

VISIONS AND PERSPECTIVES

Immunocyte: the invertebrate counterpart of the vertebrate macrophage**E Ottaviani***Department of Biology, University of Modena and Reggio Emilia, Modena, Italy**Accepted December 15, 2010***Abstract**

The circulating phagocytic immune cell is considered to be the main effector of the invertebrate defense system, involved in both immune and neuroendocrine responses, showing the functional characteristics of vertebrate macrophage. Various names have been used to define this cell in different taxa *i.e.*, hemocyte, celomocyte, amebocyte, plasmatocyte, etc. However, regardless of the terminology, these cells perform the same immune function, and possess very similar morphology. For these reasons, it is suggested that the general term immunocyte be used to describe these cells in invertebrates.

Key Words: immunocyte; invertebrates; immune system**Introduction**

Elie Metchnikoff was the first to suggest an evolutionary mechanism devoted to protecting organisms. He demonstrated the phagocytic role of certain cells of the freshwater crustacean *Daphnia magna* infected by the parasitic fungus *Monospora bicuspidata* (Metchnikoff, 1884), and described in vertebrates two phagocytic cell types, microphages (polymorphonuclear leucocytes) and the mononuclear macrophages (Metchnikoff, 1901).

The discovery of phagocytic cells in humans has diverted attention of the cellular mechanisms present in invertebrates to those found in vertebrates. However, in the last few decades several papers and reviews have been dedicated to phagocytic cells in invertebrates. Different names have been used to describe invertebrate immune cells that exhibit phagocytic activity. Regrettably, this has promoted more confusion than clarification of cell function. There currently is no information on the homology of phagocytic cells in different taxa, and the use of different names for cells that perform the same activities does not enhance understanding among investigators. The present contribution attempts to promote the acceptance and usage of the general name immunocyte to the specific cells in different invertebrates that perform phagocytic activity. Such acceptance would hopefully benefit especially those researches that report cross-taxa information.

Anatomical background of the immune system in non-vertebrate models

The presence of a body cavity can be used as a phylogenetic parameter for classifying different groups of metazoans. Acelomate animals lack a body cavity (Barrington, 1967; Barnes *et al.*, 1988). Pseudocelomates have a pseudocel, a cavity lacking in own walls arising directly from the blastocelic cavity filled of fluid. Celomates that have a true cavity, called celom, that develops within the endomesoderm, covered on its outer surface by the somatic mesoderm and on inner surface by the splanchnic mesoderm. The body cavity of celomates is filled of fluid and is lined by an epithelium.

An open circulatory system is present in molluscs and arthropods, in which a fluid is contained in the dominant body cavity or hemocel. The fluid, usually called hemolymph, can bathe the organs directly and there is no distinction between blood and interstitial fluid. Conversely, annelids, cephalopods and echinoderms possess a closed system characterized by the presence of blood vessels (Brusca and Brusca, 2003). It should be noted that different circulatory systems are not linked to the taxon, consequently they are not good parameters from a phylogenetic point of view. Currently, the relationships among circulating cells and open or closed circulatory systems are unknown.

Invertebrate immune cells

It is well-documented that the immune cells represent the cellular component of invertebrate immune systems (Ratcliffe and Rowley, 1981).

Corresponding author:

Enzo Ottaviani

Department of Biology

University of Modena and Reggio Emilia

Via Campi 213/D, 41125 Modena, Italy

E-mail: enzo.ottaviani@unimore.it

However, there is a general problem in defining the number of circulating immune cells present in the hemolymph, which represents a subject of great debate. There are several reasons for this unsolved problem, including different methods employed in examining invertebrate immune cells, the lacking of an hemopoietic organ in numerous models, and the inability to document cellular maturation.

In this context, an initial morphological examination of adult *Mytilus galloprovincialis* revealed the presence of two cell types in the circulating hemolymph. However, more detailed studies such as functional tests, cytochemical and enzymatic reactions and cytofluorimetric analysis revealed the presence of a single cell type in two different stages, both of which possessed phagocytic activity (Ottaviani *et al.*, 1998). Using light and electronic microscopy two cell types have been described in *M. edulis* and *M. galloprovincialis* (Rasmussen *et al.*, 1985; Renwrantz, 1990; Noël *et al.*, 1993; Cajarville and Pal, 1995). However, using monoclonal antibodies, sub-populations of a single cell type were identified in *M. edulis* (Dyrynda *et al.*, 1997).

