



Review

Immune Phenomena in Echinoderms

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Abstract. Advances in biochemistry and molecular biology have made it possible to identify a number of mechanisms active in the immune phenomena of echinoderms. It is obvious that echinoderms have the ability to distinguish between different foreign objects (pathologically changed tissues, microorganisms, parasites, grafts) and to express variable effector mechanisms which are elicited specifically and repeatably after a variety of non-self challenges. The molecular and biochemical basis for the expression of these variable defense mechanisms and the specific signals which elicit one type of effector mechanism are not, however, yet well known. The high capacity of coelomocytes to phagocytose, entrap and encapsulate invading microorganisms is a valid immune cell-mediated mechanism of echinoderms. The entrapped bacteria, discharged cellular materials and disintegrating granular cells are compacted and provoke the cellular encapsulation reaction. Moreover, humoral-based reactions form an integral part of the echinoderm defense system against microbial invaders. Factors such as lysozyme, perforins (hemolysins) vitellogenin and lectins are normal constituents of hemolymph, while cytokines are synthesized by echinoderms in response to infection.

Key words: echinoderm immunity; defense molecules; effector cells; amebocyte immune reactions; cell-free immune factors.

Introduction

Echinodermata is a phylum of marine invertebrate animals that includes the 6 following classes: *Crynoidea* (sea lilies and feather stars), *Echinoidea* (sea urchins), *Holothuroidea* (sea cucumbers), *Asteroidea* (sea stars) *Ophiuroidea* (basket stars and serpent stars) and *Concentricycloidea* (sea daisies). Though echinoderms exhibit a great diversity of body forms, they all have an internal skeleton and a water-vascular system. The axial organ, a complex and elongated mass of tissue found in all echinoderms except the holothurians, represents the common junction of the perivisceral coeloma, the water-vascular system and the hemal system.

Although its functions are not yet well understood, the axial organ plays a part in the defense against invading pathogens and parasites, can contract, is responsible for the circulation of fluids and may have excretory and secretory activity. The blood system is a complex system of spaces that are neither part of the coeloma nor the true vessels. A hemal ring and 5 radial canals surround the esophagus and the radial canals of the water-vascular system. A sixth hemal space arises from the hemal ring and enters the axial organ³⁹.

Echinoderms are exclusively marine animals, with only a few species tolerating even brackish water. Echinoderms can protect themselves from predation and parasitic invasion in a variety of ways, most of which

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are passive. A few species are parasitic. Sea cucumbers may attach themselves to the spines of sluggish Antarctic echinoids. The crinoids are hosts for specialized parasitic worms. Without any doubt, bacterial invaders, parasitic intruders and predators can initiate immune responses and, in a variety of ways, interact with the protective mechanisms of echinoderm hosts.

The immune phenomena occurring in echinoderms are the recognition of foreign material (non-self) which has entered the body, expelling non-self or rendering it inoffensive (non-dangerous), and wound healing. These key defense mechanisms are mediated by cellular and cell-free (humoral) immune responses.

Cellular responses are realized by a few types of coelomocytes, the cells that circulate in the coelomic fluid and that form the immune effector cell system of echinoderms, and by humoral responses, which depend upon molecules present in the coelomic fluid. Infection of the body cavity of echinoderms by microorganisms or parasites, or the transplantation of grafts (cells or tissues) induce a process that involves humoral molecules that recognize and attack foreign material or stimulate coelomocyte cell proliferation.

The investigation of the immune system of echinoderms should help in understanding the cellular and humoral interactions that regulate host defense in this group of marine animals. These investigations are also important from a phylogenetic point of view because they may contribute to the understanding of the immune phenomena of higher animals, including mammals. Moreover, they may lead to possible analogies or homologies with immune mechanisms operating in vertebrates.

Effector Cells of the Echinoderm Immune System

In echinoderms, coelomocytes can be defined as the effector cells of the immune system. They synthesize and secrete upon stimulation a number of specific molecules such as lectins, perforins, lysosomal enzymes, serum proteins and profilins. Most of these immune molecules are not fully characterized, since they have only been reported in terms of their activities. The coelomocytes are the first line of defence in the echinoderms against infections and injuries. They are found in the coelomic fluid and in the hemal and water vascular system. At least 6 morphologically distinguishable cell types can be found in the coelomic fluid: progenitor cells, phagocytic amebocytes, colored and

colorless spherula cells, vibratile cells, hemocytes and crystal cells³⁹. Not all 6 cell types are present in every species of the echinoderms³⁰. Amebocytes play an important role in initiating the healing of internal and external wounds. Their action includes accumulation, phagocytosis of cellular debris and the formation of a layer of cells at the site of injury²⁷.

Now it is widely accepted that the main source of coelomocytes is the axial organ, which therefore may represent an ancestral primary lymphoid gland. The axial organ of *Asteria rubens* (LINNAEUS) produces 2 populations of cells: the adherent cells (B-like cells), resembling mammalian B lymphocytes, and non-adherent cells (T-like cells), resembling mammalian T lymphocytes²⁸.

