ISJ 8: 56-58, 2011 ISSN 1824-307X

VISIONS AND PERSPECTIVES

Stress-based modulation of the immune response in molluscan hemocytes: a two-receptor model

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Accepted March 23, 2011

Abstract

In molluscs, hemocytes perform the molecular mechanisms related to immunity. These cells have the ability to respond to the different varieties of stress by modulating their responses. The stressors may be bacterial toxins, cytokines or growth factors, and even physical agents such as changes in temperature or oxygen partial pressure. In the first place, hemocytes synthesise catecholamines, which, in turn, modify the immune response in terms of phagocytosis or nitric oxide synthesis. According to studies on the hemocytes of the mussel *Mytilus galloprovincialis*, we propose a model for a sequential action where the IL-2 receptor and its wide agonist specificity play an important role. Also, α and β-adrenergic receptors suggest the functioning of a return-to-hemocyte mechanism. The model is proposed taking into account the possible relationship between the pathways mediated by cAMP-activated protein kinase and protein kinase C in hemocytes.

Key Words: molluscs; *Mytilus galloprovincialis*; stress; endocrinology; immunology

Introduction

In molluscs the immune response is performed by specialized cells termed hemocytes. Because molluscs lack an adaptative immune system, the response against toxic agents of diverse origin is performed through an ancestral process that was preserved along the evolution until vertebrates and is known as innate immunity (Medzitov and Janeway, 1997). Up to date, the system has been admitted to involve a rigid response; however, new data regarding the structures of the recognition elements suggest a certain selective ability, which allows guessing the elaboration of a possible molecular memory (Brehélin and Roch, 2008; Ottaviani, 2011).

The immune response of molluscan hemocytes is modulated by stress in a way apparently similar to that described in vertebrates as "hypothalamus-hypophysis-adrenal axis" (HPA axis), constituting a proto-response to stress centered on these cells, which can be qualified as an "immune-mobile brain" (Ottaviani *et al*. 1993).

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List of abbreviations:

ACTH: Adrenocorticotropic hormone; CRH: Corticotrophin-releasing hormone; ERK: extracellular signal-regulated kinase; IFN: Interferon; IL-2: Interleukin-2; (IL-2R: IL-2 receptor; JNK: c-Jun N-terminal kinase; LPS: Lipopolysaccharide; MAPK: Mitogen-activated protein kinase; NO: nitric oxide; PDGF: Platelet-derived growth factor; PKA: cAMP-activated protein kinase; PKC: protein kinase C; ROS: oxygen radicals; STAT: Signal Transducers and Activators of Transcription protein; TNF: Tumor necrosis factor.

The receptor-based modulation of molluscan immunity

When a foreign element enters the organism, the immune response increases the phagocytic activity, among other actions. Phagocytosis develops in several stages, and one of them is the synthesis of ROS and NO, leading to the generation of peroxynitrite. When molluscan hemocytes are incubated with agonists so different in structure as growth factors (PDGF, TNF), peptidic hormones (ACTH, CRH), interleukins (IL-2), or even bacterial toxins (LPS), catecholamine and NO synthesis is induced (Ottaviani and Franceschi, 1996). The

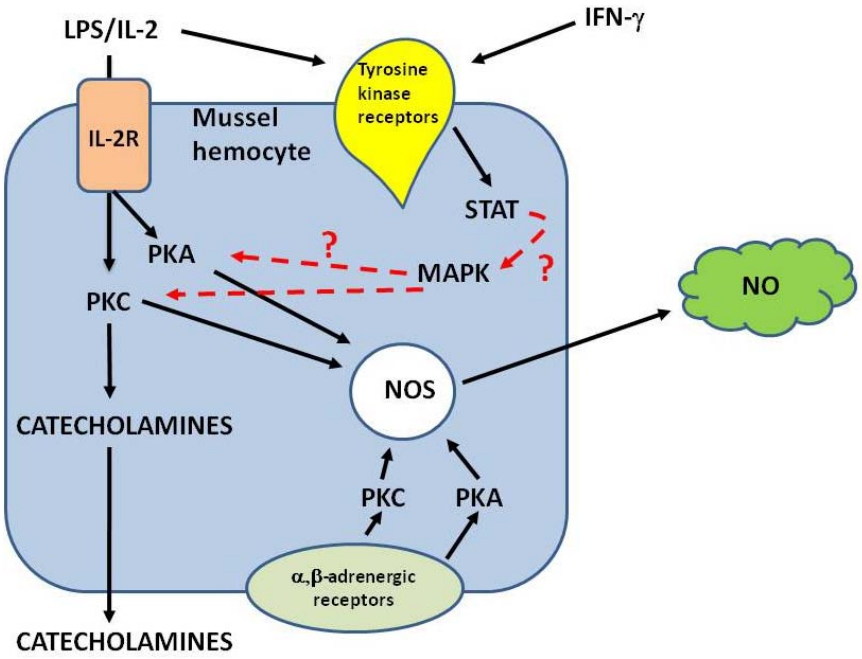

Fig. 1 Hypothesis on the stress-based molecular mechanisms modulating the immune response in hemocytes of *Mytilus galloprovincialis* Lmk. Nitric oxide synthase (NOS). Other abbreviations, in the text. The red lines suggest non demonstrated pathways.

