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Critical review

Comparative

New insights into innate immune system of sea urchin: coelomocytes as biosensors for environmental stress

PC Branco[1*], DAL Figueiredo[1], JRMC da Silva[1,2]

Abstract

Introduction

In the last decade several studies reinforced the use of sea urchin as a model for immunological purposes due to their phylogenetic proximity to chordates and also due to the diverse immune genes common to vertebrates. Since the year of 2000, the use of sea urchin as bioindicators for environmental stress has been suggested, especially regarding the use of their immune cells, also referred to as coelomocytes. In this critical review, we provide a brief but consistent review covering the most studied topics of the innate immune system of sea urchin emphasizing the use of coelomocytes as biosensors for environmental disorders.

Conclusion

The use of coelomocytes as biomarkers is a very useful and sensitive tool to evaluate marine environmental stress. However the mechanisms by which these conditions lead to an upregulation of coelomocytes, either increasing its number or increasing the proteins or genes expressed by them, are poorly understood. We reinforce that further studies aiming to answer these questions are necessary.

Introduction

Sea urchins belong to the deuterostome clade, sharing a common ancestry with chordates. This feature has been explored more since 2006, when the genome of S. purpuratus was sequenced. In this pioneer study, a sophisticated immune system mediated by an immense repertoire of innate pathogen recognition proteins has been described, containing more than 200 members of Toll Like Receptors (TLR), NOD-like receptors and Scavenger Receptors[1].

A gene family that plays an important role in immunity for sea urchins is the Sp185/333 that encodes a diverse array of putative immune response proteins. This family is readily induced in immune cells of the sea urchin in response to immune challenge[2].

Another important issue that justifies the use of sea urchins in scientific research is their use as bioindicators for environmental stress. The aim of this critical review is to provide a general view of innate immune system of sea urchin and assess the use of their coelomocytes as biosensors for environmental stressful conditions.

Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. Animal care was in accordance with the institution guidelines.

Sea urchin: basic characteristics and model for scientific studies

Sea urchins are benthonic animals that belong to the Echinodermata phylum, constituting the Echinoidea class. The word Echinodermata comes from the Greek Echino=spine and Derma=tegument.

They present pentameric radial symmetry during their adult phase. They possess an aquatic vascular system, a simple and radial nervous system, and sexual reproduction with external fecundation[3]. They are exclusively marine and are endowed with very limited capacity of locomotion, justifying their use as environmental bioindicators[4].

The classification of the Echinodermata has suffered many alterations and, in 1875, Huxley proposed the Deuterostomata clade and four phyla were introduced: Chaetognatha, Echinodermata, Hemichordata and Chordata[5]. At this time the phylogenetic proximity of echinoderms and chordates was evidenced (Figure 1).

The role of sea urchins for early development studies is well known, however they became a model for other areas of scientific studies such as: efflux transport[6]; autophagy and apoptosis[7] and, due to its phylogenetic proximity to chordates, they became a very interesting model for the study of immune system[8,9,10] (Figure 1).

Innate immune system in sea urchin: general aspects

The word "immune" was used to indicate that the Catholic Pope (and nowadays to the politicians during their mandates) was not liable to men's laws, being protected and free from penalties. The concept was transferred to organisms, and refers to their effort to maintain homeostasis after injury or infection of microorganism/parasites or even allografts[11].

In the late nineteenth century, the Russian biologist Elie Metchnikoff (1854–1916) observed that some cells present in the perivisceral coelom of echinoderms were able to move and engulf inert or even live particles. Metchnikoff observed this phenomenon in various taxa, although not in cephalochordates[11]

Metchnikoff is the creator of the metaphysical concept (as we use today) of the immune system as an active

*Corresponding author
Email: pbranco@usp.br

[1] University of Sao Paulo, Sao Paulo, Brazil
[2] Marine Biology Center of University of Sao Paulo, Sao Paulo, Brazil

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

FOR CITATION PURPOSES: *Branco PC, Figueiredo DAL, da Silva JRMC. New insights into innate immune system of sea urchin: coelomocytes as biosensors for environmental stress. OA Biology 2014 Jan 18;2(1):2.*

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process mediated by some animal cells[12].

Sea urchins have an innate immune system that works through cellular and humoral response. Cellular response is mediated by coelomocytes, cells with free circulation in the coelomic cavity which can infiltrate tissues and organs besides being the first mediators of allograft rejections; they act in response to host invasion, injury and cytotoxic agents[13]. Cellular components will be described in the next section.

