Using sea cucumber coelomocytes as stress indicators? The case of lipopolysaccharide endotoxin

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Marine invertebrates make up the vast majority of the biomass of marine metazoans. Among them, sea cucumbers (holothuroids) occupy a special place in marine ecosystems, performing much the same function as earthworms but in the sea. More specifically, by ingesting sediments, they help to recycle sedimented organic matter and oxygenate soils. This ecological position makes holothuroids ideal organisms for studying the effect of pollution on marine fauna, as they are directly exposed to the disturbing elements that settle on the seabed [1]. In addition, certain species of holothuroids are of great economic interest because they are exploited as seafood in Asian countries. In recent decades, as a result of the growing demand for their consumption, numerous holothuricultures (i.e. holothuroid aquacultures) have been developed around the world. Gaining knowledge of how these organisms respond to various stresses in their aquaculture facilities has also become an important issue in the development of this green economy.

The response of sea cucumbers to stress factors is mainly ensured by the equivalent of vertebrate leucocytes, called coelomocytes in echinoderms. These cells are involved in a wide range of mechanisms, including the humoral response, wound healing and the clearance of biotic and abiotic foreign materials by phagocytosis or encapsulation processes [2]. But while we know that these cells have a global immune function, how they recognise and respond to stress factors remains poorly understood. To better appreciate these processes and assess the potential value of coelomocytes as sentinels of environmental stressors, we studied the response of these cells to lipopolysaccharide (LPS) exposure, an endotoxin produced by Gram-negative bacteria. Two species were investigated: the first species is Holothuria forskali, a temperate species living on European rocky shores; the second species is Holothuria scabra, a tropical species living in Indo-Pacific waters and aquacultivated for export to Asian countries. More concretely, 24 hours after injections of LPS, coelomocytes were collected from the body fluids, the proportion of coelomocytes was assessed to compare with that of control individuals, and differential RNA-sequencing analyses were carried out to identify marker genes that are up-regulated in individuals that have been exposed to the toxin. In parallel, morphological characterisation of coelomocytes was carried out to better define the coelomocyte cell types in these two species.

Our results revealed 6 main cell types in H. forskali and 5 in H. scabra. Changes in the coelomocyte concentration and proportion were highly variable between individuals, making it difficult to identify a clear stress response at the cellular level. Nevertheless, for both species, it was possible to discern a certain increase or decrease in the proportion of cells, suggesting a clear response for at least part of the cell populations, and in particular in the hemocytes of H. forskali. Finally, differential gene expression analyses revealed a wide diversity of immune genes that were overexpressed following stress. In particular, NOD-like receptors (NLRs) were particularly abundant, with around 300 genes annotated as NLRs and around 10% of them differentially expressed in endotoxin-exposed individuals. NLRs are important pathogen recognition receptors that induce inflammation and stimulate the immune response.

In summary, this study identifies the main molecular and cellular actors in the response to lipopolysaccharide exposure in holothuroids and could serve as a basis for developing tools for monitoring stress in these ecologically and economically valuable organisms.

References

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