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Antiviral immune responses to IPNV in Atlantic salmon cells

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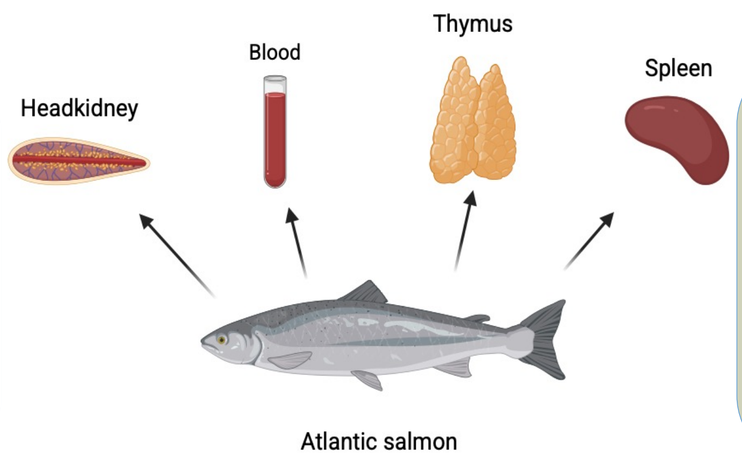
Abstract:

In this project, we investigated immune responses in leukocytes isolated from Atlantic salmon following exposure to Infectious pancreatic necrosis virus (IPNV), the causative agent of IPN. We have looked at leukocytes from the head kidney, blood, thymus and spleen. Furthermore, the virus was detected by immunostaining and presence of neutralizing antibodies upon vaccination was determined. The aim of this project was to examine how salmon leukocytes responded to IPNV, with particular focus on antiviral and cytotoxic activity, and to establish a protocol for virus neutralization.

Why IPNV?

Infectious pancreatic necrosis virus (IPNV) is a virus infecting salmonid fish. The virus can cause significant health problems and mortality, especially in young fish, and is therefore an important concern in aquaculture. Understanding how salmon cells respond to IPNV is important for improving vaccines and evaluating protective immune responses.



Methods:

- Isolation of leukocytes and RNA
- CHSE cell culture
- Virus propagation and titration
- Agarose gel electrophoresis
- cDNA synthesis
- Measurement of immune responses by qPCR
- Immunostaining with antibodies to visualize virus
- Establishment of a neutralization assay protocol

Results and discussion:

Leukocytes isolated from head kidney, spleen, thymus and blood showed visible differences in cell morphology (Figure 1). PCR products for immune-related genes were confirmed by agarose gel electrophoresis (Figure 2), and qPCR showed measurable expression of IFNα, MX, IFNγ and Granzyme A (Figure 3). In CHSE cells, IPNV antigen was detected by fluorescence microscopy (Figure 4), while serum from vaccinated fish showed stronger antibody recognition of IPNV antigen than the PBS control (Figure 5).

Leukocytes:

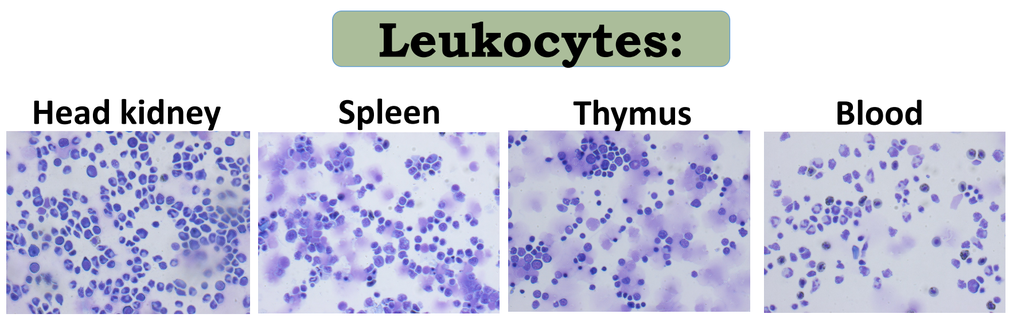


Figure 1: Cytospin preparations of Atlantic salmon leukocytes. Representative cytospin images (40× objective) of leukocytes. Fractions of different cell types varied among leukocytes isolated from head kidney, spleen, thymus and blood.