In synthesizing the different interpretations of circulating immune cells in bivalves, Cheng (1981) concluded that only two cell types are present: hyalinocytes and granulocytes. Conversely, Mix (1976) suggested that hyalinocytes are an intermediate proliferative stage that matures to become granulocytes.

Another notable example is the classification of immune cells in insects. Rowley and Ratcliffe (1981) reported the presence of the following cell types in the hemolymph: prohemocytes, plasmatocytes, granular cells, cystocytes, spherule cells and oenocytoids. While Brehélin and Zachary (1986) proposed another classification of insect blood cells where nine cell types are described: prohemocytes, plasmatocytes, oenocytoids, spherule cells, thrombocytoids and four types of granular hemocytes. Three basic cell types are observed in the hemolymph of adult insect *Calliphora vomitoria*: prohemocytes, plasmatocytes and granular cells (Franchini *et al.*, 1996). In *Drosophila melanogaster* plasmatocytes, lamellocytes and crystal cells have been described as cells derived from progenitors originated in the larval lymph gland (Nappi *et al.*, 2004).

As previously noted, the lack of an hematopoietic organ presents a problem in classifying blood cell types. However, even when this organ is present problems occur. Indeed the different species of Planorbids and *Lymnaea palustris* (Kinoti, 1971; Lie *et al.*, 1975; Rachford, 1976; Jeong *et al.*, 1983; Ottaviani, 1988) in which a hemopoietic organ is present, it is insufficient to produce the quantity of immune cells that need to the animals, and the majority of the cells comes from the hemolymph.

In annelids, echinoderms and tunicates, a terminology for immune cells includes celomocytes, amebocytes among others (Smith *et al.*, 2006; Ballarin, 2008; Lefebvre *et al.*, 2008; Arizza and Parrinello, 2009; Vetvicka and Sima, 2009). In the leech *Theromyzon tessulatum* three distinct celomic cell populations are reported: the chloragocytes

which were initially defined as large celomocytes, the granular amebocytes and small celomic cells (Lefebvre *et al.*, 2008).

In general, the various annelid cell types are usually characterized as amebocytes, elocytes, erythrocytes and hemocytes in Polychaeta, celomocytes, amebocytes, vascular lymphocytes, elocytes and macrophages in Oligochaeta and amebocytes and chloragocytes in Hirudinea (Vetvicka and Sima, 2009).

In the sea urchin *Strongylocentrotus purpuratus* are described the following celomocyte types: type 1 (discoidal phagocyte), type 2 (polygonal phagocyte), small phagocyte, red spherule cell, colorless spherule cell and vibratile cell (Smith *et al.*, 2006).

In colonial botryllid ascidians and in particular in the species *Botryllus schlosseri* the circulating immune cells are grouped into three main categories: undifferentiated cells, immunocytes, and storage cells (pigment cells and nephrocytes). Immunocytes are represented by cytotoxic morula cells and phagocytes, the latter including hyaline amebocytes and macrophage-like cells (Ballarin, 2008).

In the solitary ascidian *Ciona intestinalis* various cell types are reported: agranular hemocytes, including hemoblasts, circulating lymphocyte-like cells, hyaline amebocytes; granular hemocytes including granulocytes with small granules, granulocytes with large granules, unilocular refractile granulocytes and morula cells (Arizza and Parrinello, 2009).

Invertebrate phagocytic immune cells

As noted above, in different taxa numerous cell types are described and various functions are assigned. Although these cells play a fundamental role in immunity, they unfortunately are designated with different names, and no information is available on their homology.

In the mollusc *Planorbarius corneus* the phagocytic cell has been called spreading hemocyte (Ottaviani, 1983; Ottaviani and Franchini, 1988). These cells show the same function of the spreading amebocytes of *Lymnaea stagnalis* (Stang-Voss, 1970; Sminia, 1972), of the granulocytes of *Bulinus guernei* (Krupa *et al.*, 1977) and the granulocytes of *Biomphalaria glabrata* (Harris, 1975; Joky *et al.*, 1983).