Humoral Factors

A wide range of immune molecules are expressed by sea urchin coelomocytes and released in the coelomic fluid; they are subject of extensive research and most molecules are homologous to the vertebrate immune system, such as lectins, cytokines, profilins and others. These humoral components are capable of destroying or damaging invading microorganisms.

Each molecule is associated with a specific function like neutralizing pathogens, recognizing foreign material, opsonization (enhance of cellular response) and wound healing[13].

Altogether, these molecules contribute to a complex and efficient defence mechanism.

Agglutinins are humoral factors involved in phagocyte aggregation to form clots and encapsulation of foreign material, besides participating in wound repair. A study in P. lividus showed that the presence of hemagglutin enhanced in vitro adhesion of autologous coelomocytes and appears to be involved in cell-cell and cell-matrix interactions[13].

Hemolysins are molecules capable of interacting with membranes and recognizing self from nonself particles like lipopolysaccharides (LPS), zymosan and erythrocytes[14].

Lectins are important defence molecules responsible for identification and opsonization of foreign cells; these molecules are also related to clot formation, and wound repair. They recognize carbohydrate fragments on cell surface (self and non-self) and on extracellular matrix[14].

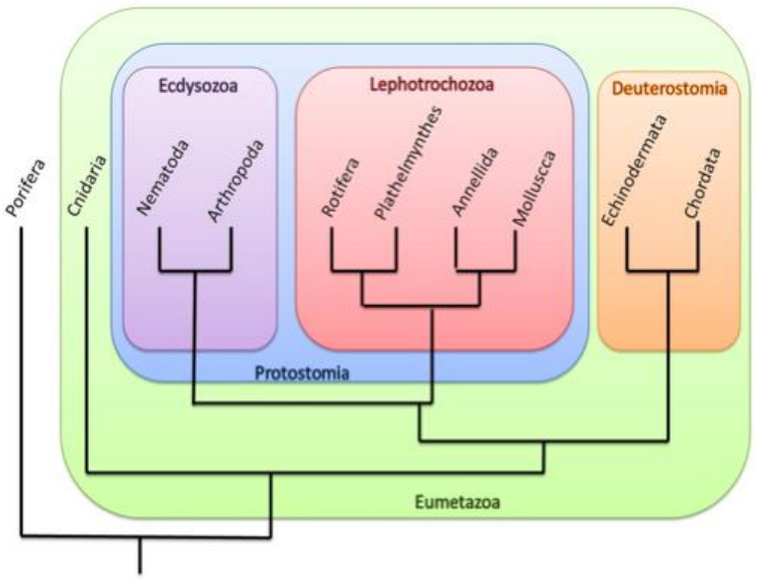

Figure 1: Cladogram demonstrating the phylogenetic position of the Deuterostome group (Orange box). The phylogenetic proximity of sea urchin (echinoderm) to chordates shall be noted.

