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MINIREVIEW

# The case of the "serfdom" condition of phagocytic immune cells

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## Abstract

In a modern immunological perspective, it may be asserted that the phagocytic cell should not be considered as the "serfdom", but rather the pivot of the immune system. Indeed, the invertebrate immunocyte as well as the vertebrate macrophage play a central role in immunity, inflammation and stress response. The evolutionary conserved immune-neuroendocrine effector system have remained of fundamental importance in defense against pathogens, and its efficiency increased through synergy with the new, clonotipical recognition repertoire in vertebrates.

**Key Words**: invertebrate immunocyte; vertebrate macrophage; immune and neuroendocrine responses; evolution

## Introduction

The historical term "serfdom" indicates the low socio-economic status of several peasants during feudalism. As a paradox, it may be stated that a very low level in the functioning of immune system has been attributed for a long time to the vertebrate macrophage and, for extension, also to invertebrate phagocytic immune cells. Indeed, expecially among vertebrate immunolgists' attitude, it appears that these cells do not have the "royal" position occupied in the immune system by the lymphocytes. Despite this obsolete point of view, the invertebrate immunocyte/vertebrate macrophage plays a crucial role in immune and neuroendocrine responses in multicellular organisms, and its importance has been neglected for a long time even though it is obviously the pivotal immune cell in forms of life endowed of only innate immunity, as invertebrates. Beside this wrong opinion, also the concept that the invertebrate recognition is gross and far less sophisticated than vertebrates was quite diffused before the discovery of molecules such as Dscam, 185/333 and FREP (Watson *et al*., 2005; Bowden *et al.,* 2007; Ghosh *et al.*, 2010; Romero *et al.*, 2011). Before the advances of the last decade in invertebrate immunity, several vertebrate immunologists underestimated the power of invertebrate immune system, since their scientific interest was mainly focused on mammalian models. Despite the difficulty of debunking this old concept among human immunologists, every day new evidence emerge about the role played by phagocytic immune cells.

The root of the misconception may be linked to the attitude of the two scientists who formulated the two theories of the immunity, the cellular (Elie Metchnikoff) and humoral (Paul Ehrlich). Not surprisingly, both of them thought that one component was more significant than the other, but the humoral theory for a long time has played a dominant role as giving responses to unanswered questions on the immunological mechanisms and related diseases.

In this minireview we recapitulate the findings that justify why the pahogytic immune cells should be considered the major players of the immune system.

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## Phagocyte: morpho-functional characteristics

In mammals, macrophage appears to derive from a common progenitor in the bone marrow which gives rise to a separate lineage. All the professional phagocytic cells in humans derive from circulating monocytes and acquire new morphological and physiological characteristics according to the organs and microenvironments in which they settle. This unitarian origin is uncertain for circulating and tissue phagocytes in invertebrates, so we proposed the term "immunocyte" to indicate circulating cells endowed of phagocytic activity, irrespective of their developmental origin (Ottaviani, 2011). In our view, the most general characteristic shared by immunocytes and macrophages throughout metazoans is the ability to be activated by not-self material and to react through the release

