Fish & Shellfish Immunology xxx (2015) 1–15

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

The new insights into the oyster antimicrobial defense: Cellular, molecular and genetic view

Evelyne Bachère [a, *], Rafael Diego Rosa [a, b, 1], Paulina Schmitt [a, c, 1], Aurore C. Poirier [a], Nicolas Merou [a], Guillaume M. Charrière [a, 1], Delphine Destoumieux-Garzón [a]

[a] Ifremer, UMR 5244, IHPE Interaction Host Pathogen Environment, UPVD, CNRS, Université de Montpellier, CC 80, F-34095 Montpellier, France
[b] Laboratory of Immunology Applied to Aquaculture, Department of Cell Biology, Embryology and Genetics, Federal University of Santa Catarina, 88040-900 Florianópolis, SC, Brazil
[c] Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad, Católica de Valparaíso, Avenida Universidad 330, 2373223 Valparaíso, Chile

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Mollusk
Immunity
Antimicrobial peptide
Hemocyte
Defensin

ABSTRACT

Oysters are sessile filter feeders that live in close association with abundant and diverse communities of microorganisms that form the oyster microbiota. In such an association, cellular and molecular mechanisms have evolved to maintain oyster homeostasis upon stressful conditions including infection and changing environments. We give here cellular and molecular insights into the *Crassostrea gigas* antimicrobial defense system with focus on antimicrobial peptides and proteins (AMPs). This review highlights the central role of the hemocytes in the modulation and control of oyster antimicrobial response. As vehicles for AMPs and other antimicrobial effectors, including reactive oxygen species (ROS), and together with epithelia, hemocytes provide the oyster with local defense reactions instead of systemic humoral ones. These reactions are largely based on phagocytosis but also, as recently described, on the extracellular release of antimicrobial histones (ETosis) which is triggered by ROS. Thus, ROS can signal danger and activate cellular responses in the oyster. From the current literature, AMP production/release could serve similar functions. We provide also new lights on the oyster genetic background that underlies a great diversity of AMP sequences but also an extraordinary individual polymorphism of AMP gene expression. We discuss here how this polymorphism could generate new immune functions, new pathogen resistances or support individual adaptation to environmental stresses.

1. Introduction

Oysters are bivalve mollusks belonging to the Ostreidae family (Mollusca, Bivalvia, Lophotrochozoa). They are sessile filter-feeders living in shallow water from intertidal zones of bays, lagoons and estuaries. In these habitats, oysters are confronted and adapted to great changes in biotic and abiotic environmental conditions. Abiotic factors include temperatures and salinity fluctuations but also exposure to xenobiotics and water acidification due to human activities. Biotic factors include abundant and diverse populations of microbes. As filter feeders, oysters are in permanent contact and exchanges with microorganisms. Thus, they harbor on their surfaces and inside their body cavities and hemolymph a dense microbiota which has been shown to be greatly dominated by *Vibrio* species [1,2]. Indeed, a large attention has been paid over the past years, to populations of vibrios as they are among the most abundant cultivable bacteria isolated from oyster tissues [3]. In healthy oyster, bacteria load, including vibrio, has been shown to vary over time according to individuals and to temperature, with hemolymph average concentrations of 5,7 colonies forming unit (CFU) per μL [2,4]. Hence, oyster must be seen as an organism associated to a microbiota (including mutualistics, opportunists and pathogens), that has adapted effective cellular and molecular mechanisms for maintaining homeostasis and health status in stressful and changing environments. The multifactorial diseases affecting *Crassostrea gigas* oysters worldwide [5] are the outcome of an equilibrium collapse in the interplay between the biotic and abiotic environmental factors such as microorganisms and

* Corresponding author. Tel.: +33 4 67 14 47 10.
E-mail address: ebachere@ifremer.fr (E. Bachère).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.fsi.2015.02.040
1050-4648/

temperature [6], on the one hand, and the oyster physiology and immune responses on the other hand [7].

By focusing on *C. gigas*, we propose here to give cellular and molecular insights into the oyster antimicrobial defense system considering the genetic background of individuals.

2. Effectors of the antimicrobial defense

Oyster immunity involves not only hemolymph-mediated reactions, but also immune effectors produced by epithelial cells from various organs, including gills, mantle, digestive gland and intestine, which participate in the antimicrobial defense mechanisms.

2.1. Plasma proteins

As oysters have a semi-open circulatory system, hemolymph is typically an important interface between the immune system and the microorganisms that enter the oyster body. The oyster hemolymph is devoid of clotting reaction by means of plasma gelation but the hemocytes display remarkable spontaneous reaction of aggregation resulting in cellular clot [8]. Aggregation is reversible, the hemocytes can further disperse and re-enter the circulating system. *In vitro* hemocyte aggregation has been shown to be inhibited by recombinant tetraspanin [9] which participates to cell-adhesion molecular complexes of mammalian white cells. To date, no respiratory protein has been characterized in Ostreoidae bivalves but strikingly, the oyster plasma is characterized by the over representation of proteins that present homologies with extracellular metalloenzyme Superoxide Dismutases (EcSODs). Named dominin [10], cavortin [11] or EcSOD [12,13], these proteins could belong to a complex family of multifunctional molecules [5]. For instance, one member of this family, *Cg*-EcSOD, has LPS-binding properties and acts as an opsonin for pathogenic vibrios (see below) [13,14]. To date, there is no compelling evidence that all SOD-related proteins retained SOD activities [10,11]. Thus, these circulating proteins may play major roles, even now underexplored, in the interplay with the oyster hemolymph microbiota but also in the antimicrobial defense reactions, by mediating microbe recognition and promoting phagocytosis.