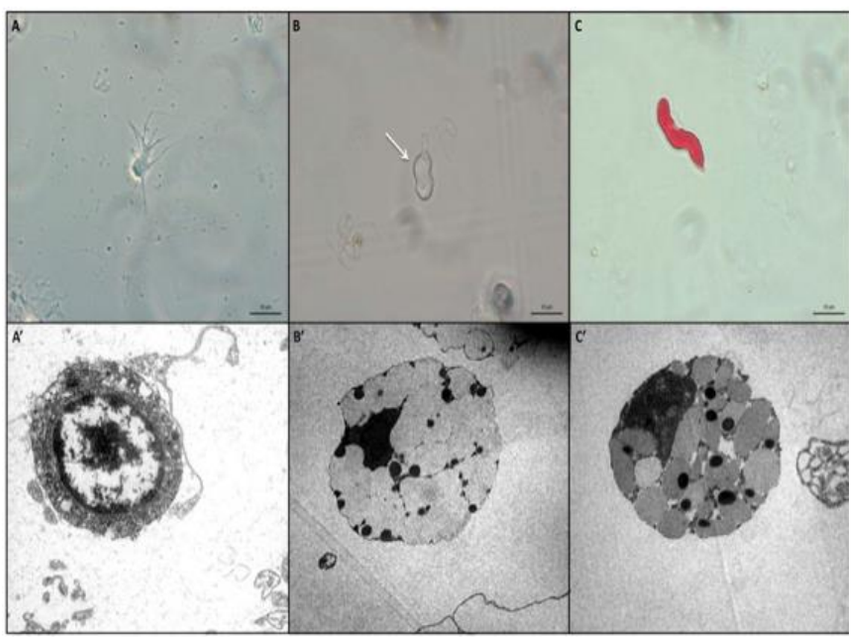

Figure 2: Coelomocytes morphology. A. Phagocytic amoebocyte: cell with a large and central nucleus with loose chromatin and cytoplasm with quantity of endoplasmic reticulum indicating an intense synthetic activity. B. spherical cell with a small, peripheral nucleus with dense chromatin and cytoplasm filled with homogenous vesicles. C. Morphology similar to red spherule cell varying only in its vesicles density, which are more heterogenous.

Complement system

This system is basically composed by the alternative and the lectin pathways and its function is related to opsonization. Homologue of C3 (SpC3) has been identified[15]. They are produced by phagocytes and can be induced by LPS stimulation, which was the first complement molecule identified in an echinoderm[14]. Another complement protein discovered is SpBf (B Factor), also expressed by coelomocytes. It is a complement receptor and its function is thought to

Competing interests: None declared. Conflict of interests: None declared.
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be similar to the human Bf which interacts with C3 to form the enzyme C3 convertase[13,15].

AMPs – Antimicrobial peptides

AMPs are molecules with short amino-acid sequences and their composition can present different conformations and molecules[16,17].

In echinoderms AMPs are found mainly in coelomocytes, and their functions are associated with the capacity to kill bacteria and act in immunoregulatory mechanisms like the production and release of cytokines. They can act both in cell membrane and in internal sites and have the capacity to permeabilize and form pores in membranes, and thus have the potential to act in slow or non-growing bacteria; besides, they can alter the adhesive features of abiotic surfaces affecting initial adhesion of microbial cells[17].

The first AMP described for sea urchin was a cysteine-rich AMP, from the green sea urchin S. droebachienses, the so called Strongylocins (1 and 2)[16], with potent activity against Gram-positive and Gram-negative bacteria.

The same group found two recombinant peptides in S. purpuratus: SpStrongylocin 1 and SpStrongylocin 2, and two new peptides in S droebachienses: Centrocins (1 and 2) with either activity against Gram-positive and Gram-negative bacteria beyond the fungal activity of centrocins.

These findings demonstrate the diverse immune molecules present in the sea urchin immune system; these molecules are responsible for different response mechanisms to different pathogens, suggesting that they are extremely important for host defence.

Coelomocytes: morphology and citophysiology

Sea urchins have four coelomocyte types in the coelomic fluid: phagocytic amoebocytes (PA), red spherule cells (RSC), colourless spherule cells (CSC) and vibratile cells (VC) (Table 1 and Figure 2).