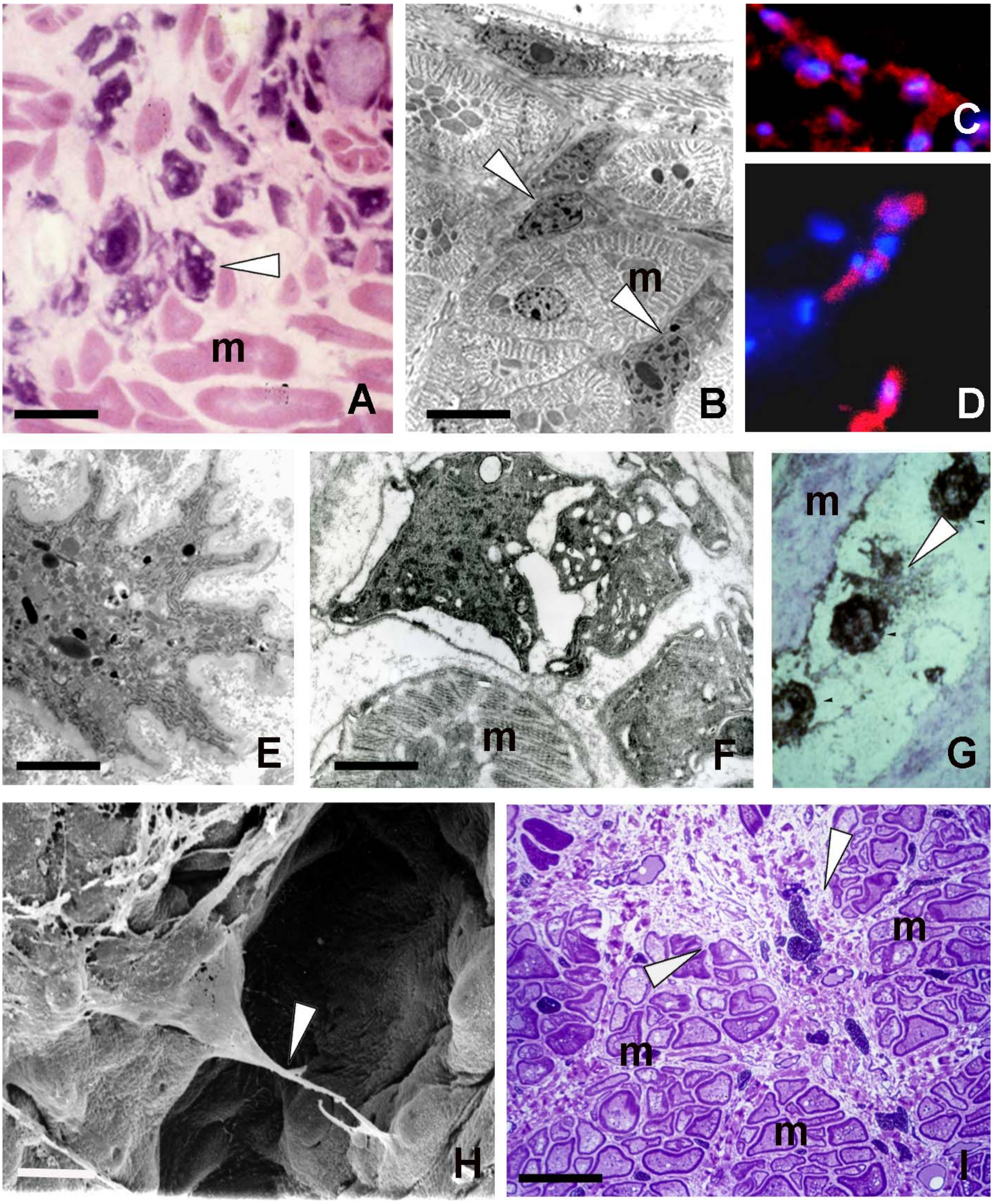


**Fig. 1**. Leech body wall after injection with LPS. **A**) Semi-thin section of leech body wall. The adjacent fields of muscle fibers (m) usually in close contact are separated by numerous migrating cells (arrowhead). **B**) Thin section of leech body wall. Under the epithelium, among the muscle fibers (m) forming the muscular sac, migrating cells (arrowheads), irregularly shaped (their dimensions vary due to emission of pseudopodia) are recognizable. **C**, **D**) Cryosections, Indirect immunofluorescence staining of migrating cells using anti-D14 (**C**) and anti-CD68 (**D**). CD14 and CD68 are typically expressed by macrophages in a wide variety of tissues. **E**, **F**) TEM. Macrophage-like cells moving in the connective tissue show ruffled surface and projections. **G**) Cryosection. Cathepsin B (arrowhead), indicated by dark brown signal, is localized in macrophage-like cells migrating among muscle fibers (m). **H**) SEM. Area of lesion where macrophage-like cell, showing thin pseudopodia (arrowhead), bridges the epithelial edges. **I**) Semi-thin section of lesioned leech body wall. Macrophage-like cells are involved in the obstruction of the wound (arrowheads). Migrating cells form a wedge-shaped cluster between muscle fibers (m).
Bars: A: 25 μm; B: 10 μm; E: 2 μm; F: 5 μm; H: 20 nm.

