds to annelide basic body plan. However, these animals endured substantial modification when separate coleomic and vascular spaces were fused together into common hemocoel. These modifications of the vascular system and coelomic cavity led to the flow of hemocoelomic fluid through a highly complicated network of coelomic sinuses and channels. *Hirudidae* can be characterized by the presence of the botryoidal tissue located within the parenchyma adjacent to the body wall. Besides being involved in angiogenesis, this celothelium-derived tissue display myelo/erytroid function. It also can change its shape from a solid cord of cells to a prevascular structure when groups of closely associated cells became evident in the centre of the immature lumen. As the vessel growth, the precursors of circulating cells loose the cell-cell attachment and move freely within the lumen.

Unfortunately, cytology of hirudineans has attracted little attention, so our knowledge of the origin and function of their free cells are still incomplete. Cell populations within the hemocel are commonly divided to amoebocytes and chloragogen cells, similar to the previous annelide classes. Amoebocytes denominated also leukocytes or lymphocytes are usually of homogeneous size and morphology, exerting phagocytosis as the main defense activity (Sawyer and Fitzgerald, 1981). These cells are either free or attached to the hemocel wall lining. Hypotheses about their origin are rather speculative. The size of chloragogen cells differs among individual species. Their origin is clear, they arise from the hemocelomic epithelia (Oka, 1894) and from the cilio-phagocytic organ of the nephridia (Abeloos, 1925). Recently, using human monoclonal antibodies, three cell populations, the macrophage-like, NK-like, and granular cells, were identified in *Glossiphonia complanata* (de Eguileor *et al.,* 2000). Similarly, immunohistochemical and ultramicroscopic studies demonstrated more precisely the presence of three basic cell populations in *Theromyzon tessulatum* (Lefebvre *et al.,* 2008) (Table 2). We must be also aware that free cells may be derived from different tissue sources in different species. On the contrary to family *Hirudidae*, the celothelial botryoidal tissue in some species of the *Glossiphoniidae* plays besides the angiogenetic functions the important role in production of free hemocelomic cells. Prevascular structures are tightly associated with solid cell cords, from which circulating cells release loosing mutual contacts and pass freely into the vascular lumen (de Eguileor *et al.,* 2001). An increasing body of evidence indicates that in the leech *Hirudo medicinalis* the angiogenic process is regulated and coordinated by the botryoidal tissue. *Hirudo medicinalis* subjected to an angiogenic stimulus (e.g., wounding) responds with an extensive angiogensis that is accompanied by the production of free cells. These processes, moreover, could be influenced by means of mammalian activators of vascular cell growth, anti-angiogenic peptides (angiostatin and endostatin) and even proliferation-inducing mitomycin. The surprising degree of similarity of invertebrate angiogenesis with neovascularization in vertebrates, both at the biochemical and cellular levels, suggests that both involve similar growth factors and their receptors, and common cell-cell or cell-extracellular matrix interactions (Grimaldi *et al.*, 2006, Grimaldi *et al.*, 2008). The reviewed data confirm phylogenetic kinship between hirudinean and vertebrate processes in wound healing. It suggests that cytogenesis of free cells within the vascular system in such remote phyla as protstomian annelids and deuterostomian chordates may share evolutionary common basic mechanisms.

Summary

Defense reactions of annelids may be clearly defined as the two compound systems developed from and housed in the coelom in which cooperate the cellular (celomocytes) and humoral (celomic and vascular fluid) part of immunity. Individual types of celomocytes play a role similar to vertebrate lymphocytes, leukocytes and macrophages. Together, both parts are sufficiently effective in disposing of foreign material and pathogenic invaders.

References

Abeloos M. Recherches histochimique et physiologiques sur le parenchyme et les nephridies des Huridinees Rhynchobdelles. Bull. Biol. Fr. Belg. 59 : 436-456, 1925.

Baskin DG. The coelommocytes of nereid polychaets. Contemp. Topics Immunol. 4: 55-64, 1974.

Bilej M. Cellular defense mechanisms. In: Vetvicka V, Sima P, Cooper EL, Bilej M, Roch P (eds), Immunology of Annelids, CRC Press, Boca Raton, pp 167-200, 1994.