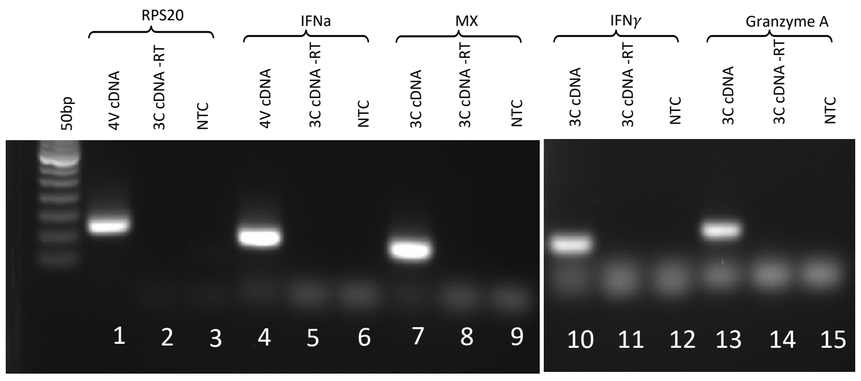


Figure 2: Validation of PCR products by agarose gel electrophoresis. Agarose gel electrophoresis of PCR products for RPS20 (122 bp), IFNa (104 bp), MX (70 bp), IFNγ (55 bp) and Granzyme A (81 bp). Bands of the expected size were detected for the target genes, while no amplification was observed in the no-template controls (NTC). The -RT controls were included to assess potential genomic DNA contamination.

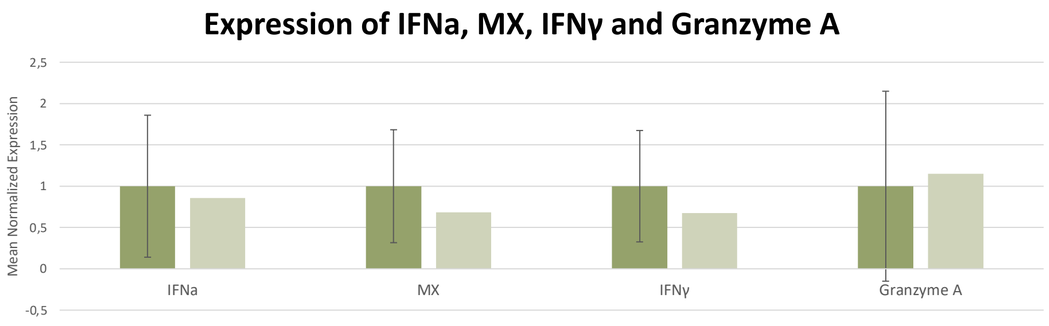


Figure 3: Relative gene expression of IFNa, MX, IFNγ, and Granzyme A. Gene expression was measured by qPCR and calculated using the ΔΔCt method and normalized to the reference condition. Bars represent mean relative expression, and error bars indicate variation between replicates.

CHSE cells:

