ISJ 1: 2-4, 2004 ISSN 1824-307X

Visions and Perspectives

The mollusc as a suitable model for mammalian immune-neuroendocrine investigations

E Ottaviani

Department of Animal Biology, University of Modena and Reggio Emilia, Modena, Italy

Accepted June 30, 2004

Abstract

The same or relatively similar molecules seen in molluscan defense responses are also found in mammals, where their functions remain basically similar. The mollusc immunocytes are able to recognize a variety of stimuli and to set up correspondingly complex responses, in which primitive, but very efficient, forms of immune and neuroendocrine responses are intermixed. Thus, invertebrates could represent an ideal alternative in studying mammalian complexity from an immuno-neuroendocrine point of view.

Key words: immune-neuroendocrine responses; molluscs; mammals

Immune responses

Molluscs are characterized by a coelomatic cavity, which makes it possible to distinguish a well-defined cellular and humoral component in the immune system (for review, see Ottaviani, 1992). In the majority of molluscs, there are two circulating immunocytes, which control the main immune responses, i.e. phagocytosis, cell shape changes (the expression of cell motility), chemotaxis (the expression of cell migration), and cytotoxicity.

The humoral factors are represented by agglutinins, lectins, nitric oxide, cytokine-like molecules (lm), corticotropin-releasing hormone (CRH)-lm, enkephalin-lm, pro-opiomelanocortin (POMC)-derived peptide-lm, e.g. adrenocorticotropin hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH) and β-endorphin (for reviews, see Stefano *et al.*, 1989; Ottaviani, 1992; Ottaviani *et al.*, 1997). There is a close relationship between the two components, and it has been observed that the exogenous humoral factors increase cell motility, cell migration and phagocytosis (for reviews, see Stefano *et al.*, 1989; Ottaviani *et al.*, 1997). Exogenous molecules, such as CRH, ACTH, interleukin (IL)-8, platelet-derived growth factor (PDGF)-AB and transforming growth factor (TGF)-β1, induce cell shape changes in the immunocytes via an adenylate cyclase/cAMP/protein kinase A pathway, as well as the activation of protein kinase C (Sassi *et al.*, 1998; Malagoli *et al.*, 2000; for review, see Ottaviani *et al.*, 2001). As other invertebrates, molluscs are able to discriminate between self and not-self. In a freshwater mollusc, an autograft was accepted, while an allograft and xenograft were rejected. These latter elicited an initial inflammatory reaction followed by the encapsulation of the foreign tissue (Ottaviani and Vergine, 1990). Humoral and cellular experiments, bacterial clearance studies, and the specific responses found in molluscan transplantations suggest the presence of a memory-type response of short duration (Ottaviani *et al.*, 1986; Ottaviani, 1990; Ottaviani and Vergine, 1990). With regard to cytotoxicity, a natural killer (NK)-like activity has been observed which is modulated by IL-2 (Franceschi *et al.*, 1991). Hubert *et al.* (1997) isolated in the mussel a cytotoxic protein complex able to kill eukaryotic cells, tumor cells and protozoan parasites. Altogether, the molluscan immune system presents the three functional components identified by Hildemann *et al.* (1979) as minimal criteria for immunological competence, i.e. specificity, cytotoxicity and memory.

Corresponding Author:
Enzo Ottaviani
Department of Animal Biology, University of Modena and Reggio Emilia, Modena, via Campi 213/D, 41100 Modena, Italy
E-mail: ottaviani.enzo@unimo.it

Neuroendocrine responses

It is well known that CRH and ACTH are the main mediators of stress response in vertebrates. This phenomenon involves several organs. The release of ACTH by the pituitary, which is modulated by hypothalamic CRH, guides the glucocorticoids by means of the adrenal gland, which, in turn and together with the sympathetic nervous system, induces the release of catecholamines from adrenal medulla. Molluscs present a stress response superimposed on that observed in mammals, in which the key mediators are the same, i.e. CRH and ACTH, and the series follows the same order and pattern, i.e. CRH > ACTH > biogenic amines. Together with POMC-products and CRH-lm, the presence of biogenic amines and cortisol-lm has also been reported in molluscs (Ottaviani *et al.*, 1998; for review, see Ottaviani and Franceschi, 1996). However, unlike in vertebrates, the stress response in invertebrates does not require the intervention of numerous organs and cells. It is rather concentrated in a single immune-neuroendocrine cell, which is also able to perform fundamental immune functions (for review, see Ottaviani and Franceschi, 1996).