a variety of biologically active molecules, such as cytokines, nitric oxide, reactive oxygen species, hydrolytic enzymes, adrenocorticotropic hormone (ACTH), β-endorphin and corticotropin-releasing hormone (CRH) that affect the immune and the neuroendocrine responses (Ottaviani and Franceschi, 1997; Ottaviani *et al.*, 1998, 2004, 2007). As far as the immune role of the reported molecules is concerned, it has been found that mammalian cytokines, ACTH peptide fragments and CRH are able to influence the chemotactic and phagocytic activity of invertebrate immunocytes and vertebrate macrophages (Ottaviani *et al.*, 1990, 1992, 1994, 1997, 2004; Genedani *et al.*, 1994). These findings revealed that the pro-chemotactic and pro-phagocytic effects of the above reported mammalian molecules on invertebrate immunocytes are not straightforward being specie-specific and dose-correlated. The effects on phagocytosis are more uniform than those registered on chemotaxis, making the general assumption that chemotaxis and phagocytosis are strictly correlated, not valid for all invertebrates. The discrepancies observed for the effects of different heterologous peptides on diverse invertebrate immune functions may be attributed to several factors, not excluded the possibility that the immunocyte responds to the same stimulus on the basis of contingency (Ottaviani *et al*., 1997, 2004; Malagoli and Ottaviani, 2010a).

As far as the neuroendocrine responses are concerned, it has been demonstrated that the principle mediators of stress response in invertebrates are fundamentally comparable to those known in vertebrates and have been conserved during evolution (Malagoli *et al.,* 2011). We have found that molecules similar to the mediators of mammalian stress, CRH, ACTH, biogenic amines, glucocorticoids and cytokines, are present in and/or released by invertebrate immunocyte (Ottaviani and Franceschi, 1996; Ottaviani *et al.*, 1998; Malagoli *et al.,* 2007). It is to underline that even though in invertebrates the molecules are those that participate also in vertebrate stress response, in invertebrates the framework is simpler. In vertebrates, various organs are involved, while all the molecules determining invertebrate stress response are harboured into the immunocyte. In other words, the prototypical response in invertebrates appears to be concentrated into a single, multifunctional cell, representing the best example of an immune-neuroendocrine cell observed so far.

During animal diversification, four fundamental evolutionary events that have influenced the formation of the increasingly complex vertebrate immune system, can be recognized. The first evolutionary event is represented by the formation of multicellular organisms, not so much the formation of colonies where the cells are more or less identical to one another, but the association of cells that differ and are able to perform different integrated functions. This series of events is observed for the first time in Porifera. The second event is the appearance of a body cavity, *i.e.*, a place in which the circulating cells may be retrieved. The third evolutionary event, is represented by deuterostomia (the blastopore becomes the anus) characteristic of some invertebrate (*e.g.*, echinoderms) and the vertebrate embryos. The fourth event coincides with the transition from the aquatic to the terrestrial area by tetrapode vertebrates. By examining the different species included in the four evolutionary events reported, the key role played by the phagocyte emerges (see Turner, 1994). It should be underlined that in animals without a digestive tract, such as Porifera and Coelenterata, the phagocyte plays a double role *i.e.,* defense and nutrition. Hildemann *et al.* (1979) identified in the immune system of the sponge *Callyspongia diffusa* (Porifera)*,* the three functional components as minimal criteria for immunological competence: cytotoxicity, specificity and memory.

