Evaluation of immune response after LCDV-Sa infection in DNA-vaccinated gilthead seabream.

Rocío Leiva-Rebollo, Juan Gémez-Mata, Dolores Castro, Juan J. Borrego, Alejandro M. Labella

Universidad de Málaga, Instituto de Biotecnología y Desarrollo Azul, IBYDA, Departamento de Microbiología, Málaga, Spain

Introduction: The immune-related gene expression in vaccinated gilthead seabream after Lymphocystis Disease Virus 3 (LCDV-Sa) infection was analysed by using an OpenArray® platform based on TaqMan quantitative PCR. The DNA vaccine used in this study (pcDNA-MCP) encodes the viral major capsid protein (MCP) and confers protection against LCDV-Sa infection in juvenile gilthead seabream.

Methodology: Gilthead seabream juveniles were distributed into four experimental groups and intramuscularly injected with the vaccine (vaccinated group), the empty-plasmid (mock-vaccinated group), or PBS (control groups). Thirty days after vaccination, vaccinated and mock-vaccinated fish, as well as one of the control groups, were injected intraperitoneally with LCDV-Sa (10^6 $TCID_{50}$/fish). Samples of head-kidney (HK) from 6 fish were individually collected 1 and 3 days post-infection (dpi). The relative expression levels of 49 genes related to the immune response, 4 reference genes (*ef1α*, *actβ*, *rps18*, and *ub*), and 3 viral genes (*mcp*, *mmp*, and *rpb*) were analysed using an OpenArray. Samples from the non-infected control group were collected at the same time points and used as calibrator.

Results: The number of genes differentially expressed (DEG) in HK at 1 dpi was higher in vaccinated fish compared with both mock-vaccinated and non-vaccinated animals. Furthermore, 94% of those DEG were downregulated in vaccinated fish, whereas 80 and 75% of DEG showed downregulation in mock-vaccinated and non-vaccinated fish, respectively. At 3 dpi, most DEG were upregulated, and the differences in their number among groups were minimized. The recombination-activating gene 1 (*rag1*), a mediator of development of B and T lymphocytes, was the only gene upregulated in HK samples at 1 dpi. This gene was also upregulated in non-vaccinated animals but at 3 dpi. In contrast, early *mx* induction was observed in non-vaccinated animals (upregulation of *mx2* at 1 dpi) in comparison to vaccinated seabreams (upregulation of *mx1* and *mx2* at 3 dpi).

Conclusions: The results that will be discussed could evidence the role of the DNA vaccine as regulator of the primary lymphoid tissues (HK) in gilthead seabream against LCDV-Sa infection, through downregulation of inflammation related-genes, early upregulation of *rag1*, and a later expression of interferon stimulated genes.

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