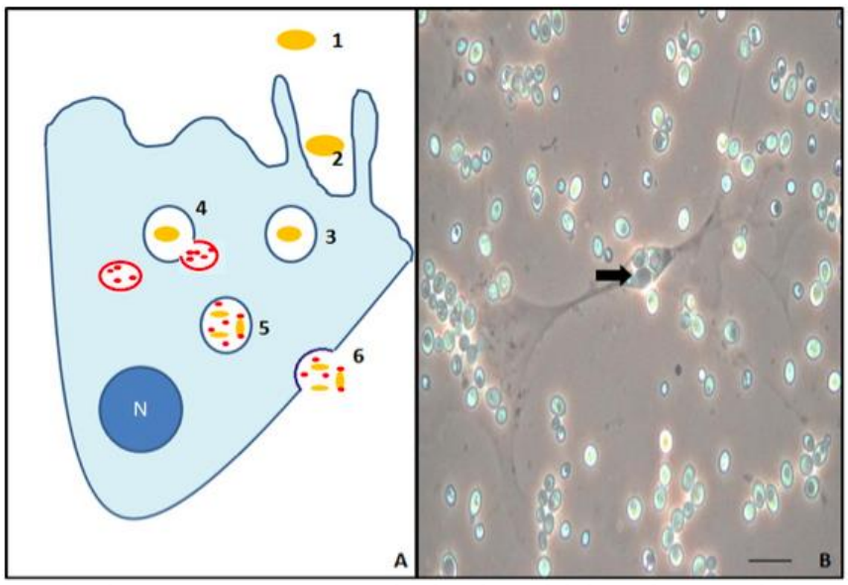

Figure 3: Phagocytosis. It represents the first line of defence for invertebrates and vertebrates. In sea urchins phagocytic amoebocyte is the only cell type that is able to perform phagocytosis. A. Schematic drawing demonstrating steps of phagocytosis. 1. Chemotaxis to the foreign particle (through chemotactic elements like LPS, complements and damaged cells) culminating in adhesion of phagocyte to the particle. 2. Cytoskeleton remodelling leading to pseudopodia extension around the particle. 3. Formation of phagosome in which the particle is completely internalized. 4. Fusion of phagosome to lysosomes aiming to digest the phagocytic particle. 5. Phagocytized material is then digested by lysosomal enzymes. 6. Discharge of digested material that cannot be recycled by the cell. B. Phagocytosis observed under phase contrast microscopy. A phagocytic amoebocyte is visualized spread onto glass slide. Inside its cytoplasm is possible to observe some yeast cells internalized (arrow).

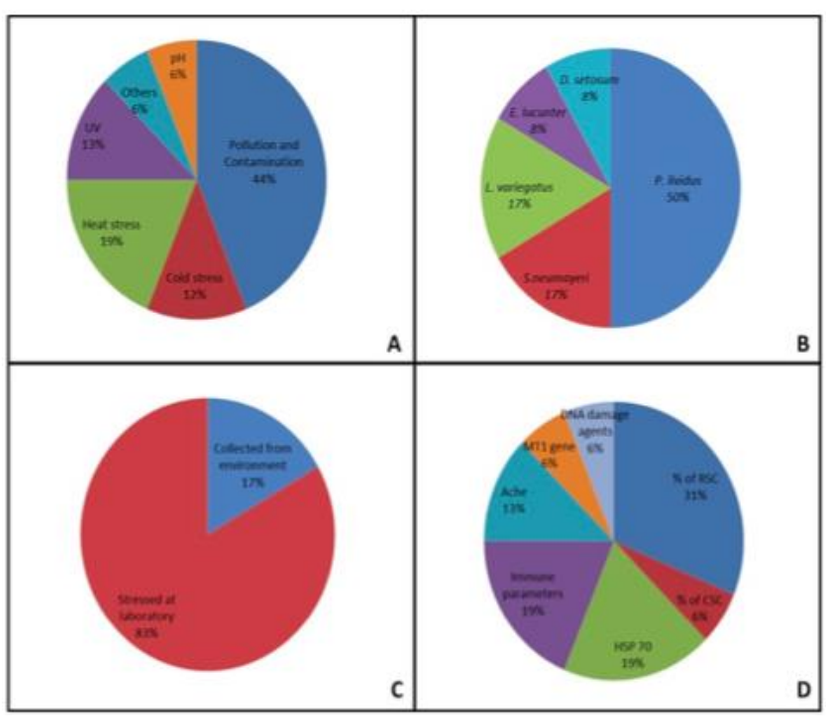

Figure 4: Graphics drawn based on indexed researches from 2000 to 2013 involving environmental stress on sea urchin coelomocytes. For this search either the proportion of coelomocytes or the genes upregulation and immune response were considered. A. Proportion of stressors capable to induce coelomocytes alterations. B. Proportion of sea urchin species studied. C. Proportion of studies performed on laboratory stress conditions or collected at field work. D. Proportion of biomarkers reported for sea urchin environmental stress.

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Table 1: Sea urchin coelomocytes: morphological characteristics according to phase and transmission electron microscopy, nomenclatures used, type of migration of proportion of each cell type.

