

11)-Fish granzymes and their role in the innate cell-mediated cytotoxicity against nodavirus-infected cells.

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Topic: Vertebrate innate immune cells: receptors and effector mechanisms

Keywords: granzymes, cytotoxic activity, fish, nodavirus

Abstracts:

Granzymes (Gzm) are granule-associated serine proteases, which are important effector molecules in cell-mediated cytotoxicity, classified by its proteolytic activity in: tryptase (GzmA and GzmK), Asp-ase (GzmB), Met-ase (GzmM) or chymase (others). In fish, even at gene level, very few studies have partially identified and characterize them. In this work we have identified the activity of these four granzymes, using colorimetric assays, in leucocytes from gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), and how they are increased upon cytotoxic activity against nodavirus-infected tumor cells. The results also showed that GzmA was the most abundant and GzmB the lowest. Deeper characterization of the GzmB led us to identify that seabream leucocytes under cytotoxic response against nodavirus-infected cells showed significantly increased GzmB activity whilst this pattern failed to do so in sea bass leucocytes. Moreover, flow cytometry studies also suggested the presence of GzmB into the cytoplasm of effector cells. At gene level, seabream GzmB gene was found to be up-regulated in leucocytes under cytotoxic activity, mainly if the target cells were infected with nodavirus. These data will through some light in the importance of granzymes in the cytotoxic response of fish and their defense against nodavirus infection.

Financial support by grants AGL2010-20801-C02-02 and AGL2013-43588-P (MINECO and FEDER), RYC-2009-05441 (MINECO), 04538/GERM/06 (*Fundación Séneca de la Región de Murcia*) and fellowship to Y. Valero (*Instituto Español de Oceanografía*) is gratefully acknowledged.