2.2. Oyster hemocytes

The hemocytes (blood cells) are immunocompetent cells but they are also involved in many physiological processes such as wound and shell repair, nutrient transport and digestion, gonad resorption. As the oyster circulatory system is semi-open, hemocytes are not confined to the vessels and they invade or reside in many other tissues [15]. Thus, infiltrating hemocytes are present in all cavities, tissues and epithelia of oyster body where they can also fulfil defense functions. The term hemocytes refers to a diversity of circulating cells that is best highlighted by the lack of unified classification, although they have been extensively studied since early 1970's. Indeed, the lack of molecular and functional genetic tools has precluded any in-depth characterization of cell lineage ontogeny and discrimination of functionally distinct cell types.

2.2.1. Hemocyte lineage

Although a clear definition of distinct cell types is still missing, a consensus about three main cell populations, i.e. blast-like cells, hyalinocytes and granulocytes, appears in the literature as they can be distinguished either by microscopy or flow cytometry analyses [15,16]. Among hyalinocytes, also named agranular cells, a subset of professional phagocytes, which are sometimes described as macrophage-like cells, harbor a potent phagocytosis activity; they account for 30–40% of the total populations of hemocytes [14]. Attempts to define cell lineages and functional subsets have been reported using classical May-Grünwald Giemsa (MGG) staining, peroxidase or phosphatase staining, electron microscopy, or flow cytometry analyses [16–19]. From works in the different oyster species, some authors reported basophile and eosinophile granulocytes similar to their mammalian counterparts [16]. Other authors have drawn hypothesis of a cell maturation process in one single lineage from blast-like cells, hyalinocytes to granulocytes [20]. Hence the different hypotheses range from one unique lineage to numerous functionally distinct cell types from different lineages [16,20]. One caveat for most of these different reports is that most of the tools that have been used so far for hemocyte characterization were developed to analyze mammals blood cells (like the MGG staining for example) and thus have to be interpreted with caution in other species. Another caveat is that depending on the maturation stage or the functional activities of one cell type, the cell morphologies and physic-chemical characteristics may change, as for example the internal complexity of professional phagocytes that increases upon phagocytosis when analyzed by flow cytometry. Altogether, the actual number of cell lineages and functionally specialized subsets of hemocytes remain to be carefully examined and accurately determined.

2.2.2. Hematopoiesis

As for the distinction of the different cell types, the ontogeny of oyster hemocytes remains to be fully characterized. Different hypotheses about their hematopoietic origin have been elaborated over the past decades. Cheng (1981) proposed that hemocytes could originate from the differentiation of connective tissue cells [17]. Tirapé and colleagues (2007) described that the expression of *Cg-tal* (Tal1/SCL) [21], a family of transcription factors involved during embryonic hematopoiesis in vertebrates [22] was only detected in cells emerging from blood vessel endothelium, which is reminiscent of the hematopoietic cell emergence from the hemogenic endothelium in vertebrate embryos [23,24]. More recently, a study from Jemaà and colleagues (2014) using BrdU to localize mitotic cells within the oyster tissues suggested that some hemocyte progenitors emerge from particular structures at the basement of the gill epithelium [25], which is reminiscent of assumptions made earlier by Cuénot in 1891 [26]. Altogether, this sum of potentially contradictory pieces of work highlights the lack of knowledge about hematopoiesis in oysters and more largely in bivalves. Although the recent progress in molecular biology has dramatically advanced our knowledge on the immune-function of hemocytes, little is known about oyster hemocyte life cycle and cell lineage origin. This discrepancy is probably due to the lack of dedicated molecular tools for cell lineage analysis and/or the lack of long-term cell culture systems for studying cell differentiation and maturation. Such tools gave access to a comprehensive knowledge of hematopoiesis in other animals from drosophila to human. The recent release of the full *C. gigas* genome should help to develop the required tools.

2.3. Antimicrobial peptides/proteins (AMPs)

Several gene-encoded antimicrobial peptides and proteins (AMPs) sharing common molecular features with AMP families described in other kingdoms of life have been characterized in oysters (Table 1). These host defense effectors are usually small cationic (less than 10 kDa), amphipathic peptides showing a broad diversity of amino acid sequence and structural conformations [27,28]. However, some large cationic proteins with antimicrobial properties have also been described (Table 1). Based on common structural features or conserved sequence motifs characteristics for AMP families, oyster antimicrobials have been classified as

Please cite this article in press as: E. Bachère, et al., The new insights into the oyster antimicrobial defense: Cellular, molecular and genetic view, Fish & Shellfish Immunology (2015), http://dx.doi.org/10.1016/j.fsi.2015.02.040