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Sea urchin immune cells as sentinels of environmental stress ☆

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ABSTRACT

Echinoderms, an ancient and very successful phylum of marine invertebrates, play a central role in the maintenance of ecosystem integrity and are constantly exposed to environmental pressure, including: predation, changes in temperature and pH, hypoxia, pathogens, UV radiation, metals, toxicants, and emerging pollutants like nanomaterials. The annotation of the sea urchin genome, so closely related to humans and other vertebrate genomes, revealed an unusually complex immune system, which may be the basis for why sea urchins can adapt to different marine environments and survive even in hazardous conditions. In this review, we give a brief overview of the morphological features and recognized functions of echinoderm immune cells with a focus on studies correlating stress and immunity in the sea urchin. Immune cells from adult *Paracentrotus lividus*, which have been introduced in the last fifteen years as sentinels of environmental stress, are valid tools to uncover basic molecular and regulatory mechanisms of immune responses, supporting their use in immunological research. Here we summarize laboratory and field studies that reveal the amenability of sea urchin immune cells for toxicological testing.

1. Introduction

Echinoderms, an ancient and very successful phylum of marine invertebrates, represent a morphologically varied group consisting of around 7000 living members with unique shapes and colours. The extant phylum is divided into five main classes: crinoids (feather stars), asteroids (sea stars), ophiuroids (brittle stars), echinoids (sea urchins) and holothurians (sea cucumbers). Crinoids are considered the most primitive class, while echinoids and holothurians the most advanced. However, recent molecular studies support an ophiuroid/asteroid clade (Asterozoa) based on either convergent evolution of the pluteus or reversals to an auricularia-type larva occurring in asteroids and holothurians (Telford et al., 2014). It may not be obvious how animals like sea stars, sea urchins, sand dollars or sea cucumbers are all related, but despite their various shapes they possess common characteristics: i) adult radial symmetry, ii) a water vascular system, iii) a calcite endoskeleton with a specific three-dimensional structure (stereom), and iv) benthic lifestyle. Echinoderms play a key role in the maintenance of ecosystem integrity (Hereu et al., 2005) and are constantly exposed to environmental pressure, including: predation, changes in temperature and pH, hypoxia, pathogens, UV radiation, free radicals, metals, toxicants and emerging pollutants. The keys for their success include a few survival strategies, such as a spiny physical defence structure, an effective immune defence system, a toxin producing equipment, and an amazing regeneration capability, which provide them with protection, robustness, resistance and stemness. Echinoderms appeared 520 million years ago, prior to the Cambrian explosion, and are globally distributed in the oceans in almost all depths, latitudes, temperatures and environments (Bottjer et al., 2006; Iken et al., 2010). What we now call immune defence appeared early in the evolution of these marine invertebrates through the invention of the innate immune response, mediated by a vast repertoire of recognition molecules (immunome), and the stress response, mediated by a subset of stress-sensing gene families and pathways (defensome). These protective mechanisms are used by the echinoderm immune cells to recognize both biotic and abiotic stressors and to sense, transform and eliminate many potentially noxious materials.

2. Echinoderm immune cells

Echinoderm immune cells, also known as coelomocytes, are a heterogeneous population of freely moving cells found in all coelomic spaces, including the perivisceral coelomic cavities and the water-vascular system (Glinski and Jarosz, 2000; Smith et al., 2010). They are also present sparsely in the connective tissue and amongst tissues of various organs (Muñoz-Chápuli et al., 2005; Pinsino et al., 2007). Molecular studies have suggested the presence of phagocytic cells in the major organs and tissues, including the axial organ,

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pharynx, esophagus, intestine and gonads (Majeske et al., 2013b). Cell type composition has been postulated to depend on the species, as well as on patho-physiological conditions of each individual. Based on morphological criteria echinoderm immune cells have been classified into at least six cell types, but not all six have been identified in all classes/species. Names used to describe them in the past include phagocytic amoebocytes, phagocytes, amoebocytes, spherule cells, vibratile cells, haemocytes, crystal cells and progenitor cells (Smith, 1981). It is well recognized that echinoderm immune cells carry out functions similar to those of the vertebrate blood cells, such as clot formation, phagocytosis, encapsulation, clearance of bacteria or other foreign materials, oxygen transport (Matranga et al., 2005). It is not the purpose of this review to unravel the morphologies, roles and functions of the different cell types for each class of echinoderms; rather this report will centre mostly on the species of interest, namely the sea urchin *Paracentrotus lividus*, and will describe the results that correlate environmental stress and immunity (see sections 4–6). Although echinoderms have been the focus of classical studies that defined animal cellular immunity (Metchnikoff, 1891), only recent studies have addressed immune functions in the sea urchin.