Invertebrates and mammals: similarities in the immune and neuroendocrine mechanisms

The data reported above demonstrates that similarities exist in both the immune and neuroendocrine mechanisms of invertebrates and mammals (Fig. 1). These extraordinary parallelisms are also highlighted by the fact that the mechanisms appear to use the same signal molecules. Indeed, the history of all the biological sciences demonstrates the significance and contribution of invertebrate models. In brief, cytokine-lm are present in the molluscan hemolymph, immunocytes and nervous system, and they seem to act in concert with similar effects both in molluscan and human immunocytes. Another parallelism between mammals and invertebrates regards the stress response. In general, invertebrates, as mammals (for review, see Blalock, 1989), present a bidirectional interaction between the immune and neuroendocrine systems. Indeed, the various peptides found in invertebrates derive from neural tissue (for review, see Stefano, 1989), endocrine tissue (for review, see Roeder, 1995) and immunocytes, and act as messengers between the two systems, playing an important role in the autoregulatory communication between immunocompetent cells.

Thus, invertebrate organisms represent an ideal alternative to mammals, since they mirror in a simplified way the immuno-neuroendocrine activities in vertebrate cells.

Stefano's group pioneered the use of invertebrate models to analyse the complex biological mechanisms in mammals. Similar mechanisms of ACTH action have been found both in molluscan and in human immunocytes in schistosomiasis (Duvaux-Miret *et al.*, 1992). The parasite *Schistosoma mansoni* is able to avoid the immune response of the intermediate (the mollusc *Biomphalaria glabrata*) and the final host(man). POMC products such as ACTH and β-endorphin are released from the worm. During infection, the neutral endopeptidase 24.11 converts

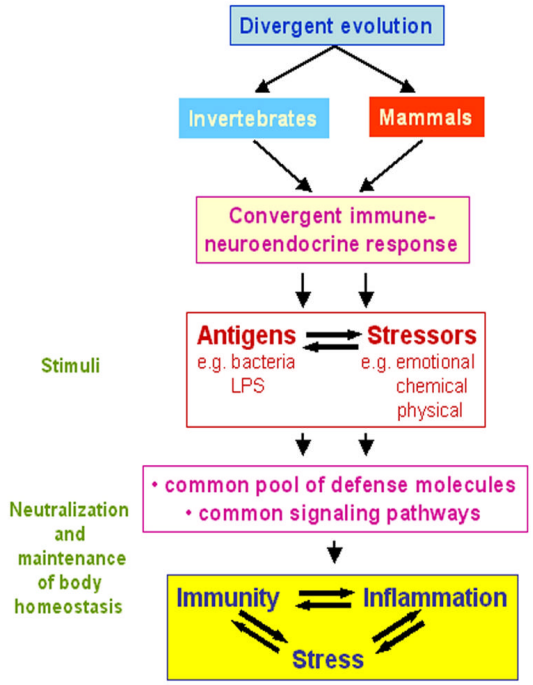

Fig. 1. Scheme of an unitarian immuno-neuroendocrine interaction during evolution.

ACTH into α-MSH which, in turn, deactivates the immunocytes of the intermediate and final hosts. It is also known that α-MSH exhibits an immunosuppressive effect on invertebrates and mammalian immunocytes (Stefano *et al.*, 1991; Van Epps *et al.*, 1992).

Another example of ACTH immunomodulation with the same characteristics in both molluscan immunocytes and human granulocytes has been demonstrated in human immunodeficiency virus (HIV) (Smith *et al.*, 1992). HIV induces the production of ACTH and MSH by H9 T-lymphoma cells. These peptides provoke the deactivation of granulocytes in 2 h by ACTH and in 20 min. by MSH. The longer period required by ACTH is because the immunosuppressive action is observed after its conversion to MSH, an action modulated by NEP. Similar experiments performed on molluscan immunocytes revealed a superimposed response by ACTH and MSH (Smith *et al.*, 1992).

The addition of a synthetic peptide fragment of HIV gp120 blocks the movement of spontaneously active human granulocytes and mussel immunocytes, while stimulates monocyte spontaneous cell motility. Moreover, when gp120 is added together with the chemotactic substances D-ala^2-D-met^5 enkephalinamide (DAMA) or IL-1, a slow and not directional cell migration of both human granulocytes and mussel immunocytes is observed, indicating that gp120 reduces the chemotatctic effect of both the opioid DAMA and IL-1. The effect provoked by gp120 seems to be due to its irreversible binding to the calcium channel (Stefano *et al.*, 1993). This would represent that an inhibitor mechanism has been conserved in molluscan and human immunocytes.

Acknowledgement
This study was supported by MIUR (Italy) grant to E.O.

References

Blalock JE. A molecular basis for bidirectional communication between the immune and neuroendocrine systems. Physiol. Rev. 69: 1-32, 1989.