In various insects, the plasmatocytes represent cells with the typical functions of a macrophage, *i.e.*, glass adhesion with the emission of pseudopodia allowing amoeboid movement, phagocytic capacity, encapsulation, nodule formation and wound repair (Rowley and Ratcliffe, 1981; Brehélin and Zachary, 1986; Franchini *et al.*, 1996). However, in Lepidoptera granulocytes are considered the phagocytic cells, whereas plasmatocytes are larger and adhesive cells that cooperate in encapsulation (Nakatogawa *et al.*, 2009)

The amoeboid celomocytes are very important cells of the immune system of annelids. 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 (Vetvicka and Sima, 2009). Smith *et al.* (2006) in their interesting review report that in echinoderms the phagocytes represent the majority of celomocytes involved in graft rejection, chemotaxis, phagocytosis, encapsulation, immune gene expression, agglutination and clotting reactions. In *B. schlosseri*, the circulating professional phagocytes are considered hyaline amoebocytes and macrophage-like cells, which represent two diverse morphologies of the same hemocyte type (Sabbadin, 1955; Ballarin *et al.*, 1993). Also in *C. intestinalis* hyaline amoebocytes are the most common cell type with phagocytic activity (Arizza and Parrinello, 2009).

In conclusion, on the basis of the reported observations, I suggest, from a conceptual point of view, to adopt the general term of immunocyte for the invertebrate cells endowed of characteristic functions of vertebrate macrophage. In this way one immediately understands the kind of cell described, regardless of the taxonomic group involved.

Acknowledgements

The author wish to thank Dr AJ Nappi, Emeritus Professor (Department of Biology, Loyola University, CA, USA) for the critical reading.

References

- Arizza V, Parrinello D. Inflammatory hemocytes in *Ciona intestinalis* innate immune response. *Inv. Surv. J.* 6: S58-S66, 2009.
- Ballarin L, Cima F, Sabbadin A. Histochemical staining and characterization of the colonial ascidian *Botryllus schlosseri* hemocytes. *Boll. Zool.* 60: 19-24, 1993.
- Ballarin L. Immunobiology of compound ascidians, with particular reference to *Botryllus schlosseri*: state of art. *Inv. Surv. J.* 5: 54-74, 2008.
- Barnes, RSK, Calow, P, Olive, PJW, Golding, DW. The invertebrates: a new synthesis. Blackwell Scientific Publications, Oxford, 1988.
- Barrington, EJW. Invertebrate structure and function. Thomas Nelson and Sons Ltd, Australia, 1967.
- Brusca RC, Brusca GJ. Invertebrates. 2nd ed. Sinauer Associates, Inc., Publishers, Sunderland, MA, USA, 2003.
- Brehélin M, Zachary D. Insect haemocytes: a new classification to rule out the controversy. In: Brehélin M (ed), Immunity in invertebrates, Springer-Verlag, Berlin, pp 36-48, 1986.
- Cajaraville MP, Pal SG. Morphofunctional study of the haemocytes of the bivalve mollusc *Mytilus galloprovincialis* with emphasis on the endolysosomal compartment. *Cell Struct. Funct.* 2: 355-367, 1995.
- Cheng TC. Bivalves. In: Ratcliffe NA, Rowley, AF (eds), Invertebrate blood cells. Vol. 1, Academic Press, London, UK, pp 233-300, 1981.
- Dyrynda EA, Pipe RK, Ratcliffe NA. Subpopulations of haemocytes in the adult and developing marine mussel, *Mytilus edulis*, identified by use of monoclonal antibodies. *Cell Tissue Res.* 289: 527-536, 1997.
- Franchini A, Miyan JA, Ottaviani E. Induction of ACTH- and TNF- α -like molecules in the hemocytes of *Calliphora vomitoria* (Insecta, Diptera). *Tissue Cell* 28: 587-592, 1996.
- Harris KR. The structure of encapsulation in *Biomphalaria glabrata*. *Ann. NY Acad. Sci.* 266: 446-464, 1975.
- Jeong KH, Lie KJ, Heyneman D. The ultrastructure of the amoebocyte-producing organ in *Biomphalaria glabrata*. *Dev. Comp. Immunol.* 7: 217-228, 1983.
- Joky A, Matricon-Gondran M, Benex J. Fine structural differences in the amoebocytes of *Biomphalaria glabrata*. *Dev. Comp. Immunol.* 7: 669-672, 1983.
- Kinoti GK. Observations on the infection of bulinid snails with *Schistosoma mattheei*. *Parasitology* 62: 161-170, 1971.
- Krupa PL, Lewis LM, Del Vecchio P. *Schistosoma haematobium* in *Bulinus guernei*: electron microscopy of hemocyte-sporocyst interactions. *J. Invertebr. Pathol.* 30: 35-45, 1977.
- Lefebvre C, Vandenbulcke F, Bocquet B, Tasiemski A, Desmons A, Verstraete M, *et al.* Cathepsin L and cystatin B gene expression discriminates immune coelomic cells in the leech *Theromyzon tessulatum*. *Dev. Comp. Immunol.* 32: 795-807, 2008.
- Lie KJ, Heyneman D, Yau P. The origin of amoebocytes in *Biomphalaria glabrata*. *J. Parasitol.* 63: 574-576, 1975.
- Metchnikoff E. A disease of *Daphnia* caused by a yeast. A contribution to the theory of phagocytes as agents for attack on disease-causing organisms. In: Brock T (ed), Milestones in microbiology, Washington, DC: American Society for Microbiology, pp 132-138, 1884.
- Metchnikoff E. Immunity in infective diseases. Cambridge University Press, Cambridge, UK, 1901.
- Mix MC. A general model for leukocyte cell renewal in bivalve mollusks. *Mar. Fish. Rev. US Natl. Mar. Fish. Serv.* 38: 37-41, 1976.
- Nakatogawa S, Oda Y, Kamiya M, Kamijima T, Aizawa T, Clark KD, *et al.* A novel peptide mediates aggregation and migration of hemocytes from an insect. *Curr. Biol.* 19: 779-785, 2009.
- Nappi AJ, Kohler L, Mastore M. Signaling pathway implicated in the cellular innate immune responses of *Drosophila*. *Inv. Surv. J.* 1: 5-33, 2004.
- Noël D, Bachère E, Mialhe E. Phagocytosis associated chemiluminescence of hemocytes in *Mytilus edulis* (Bivalvia). *Dev. Comp. Immunol.* 17: 483-483, 1993.
- Ottaviani E. The blood cells of the freshwater snail *Planorbis corneus* (Gastropoda, Pulmonata). *Dev. Comp. Immunol.* 7: 209-216, 1983.
- Ottaviani E. Histological and immunocytochemical studies on the origin of haemocytes in the freshwater snail *Planorbis corneus* (L.) (Gastropoda, Pulmonata). *Z. mikrosk.-anat. Forsch.* 102: 649-654, 1988.
- Ottaviani E, Franchini A. Ultrastructural study of haemocytes of the freshwater snail *Planorbis corneus* (L.) (Gastropoda,