The coelomocytes play a number of roles, including recognition of self from non-self, phagocytosis, allo- and xenograft rejection, cytotoxicity, cell aggregation and encapsulation and humoral defense reactions³⁶. Most of the immune responses are mediated by phagocytic amebocytes. They exhibit 2 morphologically distinct phases: the petaloid form, which is actively phagocytic, and the filopodial stage which appears to be involved in clotting. After injury, the petaloid amebocytes migrate to the site of injury, change their shape to filopodial and form a clot together with other coelomic cells^{14, 39}.

It is well documented that all phyla of invertebrates possess ameboid cells capable of recognizing parasites and other foreign bodies and reacting to them either by phagocytosis or encapsulation. These 2 responses are the basic cellular defense mechanisms of echinoderms when confronted with foreign pathogens. Bacteria and viruses are regularly phagocytized, but if foreign material such as parasitic eggs, larval stages of parasites or whole parasites penetrate the body cavity, it is encapsulated, since the parasite is too large to be phagocytized and ingested by a single cell¹⁴.

Phagocytosis of foreign particulate material is attributed to amebocytes, in which lysosomal enzymes, including lipase, peroxidase and serine protease, are constitutively present. Consequently, it seems clear that these lysosomal enzymes cause a breakdown of engulfed foreign material. Phagocytic cells act as an efficient clearance mechanism working in sea urchins due to their recognition, ingestion and efficient degradation of ingested living particles⁶. Moreover, phagocytes from the *Strongylocentrotus nudus* produce hydrogen peroxide in the resting state, and this production is enhanced after stimulation by non-self materials²¹.

Bacteria are efficiently cleared from the coelomic fluid of echinoderms, but no difference exists between

the primary and secondary clearance rates. In the 2 sea urchin species *Strongylocentrotus purpuratus* (STIMPSON) and *Strongylocentrotus franciscanus* (AGASSIZ), phagocytosis of Gram-positive bacteria was more efficient than that of Gram-negative bacteria^{23, 27}.

Both phagocytic amebocytes and spherula cells appear to be involved in cell clumping and the formation of capsules around ingested parasites. It is possible that spherula cells release bactericidal substances, including lipase, peroxidase and serine proteinase²³, that cause the breakdown of phagocytized material¹³. Among them, an arylsulphatase activity found in a coelomocyte population of *Asteroidea* and *Echinoidea*¹¹ seems to play a certain role. This enzyme detected in granules of spherula cells, is probably associated with inflammatory phenomena³³.

Hemolysin producing amebocytes, which are large, variously shaped, extremely motile cells positive for acid and alkaline phosphatases, chloroacetic esterase and for phenoloxidase cells, function as phagocytic cells, cytotoxic cells in allograft and xenograft rejection, cell aggregation and encapsulation. The mechanisms by which amebocytes accomplish cell-mediated lysis is still obscure.

The invertebrate host defense function involves first immobilizing and then phagocytizing, encapsulating or lysing the invasive microorganisms. Various entities participate in these reactions: lectins, perforins and vitellogenin, which are involved in adhesion between the cells surrounding and microorganisms, and various types of amebocytes.

Colorless spherula cells are reservoirs of vitellogenins. Coelomocytes respond to a stress condition by discharging the molecules into the coelomic fluid. A 220 kDa aggregating factor isolated from *Holothuria polii* is released by coelomocytes and is capable of agglutinating coelomocytes¹².

Cytokines, the polypeptides released by activated blood cells usually upon infection or inflammation, induce proliferation of immune effector cells. An interleukin-1-like molecule is present in the echinoderms³⁴. The sea star *Asteria forbesi* (LINNAEUS) produces so-called SSF (sea star factor) of 39 kDa. Recently, BECK and HABICHT^{1, 2} found in the coelomic fluid of *A. forbesi* a molecule with the characteristics and functions of interleukin 1 (IL-1). This molecule (29.5 kDa) stimulates the proliferation of murine thymocytes and fibroblasts, stimulates protein synthesis of fibroblasts and enhances the production of prostaglandin E. It is cytotoxic for human cell line E375. The activity of this molecule can be blocked by antibody specific for human IL-1. BECK et al.³ have isolated from echino-

derms an IL-2-like molecule. The physicochemical and biological activities of starfish IL-1 are the same as those of IL-1 in the mammalian immune system. Consequently, it is suggested that invertebrate host defense is regulated by a cytokine network analogous to that found in vertebrates¹.

Blood clotting and response to injury are prerequisite to wound healing. Coelomocytes of *S. purpuratus* can respond to minor injuries by increased expression of a gene coding sea urchin profilin¹⁸, a small protein having both actin and phosphatidyl-inositol-diphosphate binding functions. SMITH and DAVIDSON³⁸ even proposed that sea urchins have a simple alert mechanism operating after injury, inflammation and host invasion.

Clot formation in echinoderm coelomic fluid is basically brought about by the aggregation of coelomic amebocytes¹⁵. The purposes of clot formation is to prevent the loss of coelomic fluid that may occur following an injury. There may be 2 distinct types of clots formed in both asteroids and holothurians^{7, 16}. The first type of aggregate is relatively loose and the cells maintain their individual identity. The second form results in intimate cell contacts that lead to the formation of a plasmodium. A soluble tissue factor with an adhesive activity may be involved in the formation of both of these types of clots⁷.