Bilej M. Humoral defense mechanisms. In: Vetvicka V, Sima P, Cooper EL, Bilej M, Roch P (eds), Immunology of Annelids, CRC Press, Boca Raton, pp 245-261, 1994.

Boilly B. Ultrastructure des hématies anuclés de Magelona papillicornis F. Müller (Annélide Polychète). J. Microsc. 19: 47-67, 1974.

Brinkhurst RO, Jamieson BGM. Aquatic Oligochaeta of the world. Oliver and Boyd, Edinburgh, 1971

Cameron GR. Inflammation in earthworms. J. Pathol. Bacteriol. 35: 933-972, 1932.

Clark RB. Dynamics in metazoan evolution, Claredon Press, Oxford, 1964.

Cooper EL, Roch P. Immunological profile of annelids: transplantation immunity. In: Vetvicka V, Sima P, Cooper EL, Bilej M, Roch P (eds), Immunology of Annelids, CRC Press, Boca Raton, pp 201-243, 1994.

Cooper EL, Roch P. Earthworm immunity: a model of immune competence. Pedobiologia, 47: 676-688, 2003.

Cossariza A, Cooper EL, Suzuki MM, Salvioli S, Capri M, Gri G, *et al*. Eartworm leucocytes that are non phagocytic and cross-react with several human epitopes can kill huma tumor cell lines. Exp. Cell Res. 224: 174-182, 1996.

Dales RP. The basis of graft rejection in the earthworms *Lumbricus terrestris* and *Eisenia foetida*. J. Invertebr. Pathol. 32: 264-368,1978.

Dales RP, Dixon LRJ. Polychaetes. In: Invertebrate blood cells 1. Ratcliffe NA, Rowley, AF (eds), Acad. Press, London, pp 35-74, 1981.

Dales RP, Kalaç, Y. Phagoytic defense by earthworm against pathogenic bacteria. Comp. Biochem. Physiol. 101A: 487-490, 1992.

de Eguileor M, Grimaldi A, Tettamani G, Vasvassori R, Cooper EL, Lanzavecchia G. Lipopolysaccharide-dependent induction of leech leukocytes that cross-react with vertebrate cellular differentiation markers. Tissue Cell 32: 437-445, 2000.

De Equileor M, Grimaldi A, Tettamanti G, Congliu T, Protasoni M, Reguzzoni M, *et al*. Ultrastructure and functional versatility of hirudinea botryoidal tissue. Tissue Cell 33: 332-341, 2001.

Dehorne A. Histolyse et phagocytose musculaire dans le coelome des Nerédiens a maturité sexuelle. C. R. Acad. Sci. (Paris) 164: 1043-1063, 1922.

Dhainaut A, Porchet-Henneré A. XIII. Haemocytes and coelomocytes. In: The Ultrastructure of Polychaeta, Microfauna marina 4, Westheide W, Hermans CO (eds), Gustav Fischer Verlag, Stuttgart, pp 215-236, 1988.

Eckelbarger KL. Origin and development of amoebocytes of *Nicolea zostericola (Polychaeta: Terebellidae)* with a discussion of their possible role in oogeneses. Mar. Biol. 35: 169-182, 1976.

Engelmann P, Pal J, Berki T, Cooper EL, Németh P. Earthworm leukocytes feact with different mammalian antigen specific monoclonal antibodies. Zoology 105: 257-265, 2002.

Engelmann P, Cooper EL, Németh P. Anticipating innate immunity without a Toll. Mol. Immunol. 42: 931-942, 2005.

Fischer E. The myelo-erytroid nature of the chloragogenous–like tissues of the annelids. Comp. Biochem. Physiol. 106A: 449-453, 1993.

Fordham MGC. Aphrodie aculeata. L.M.B.C. Memoir, 27, Proc. Liverpool. Biol. Soc. 40: 121-131 1925.

Goodrich S. On the nephridia of *Polychaeta.* II. On *Glycera* and *Goniada*. Q. J. Microsc. Sci. 41: 439-458,1898.

Grimaldi A, Tettamanti G, Perletti G, Valvassori R, de Eguileor M. Hematopoietic cell formation in leech wound healing. Curr. Pharm. Des. 12: 3033-3041, 2006.