- Pulmonata). Acta Zool. (Stockh.) 69: 157-162, 1988.
- Ottaviani E, Franchini A, Barbieri D, Kletsas D. Comparative and morphofunctional studies on *Mytilus galloprovincialis* hemocytes: presence of two aging-related hemocyte stages. Ital. J. Zool. 65: 349-354, 1998.
- Rasmussen LPD, Hage E, Karlog O. An electron microscope study of the circulating leucocytes of the marine mussel, *Mytilus edulis*. J. Invertebr. Pathol. 45: 158-167, 1985.
- Rachford FW. Host-parasite relationship of *Angiostrongylus cantonensis* in *Lymnaea palustris*. II. Histopathology. Exp. Parasitol. 39: 382-392, 1976.
- Ratcliffe NA, Rowley, AF. Invertebrate blood cells. Vols 1, 2, Academic Press, London, UK, 1981.
- Renwranz L. In ternal defense system of *Mytilus edulis*. In: Stefano GB (ed), Neurobiology of *Mytilus edulis*, Studies in neurosciences, N. 10, Manchester University Press, Manchester, pp 256-275, 1990.
- Sabbadin A. Studio sulle cellule del sangue di *Botryllus schlosseri* (Pallas) (Asciacea). Arch. Ital. Anat. Embriol. 60: 33-67, 1955.
- Sminia T. Structure and function of blood and connective tissue cells of the fresh pulmonate *Lymnaea stagnalis* studied by electron microscopy and enzyme histochemistry. Z. Zellforsch. 130: 497-526, 1972.
- Smith LC, Rast JP, Brockton V, Terwilliger DP, Nair SV, Buckley KM, et al. The sea urchin immune system. Inv. Surv. J. 3: 25-39, 2006.
- Stang-Voss C. Zur Ultrastruktur der Blutzellen wirbelloser Tiere. III. Über die Haemocyten der Schnecke *Lymnaea stagnalis* L. (Pulmonata). Z. Zellforsch. 107: 142-156, 1970.
- Vetvicka V, Sima P. Origins and functions of annelide immune cells: the concise survey. Inv. Surv. J. 6: 138-143, 2009.