Coelomocyte	Different Nomenclatures	Sub-type	Morphology based on Phase Contrast Microscopy	Morphology based on Electron Microscopy	Migration	Proportion on CF
Phagocytic Amoebocyte	Phagocytes	Discoidal Cell Polygonal Cell Small phagocytes	This cell type is agranular and presents capacity to emit cytoplasmic projections. Also they can acquire a spread shape when in touch with a glass slide.	A large and central nucleus with loose chromatin and abundant quantity of endoplasmic reticulum indicating an intense synthetic activity (Branco et al.[28]).	Mesenchymal	60-70% (Borges et al.[22])
Red Spherule Cells	Red Amoebocyte	-	Spherical cell with red cytoplasmic granules containing a naftoquinone substance called echinochrome what gives the redish colour.	Small, peripheral nucleus with dense chromatin and its cytoplasm fulfilled with heterogeneous vesicles. (Branco et al.[28]).	Ameboid	5-10% (Borges et al.[22])
Colourless Spherule Cells	Colorless Amoebocyte	-	Spherical cell with colorless cytoplasmic granules.	Small, peripheral nucleus with dense chromatin and its cytoplasm was fulfilled with electron lucent vesicles with a more electron dense centre. (Branco et al.[28]).	Ameboid	5-10% (Borges et al.[22])
Vibratile Cell	-	-	Round cell with one single flagellum that allow it to move.		Rotational movement	15-20% (Borges et al.[22])

Phagocytic Amoebocyte

Considered the main effector of the sea urchin immune system, this cell is in the highest proportion among all coelomocytes and is known to be involved in different immunological responses such as chemotaxis, graft rejection, encapsulation, cytotoxicity, reactive oxygen species production, agglutination, clotting rejection, immune genes expression and primarily in phagocytosis (Figure 3)[18]. The proportion of this cell type can vary among different species[14]. Also, some articles have reported different subpopulations of phagocytes. Based on their morphology, they can be classified as polygonal, discoidal and small phagocytes. The main differences are related to their cytoskeleton rearrangements[19]. Posterior studies demonstrated that these subpopulations present different molecules expression such as: SpC3[20], Sp185/333[21] and different phagocytic capacities[22].

Red Spherule Cell

Spherical cells containing granule in their cytoplasm. These granules are related to antibacterial activity, containing a red naphthoquinone molecule called echinochrome A, which degranulates and acts against Gram-positive and Gram-negative bacteria; in addition, they are found in high concentration surrounding injuries, being considered to participate in wound healing[18].

Colourless Spherule Cell

Spherical cells containing granule in their cytoplasm. These granules differ from the red spherule cell in the electron density of their granules, reinforcing the hypothesis that both spherule cells are the same cell type in different physiological moments of maturation[23].

Both spherule cells are similar in shape and size, impeding their fractioning by separation gradient and cell sorting. Also, both of them present amoeboid migration[18].

Vibratile cell

Round and small flagellate cells that move in a non-directional way. For this reason, they seem to be involved in the coelomatic fluid movement and coagulation[15].

Environmental stress and innate immune parameters

Environmental stress can be defined as "an environmental factor causing a

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change in a biological system, which is potentially injurious"[24]. Thereafter, a necessity to evaluate the environmental stress has evolved, when the time has come for the use of biomarkers and biomonitoring, arising considerably the number of publications regarding this issue.
Regarding biomarkers that can be involved with coelomocytes of sea urchins, literature can be didactically divided into: 1- studies involving the proportion of coelomocytes, 2- studies involving heat shock response, 3- studies involving immunological responses, 4- studies involving molecules related to inflammatory process (Figure 4).

Studies involving the proportion of coelomocytes

Studies comprising the proportion of coelomocytes as biomarkers are mainly related to the red spherule cell. The use of RSCs was first proposed in 2000[23], in a study that demonstrated their increase in the case of polluted sea water by industries residues and cold and hot sea water. Posteriorly, studies were conducted and classified them as biomarkers for different environmental stressors such as contamination by metals[25] and by oil soluble fraction[26]. Heat stress has also been reported for Antarctic sea urchins[27] and for tropical sea urchins[28].
The increase in colourless spherule cells was also reported as biomarker for heat stress in tropical sea urchins[28]. The explanation for this can also be found in the hypothesis that this cell type can be a different maturation stage of red spherule cell[23]. This hypothesis is supported by the observations of Transmission Electron Microscopy (Figure 2) and Flow Cytometer (personal communication).

Studies involving the heat shock response

Heat shock proteins (HSPs) are a large class of proteins that have been conserved throughout evolution and are expressed in all cell types studied so far. Considered chaperones, this class of proteins helps the correct folding and unfolding of proteins aiming to achieve a correct three-dimensional conformation, which is strictly correlated to a functional protein. Studies demonstrated that their expression is up-regulated after the exposure to different stressors such as: hypoxia, heavy metal exposition and diseases and are finely correlated to immune function, playing important roles in antigen presentation and activation of macrophages[29].