Grimaldi A, Bianchi C, Greco G, Tettamanti G, Noonan DM, Valvassori R, *et al*. *In vivo* isolation and characterization of stem cells with diverse phenotypes using growth factor impregnated biomatrices. PLoS One.3:e1910, 2008.

Homa J, Bzowska M, Klimek M, Plytycz B. Flow cytometric qauntification of proliferating coelomocytes non-invasively retrieved from earthworm, *Dnedrobaena veneta*. Dev. Comp. Immunol. 32: 9-14, 2008.

Kukenthal W. Über die lymphoiden Zellen der Anneliden. Jen. Z. Naturwiss. 18: 319-333,1885.

Lefebvre C, Vandenbulcke F, Bocquet B, Tasiemski A, Desmons A, Verstraete M, *et al*. Cathepsin L and cytostatin B gene expression discriminates immune coelomic cells in the leech *Theromyzon tessulatum.* Dev. Comp. Immunol. 32: 795-807, 2008.

Liebmann E. The role of the chloragogue in regeneration of *Eisenia foetida* (Sav.). J. Mophol. 70:151-187,1942.

Metchnikoff E. Lectures on the comparative pathology of inflammation. Starling FA, Starling EH (eds), Dover Press, New York, pp 67-73, 1891.

Oka A. Beitrage zuz Anatomie der Clepsine. Z. Wiss. Zool. 58: 79-98,1894.

Porchet-Henneré E. Cooperation between different coelomocyte populations during the encapsulation response of *Nereis diversicolor*, demonstrated using monoclonal antibodies. J. Invertebr. Pathol. 56: 353-361, 1990.

Porchet-Henneré E, M'Beri M, Dhainaut A, Porchet M. Ultrastructural study of the encapsulation. Response of the polychaete annelid *Nereis diversivolor*. Cell Tissue Res. 248: 463-471, 1987.

Roch P, Cooper EL, Eskinazi DP. Serological evidences for a membrane structure related to human beta 2-microglobulin expressed by certain earthworm leukocytes. Eur. J. Immunol. 13: 1037-1042, 1983.

Romieu M, Recherches histophysiologiques sur le sang et le corps cardiaque des Annélides Polychétes. Contribution a l´histologie comparée du sang. Arch. Morph. Gener. Exp. 17: 1-17, 1923.

Rosa D. Les lymphocytes des Oligochaetes, Arch. Ital. Biol. 25: 455-475, 1896.

Salzet M, Tasiemski A, Cooper E. Innate immunity in Lophotrocozoans: the annelids. Curr. Pharm. Des. 12: 3043-3050, 2006.

Sawyer RT, Fitzgerald SW. Hirudineans, In: Invertebrae blood cells, Vol. 1, Ratcliffe NA, Rowley AF (eds), Academic Press, London, pp 141-159, 1981.

Shalev A, Greenberg AH, Logdberg L, Bjorck L. Beta 2-Microglobulin-like molecules in low vertebrates and inverteebrates. J. Immunol. 127: 1186-1191, 1981.

Sima P. A survey of the evolution of fundamental body construction in relation to immunological phenomena. In: Vetvicka V, Sima P, Cooper EL, Bilej M, Roch P (eds), Immunology of Annelids, CRC Press, Boca Raton, pp 27-39, 1994a.

Sima P. Annelid coelomocytes and hemocytes: Role in cellular immune reactions. In: Vetvicka, V, Sima, P, Cooper, EL, Bilej, M, Roch P (eds), Immunology of Annelids, CRC Press, Boca Raton, pp 115-165, 1994b.

Sima P, Slipka J. The spleen and its coelomic and enteric history. Adv. Exp. Med. Biol. 371A: 331-334, 1995.

Sima P, Bilej M, Slipka J. Perienteral chloragogen tissue and its role in defense in lumbricid worms. Adv. Exp. Med. Biol. 371A: 327-329, 1995.

Valembois P. Etude ultrastructurale de coelomocytes du lombricien *Eisenia foetida* (Sav.). Bull. Soc. Zool. (France) 96: 59-65, 1971.

Wampler JE, Jamieson BGM. Cell bound luminescence from *Pontodrilus bermudensis* and its similarities to other earthworm bioluminiscence. Comp. Biochem. Physiol. 84A: 81-87, 1986.