From invertebrates to vertebrates immune cells are capable of sophisticated performances. As far as immune and neuroendocrine functions are concerned, it is amazing to observe how phagocyting immune cells perform functions ranging from chemotaxis, phagocytosis, encaspsulation, transplantation, wound healing, cytotoxic activity to the stress response (Turner, 1994; Ottaviani *et al.*, 1997; de Eguileor *et al*., 2000a, b; Grimaldi *et al*., 2004).

On the whole, it emerges that phagocytic immune cells are endowed of a vastly dinamic morphology and, beside molluscs (Ottaviani, 2011), an interesting example is also represented by *Hirudo* immunocytes (de Eguileor *et al.*,1999, 2000a, b). In leeches, the responses to surgical wounds, grafts or LPS injection are similar to those obtained in vertebrates and involve a sequence of events triggered by inflammatory reactions (de Eguileor *et al.*, 2003, Tettamanti *et al.*, 2003a, b). After immune stimulation a large number of cells migrate through the extracellular matrix from the inner regions of the body close to the gut, towards the superficial body area (Figs 1A, B). These migrating cells have been characterized by morphological, histochemical, and immunohistochemical methods. Macrophage-like cells, NK-cells and granulocytes are the cell types more represented. In particular several migrating cells involved in leech defense system, display features and behaviour observed also in invertebrate and vertebrate activated macrophages being $CD25^+$, $CD14^+$, $CD61^+$, $CD68^+$, $CD11b^+$ and $CD11c^+$ (de Eguileor *et al*., 2000a). Furthermore, immunocytes modify their morphology in relation to the time elapsed after the immune stimulation. During the phase of migration immunocytes change their shape showing ruffled borders (Figs 1A-G) and supply enzymes (Cathepsin B) to degrade components of extracellular matrix outside the cells, ensuring the movement in the connective tissue (Fig. 1G) (Grimaldi *et al.*, 2004). In lesioned leeches immunocytes are the first cells that are also involved in closing the wound by using pseudopodia to bridge the epithelial edges (Fig. 1H). Subsequently additional immunocytes togheter with granulocytes and NK cells complete the obstruction (Fig. 1I) (de Eguileor *et al.*, 2000a). During wound healing the principal function of immunocytes is phagocitosis. Indeed, in their cytoplasm large phagocytic vacuoles containing citoplasmic debris or bacteria or any kind of foreign antigen are always visible (Fig. 1E) (de Eguileor *et al.*, 2000a).

## Concluding remarks

Overall, we sustain for the circulating and phagocytic cells the part of principal actor not only during inflammatory response and immunity, as originally suggested by Mechnikoff (Tauber, 2003), but also during stress response, at least in invertebrates. In this scenario, an unitarian view emerges in which phagocytic immune cells, the most ancestral defense cell of the body, continues to play a fundamental role throughout evolution in the most complex phenomena responsible for body homeostasis, *i.e.*, immunity, inflammation and stress response (Ottaviani and Franceschi, 1997; Ottaviani *et al*., 2007). However, without minimizing the role played by the cells of acquired immunity, *i.e.*, the vertebrate lymphocytes, they have a more restricted number of functions requiring the involvement of components of innate immunity to be completed. Furthermore, the lymphocytes play a key role in immunological memory, an immune function crucial for homeothermic vertebrates, while for the cold-blooded ones it seems less relevant (Malagoli and Ottaviani, 2010b). In this context, the bony fish have their lymphocytes suppressed at low temperatures and, in this situation, found in phagocytic cells the main actors of the immune defense (Bly *et al.*, 1990). From these examples it may be argued that in vertebrates, the conserved invertebrate immune-neuroendocrine effector system, centered on the immunocyte, remains of fundamental importance in defense against pathogens, during evolution, and its efficiency increased through synergy with the new, clonotipical recognition repertoire.

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