Taking into consideration that HSPs are evolutionarily conserved, there are strong possibilities that the function of HSPs in modulating immune response might also be present in sea urchins.
The HSP70 is a heat shock protein with 70KDa that are the most studied among the chaperones. In the pioneer study of HSP70 expression by coelomocytes, the expression of HSP 70 was demonstrated for heat shock (35°C) and cold shock (4°C) for acute periods. Heavy metal, acidic pH and UV radiation led to an increase of Hsp70 expression in coelomocytes in culture[30,31].

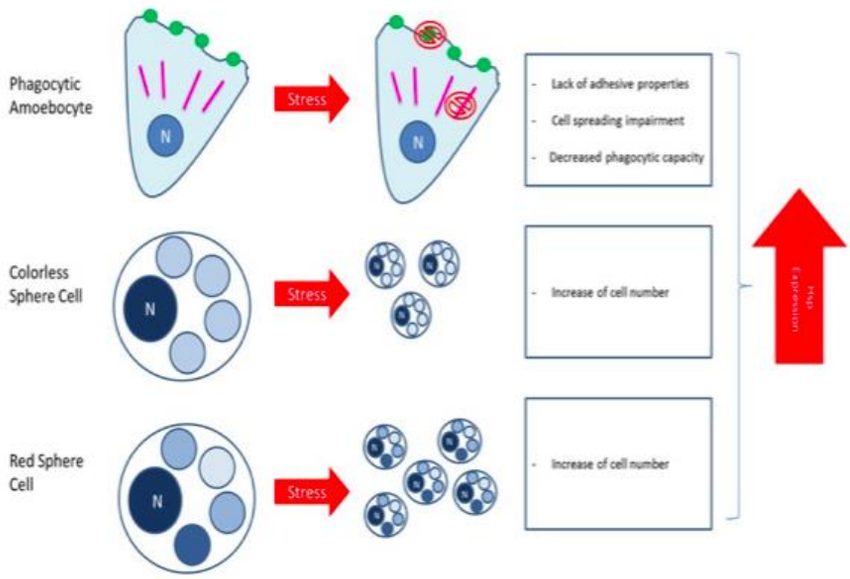

Figure 5: Schematic drawing demonstrating the effect of stress on coelomocytes. Phagocytic amoebocytes respond with impairment in the immunological function, lacking adhesive properties, presenting alterations on cell spreading and decreased phagocytic capacity. Spherule cells, either colourless or red ones, respond with increase in their cell numbers. Also, coelomocytes respond to stress increasing the expression of HSPs.

Studies involving immunological responses

Regarding immunological responses that can be affected by environmental stress, literature describes the phagocytic response of sea urchins exposed to thermal stress for different periods[27,28], in which the phagocytic response, adhesive properties and cell spreading are impaired under elevated temperatures.
Besides, the capture of metal oxide nanoparticles by phagocytes has been described as a biomarker for evaluating this nanoparticle toxicity[32].

Studies involving molecules related to inflammatory process.

The activity of cholinesterase as a good biomarker for environmental contamination of metal oxide nanoparticles was also reported[32]. The same was reported for cold stress[33].
Another stress marker already described for sea urchin coelomocytes is the methalotionein gene (MT1) described in heavy metal contamination[34].
A study revealed that coelomocytes are biomarkers for DNA-damaging agents including ultraviolet radiation and hydrogen peroxide[35].

Competing interests: None declared. Conflict of interests: None declared.
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Conclusion

The last decade was very important to elucidate the use of coelomocytes and their responses to environmental disorders, reinforcing and consecrating their use as biosensors for different environment stressful conditions (Figure 5). Despite all the efforts, many questions remain unanswered: What are the molecular mechanisms, signalling cascades and genes involved in the upregulation of coelomocytes?

How could the activation of innate immune response be positive for the homeostasis of the organism and what would be the consequences of it? These are just some examples of innumerous questions that can be raised. Much more study should be conducted aiming to better comprehend the use of coelomocytes, their regulation and the consequences for the organism after an environmental challenge.

Acknowledgment

The authors express their acknowledgments to grants 2011/06044-4 and 2011/15612-6, São Paulo Research Foundation (FAPESP), to CAPES and CNPq for the financial support and to Adam Arai Martens for the English revision of this manuscript.

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Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
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