Duvaux-Miret O, Stefano GB, Smith EM, Dissous C, Capron A. Immunosuppression in the definitive and intermediate hosts of the human parasite *Schistosoma mansoni* by release of immunoactive neuropeptides. Proc. Natl. Acad. Sci. USA 89: 778-781,1992.

Franceschi C, Cossarizza A, Monti D, Ottaviani E. Cytotoxicity and immunocyte markers in cells from the freshwater *snail Planorbarius corneus* (L) (*Gastropoda, Pulmonata*): implications for the evolution of natural killer cells. Eur. J. Immunol. 21: 489-493, 1991.

Hildemann WH, Johnson IS, Jokiel PL. Immunocompetence in the lowest metazoan phylum: transplantation immunity in sponge. Science, 204: 420-422, 1979.

Hubert F, Cooper EL, Roch P. Structure and differential target sensitivity of the stimulable cytotoxic complex from hemolymph of the Mediterranean mussel *Mytilus galloprovincialis*. Biochim. Biophys. Acta 1361: 29-41, 1997.

Malagoli D, Franchini A, Ottaviani E. Synergistic role of cAMP and IP_3 in corticotropin-releasing hormone-induced cell shape changes in invertebrate immunocytes. Peptides 21: 175-182, 2000.

Ottaviani E, Aggazzotti G, Tricoli S. Kinetics of bacterial clearance and selected enzyme activities in serum and haemocytes of the freshwater snail *Planorbarius corneus* (L.) (Gastropoda, Pulmonata) during the primary and secondary response to *Staphylococcus aureus*. Comp. Biochem. Physiol. 85C: 91-95, 1986.

Ottaviani E, Franceschi C. The neuroimmunology of the stress response from invertebrates to man. Prog. Neurobiol. 48: 421-440, 1996.

Ottaviani E, Franchini A, Franceschi C. Presence of immunoreactive molecules to CRH and cortisol in invertebrate haemocytes and lower and higher vertebrate thymus. Histochem. J. 30: 61-67, 1998.

Ottaviani E, Franchini A, Franceschi C. Pro-opiomelanocortin-derived peptides, cytokines and nitric oxide in immune responses and stress: an evolutionary approach. Int. Rev. Cytol. 170: 79-141, 1997.

Ottaviani E, Franchini A, Kletsas D. PDGF and TGF-β in invertebrate immune and neuroendocrine interactions: another sign of conservation in evolution. Comp. Biochem. Physiol. 129C: 295-306, 2001.

Ottaviani E, Vergine C. Allo-implant in the freshwater snail *Planorbarius corneus* (L.) (Gastropoda, Pulmonata). I. Histological and histochemical study. Zool. Jb. Physiol. 94: 261-267, 1990.

Ottaviani E. Immunocytochemical study on bacterial elimination from the freshwater snail *Planorbarius corneus* (L.) (Gastropoda, Pulmonata). Zool. Jb. Anat. 120: 57-62, 1990.

Ottaviani E. Immunorecognition in the gastropod molluscs with particular reference to the freshwater snail *Planorbarius corneus* (L.) (Gastropoda, Pulmonata). Boll. Zool. 59: 129-139, 1992.

Roeder T. Octopamine in invertebrates. Prog. Neurobiol. 59: 533-561, 1999.

Sassi D, Kletsas D, Ottaviani E. Interaction of signalling pathways in ACTH (1-24)-induced cell shape changes in invertebrate immunocytes. Peptides 19: 1105-1110, 1998.

Smith EM, Hughes TKJr, Hashemi F, Stefano GB. Immunosuppressive effects of corticotropin and melanotropin and their possible significance in human immunodeficiency virus infection. Proc. Natl. Acad. Sci. USA 89: 782-786,1992.

Stefano GB, Smith DE, Smith EM, Hughes TK. MSH can deactivate both TNF stimulated and spontaneously active immunocytes. In: Kits KS, Boer HH, Joosse J (eds), Molluscan neurobiology, North Holland, Amsterdam, pp 206-209, 1991.

Stefano GB, Sawada M, Smith EM, Hughes TK. Selective effects of human immunodeficiency virus (HIV) gp120 on invertebrate neurons. Cell. Mol. Neurobiol. 13: 569-577, 1993.

Stefano GB. Role of opioid neuropeptides in immunoregulation. Prog. Neurobiol. 33: 149-159, 1989.

Van Epps DE, Mason MM. Modulation of leukocyte migration by α-melanocyte stimulating hormone. In: Florey E, Stefano GB (eds), Comparative aspects of neuropeptide function, Pergamon Press, Oxford, pp 335-345, 1992.