Graft Rejection

Graft rejection is a cell-mediated process. Echinoderm phagocytes can recognize and destroy allogenic and xenogenic cells *in vitro* and *in vivo*⁴. Graft rejection has been demonstrated in the holothurian *Cucumaria tricolor*, the echinoideans *S. purpuratus* and *Lytechinus pictus* (VERILL), and in the asteroideon *Dermasterias imbricata*³⁵. The median survival time for the first-set allografts in *D. imbricata* is 213 days, whereas in *C. tricolor* it is 165 days²⁶. The transplant is infiltrated by lymphocyte-like cells and phagocytes that disrupt the cytoarchitecture of the transplants.

Phagocytes of the sea urchin *Strongylocentrotus droebachiensis* have the capability of allogenic recognition, which leads to cytotoxic reaction after 20 h. The phagocytic amebocyte is probably the effective cell involved in graft rejection¹⁹.

There is controversy regarding the existence of specific memory in echinoderms. Second-set allografts in *C. tricolor* have a median survival time of 43 days¹⁹. The third-party grafts were rejected with the same enhanced kinetics as second-set allografts. Therefore, the observed adaptative responses do not express immunological specificity.

Humoral Defense Molecules

Despite recent advances in molecular and cellular biology and genetic engineering, the molecular aspects of the immune responses of the echinoderms remain still obscure and poorly understood. The humoral components present in the coeloma of echinoderms recognize and then attack the foreign body. The specific recognition between humoral molecules and invaders takes place through lectins, carbohydrate moieties present on the surface of host cells or bacteria³⁹. The second group of humoral molecules form perforins (hemolysins). They interact with the plasma membrane, producing holes which cause the lysis of target-cells^{8, 10}. The third group forms complement-like activity, heat unstable and calcium dependent. It strongly resembles the mammalian complement activity since it can be destroyed after treatment with zymosan, pronase, trypsin²⁹. The complement-like activity is present in *S. droebachiensis*⁵. Similar lytic activity has been found in *A. forbesi*. This effect is optimal at 25°C and requires the presence of divalent cations^{5, 29}.

Lysozymes represent a class of enzymes that occur in vertebrates, invertebrates, bacteria and plants^{22, 32}. JOLLÈS and JOLLÈS²⁴ characterized them as basic proteins of low molecular weight ($15\text{--}25 \times 10^3$) with an isoelectric point of 10.5–11.0, stable at acidic pH and at a high temperature but labile at alkaline pH, capable of lysing a suspension of *Micrococcus luteus* (*M. lyso-deikticus*) and able to attack substrates with subsequent release of reducing sugar and amino sugars. The antibacterial activity found in the coelomic fluid of the sea star *A. rubens*²⁴ and in *Paracentrotus lividus* (LAMARCK) is most probably exerted by lysozyme-like molecules, peroxidase⁹, napthoquinone compounds and echinochrome-A³¹.

In echinoderms, wound repair and encapsulation of metazoan invaders requires the presence in the coelomic fluid of an adhesive activity. A calcium and magnesium dependent factor is involved in the clumping of coelomocytes after injury²⁵. In *P. lividus* and in *H. polii* coelomic fluid, a 200 kDa protein promotes adhesion of coelomocytes and their spread on the substrate¹¹. This protein in the sea urchin *P. lividus* is the precursor of toposome, which is responsible for the integrity of the sea urchin embryo. It has also been suggested that the spherula cells produce collagen in response to injuries, used in wound repair³⁸.

Echinoidin, the lectin of the sea urchin¹⁷ and the sea cucumber *Stichopus japonicus*²⁰, shows significant homology to the membrane or soluble C-type lectins from vertebrates. In the lectin from the *A. crassispina*

coelomic fluid, the carboxyl terminal half is similar to the Man-binding lectins A and C from rat liver, the chicken hepatic lectin and the rat asialoglycoprotein receptor. The participation of lectins as mediators of the immune mechanism in invertebrates is supported by evidence for their roles not only as soluble agglutinins, opsonins, but also as hemocyte associated non-self recognition and effector molecules³⁷.

Concluding Comments

It is apparent that the echinoderms provide many interesting models for studying the evolution of both non-specific and specific effector cells and their functions. The following points need further detailed study: cell-cell communication in immune response, interrelationships of cellular and humoral immunity, phylogeny, and role of the cytokines in immune response regulation.

The fact that the immune system of invertebrates is sensitive and responds even to minute quantities of environmental molecules points towards the question of how contaminants present in the environment are likely to modify immune response of echinoderms. Also, little is known about the action of xenobiotics on the immune system. Therefore, more work is required in order to determine the effects of environmental insults on a range of immune parameters. Efforts should be made to examine the biochemical and molecular aspects of the echinoderm immune phenomena and possible homologies between echinoderm immune mechanisms and the mechanisms functioning in other phyla of invertebrates and in mammals.

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