The coelomic fluid in which the immune cells reside and move is a key factor governing immunological capabilities, as it contains essential trophic and activating factors produced by immune cells themselves (Matranga, 1996; Matranga et al., 2005; Smith et al., 2010). Echinoderms lack a distinct directional closed circulatory system; on the contrary, they possess an open water vascular system (WVS), which is structurally and physiologically specialized to carry out several functions typical of the higher vertebrate vascular system (Smith, 1981) (Fig. 1). In addition, the WVS serves to generate, distribute and control the hydrostatic pressure necessary for locomotion, respiration, feeding, reproduction, and excretion (Nichols, 1972).

The coelomic fluid, which can be considered similar to seawater with a dense population of immune cells and a high concentration of factors, has functions similar to the blood of higher animals. Thus, by being in direct contact with internal cells and tissues, it can provide an overall profile of the physio-pathological state of the organism. The loss of coelomic fluid can affect the behaviour and the physiological functions of echinoderms. Thus, an efficient mechanism to plug and repair accidental or pathological leaks in the body wall becomes crucial to prevent infections and maintain homeostasis. In echinoderms, the immune system evolved as a defence strategy not only against external insults, but also against internal pathological threats. In fact, echinoderms do not show variations in metabolic functions and fertility over time, and no cases of cancer, immune and age-related diseases have been reported (Bodnar, 2009). In accordance, recent analysis of oxidative damage and proteomic studies in three sea urchin species with different lifespans revealed that the sea urchin is a promising tool for investigations of oxidative cell damage, senescence, and longevity (Bodnar, 2013; Du et al., 2013).

3. The relationship between stress and immune response

A less restrictive definition, perhaps more applicable to invertebrates in general and to echinoderms in particular, defines an antigen as any chemical substance capable of stimulating the immune system to respond by one or a combination of several reactions, including phagocytosis, cell-mediated immune responses, and the cell stress response. Recent studies have shown that proteins eliciting the cellular stress responses, including heat shock-, ER stress- and DNA damage-responses, interact with and regulate the signalling pathways involved in the activation of both innate and adaptive immunity (Muralidharan and Mandrekar, 2013). In humans, the regulation of innate immune cell activation by cell stress pathways is essential in host defence. In fact, this interaction is relevant to the control of diseases that are characteristic of aberrant immune responses, such as chronic inflammatory diseases, autoimmune disorders, allergic disorders and cancer. The immune-signalling cascades that are linked to cellular stress responses are stimulated by an accumulation of unfolded proteins within the immune cells (Fig. 2), which serves as a signal amplification cascade favouring cytokine production (Cláudio et al., 2013).

The induction of proteins related to the cellular stress responses does not necessarily indicate response to a stress. Instead, it can be an integral part of a selective transcription programme controlled by innate immune receptors (Hetz, 2012). For example, the extra-cellular 70-kDa heat shock protein (Hsp70), a cognate of the first stress protein described in the literature to respond to an increase in the temperature of the organism (De Maio et al., 2012; Ritossa, 1962), can function as a cytokine that acts on human monocytes, showing the ability to: i) bind with high affinity to the plasma

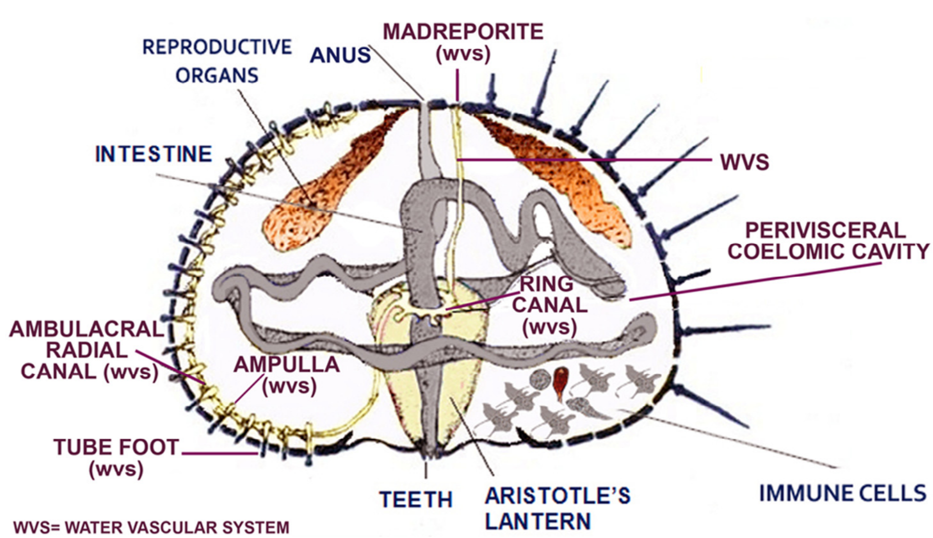

Fig. 1. Basic anatomy of the sea urchin. The schematic illustration points to the complex open water vascular system (WVS), captions in purple colour. Seawater enters through the madreporite on the aboral surface into a short straight canal, connected to a circular canal, the ring canal, which in turn is linked to the radial canals. Radial canals bring the seawater to each ampulla and thereafter to the tube feet. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)