effects are detectable, both in freshly extracted hemocytes (therefore, stressed because of extraction procedure) and in *Mytilus* hemocytes stabilized after three days in culture (Cao *et al*., 2004, 2007a). Depending on the agonist assayed, differences in the expression of the subunits forming the IL-2 receptor were detected, mainly of the α subunit (Cao *et al*., 2004, 2007a). This agrees with the hypothesis about the presence of a unique ancestral receptor with wide specificity (Ottaviani and Franceschi, 1996). On the other hand, the same agonists also provoke remarkable increases in catecholamine production, which evidencing a stress on these cells (Cao *et al.*, 2004, 2007a, b). The catecholamines that the hemocytes or other cells secrete might take a return-to-hemocyte pathway, or else, act directly as suggested by the presence of α and β adrenergic receptors in *Crassostrea gigas* (Lacoste *et al*., 2001).

The signals generated by catecholamines are internalized through PKA and PKC, as observed in *Crassostrea* hemocytes (Lacoste *et al*., 2001, 2002). Also, the signals of LPS and other bacterial factors involve the activation of stress kinases, such as p38-MAPK, JNK o ERK (Canesi *et al.,* 2002, Betti *et al*., 2006, Ciacci *et al*., 2010). At the same time, PKA and PKC mediate NO synthesis (Barcia and Ramos-Martínez, 2008, Gonzalez-Riopedre *et al*., 2009). A common intermediation of some protein kinases related to stress (MAPK) in phagocytosis and in synthesis of thermal shock proteins has been reported in *Mytilus* hemocytes incubated with toxins and bacteria (Canesi *et al*., 2002; Gaitanaki *et al.*, 2004; Kefaloyianni *et al.,* 2005), and MAPK is known to mediate processes activated through G protein receptors. Therefore, there it seems to be a network between the different protein elements involved in the internalization of the stress processes and those evidencing infective or inflammatory actions.

Canesi and colleagues also reported that the incubation of *Mytilus* hemocytes with the macrophage activator IFN-γ induced STAT phosphorylation, which proves the convergence towards MAPK-like phosphorylation mediators (Canesi *et al*., 2003). This result leaves open the possibility of the existence in hemocytes of two types of receptor for immune response activating agonists. One of them with wide specificity, similarly to IL-2R of vertebrates, and another one similar to the tyrosine kinase receptors, which would internalize STAT activating signals. In addition, at least other two adrenergic receptors should operate the modulation of the catecholamine-generated response.

The differences in the timing of signal internalization account for the operability of the different types of receptors and suggests a possible consecutive connection of their actions. In this sense, IL-2 and LPS increase catecholamine synthesis quickly (30 - 60 min) and then, a return to basal values is detected with longer incubation times (Cao *et al.,* 2004, 2007a, b; Gonzalez-Riopedre *et al*., 2009). On the contrary, NO synthesis requires a more prolonged incubation with the agonists (Novas *et al*., 2004). The results obtained suggest that IL-2 and LPS make PKC activity to increase rapidly (Barcia and Ramos-Martínez, 2008; Gonzalez-Riopedre *et al.*, 2009). Catecholamine secretion and later binding to

adrenergic receptors may trigger late NO synthesis (24 h).

This hypothesis would justify the early PKA and PKC increase induced by IL-2 or LPS (Barcia and Ramos-Martínez. 2008; Gonzalez-Riopedre *et al*., 2009). The reduction of NO synthesis detected at 30 min of cell incubation with IL-2 or LPS would confirm an early kinase action (Novas *et al*., 2004). This ensemble of actions can be summarized in the hypothesis shown in Figure 1.

The timing of successive actions of cytokines must play an important role in the activation of the different MAPKs. In this sense, and taking into account the implication of MAPKs in the synthesis of thermal shock proteins (Cellura *et al*., 2006; Gourgou *et al*., 2010), the biphasic expression of the HSP70 when incubating the cells with IL-2 or LPS confirms the hypothesis unfolded in the present text (Gonzalez-Riopedre *et al*., 2007) and opens new perspectives in the study of these cells and their function in cell signalling in molluscan neuro-immunology.

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