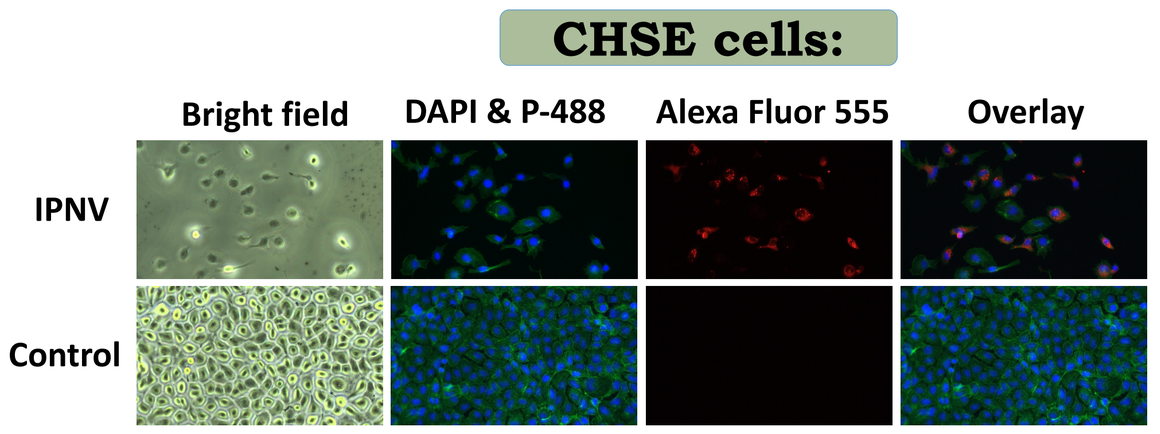


Figure 4: Fluorescence microscopy of IPNV-infected CHSE cells. Alexa Fluor 555 signal (red) is only observed in the virus infected cells, indicating detection of IPNV antigen. P-488 = Phalloidin-488.

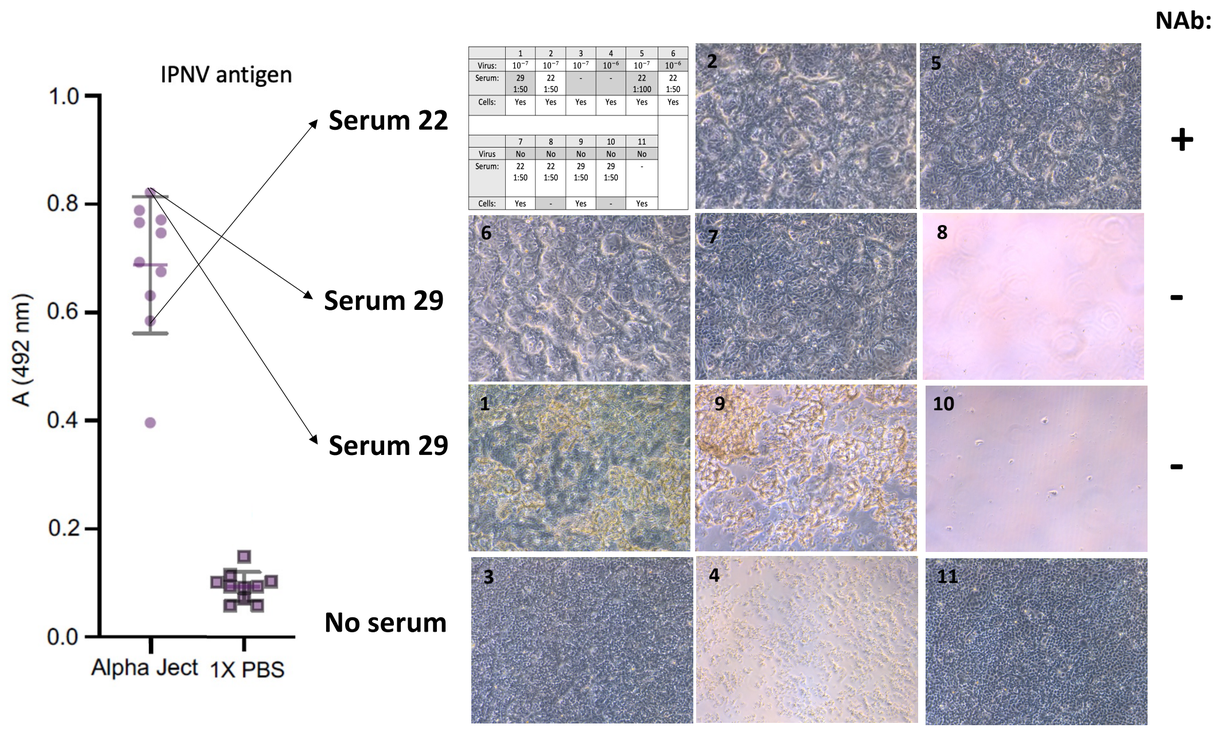


Figure 5: **Detection of IPNV antigen in infected cell cultures.** Serum from Alpha Ject-vaccinated fish showed higher absorbance at 492 nm compared with the PBS control group, indicating a stronger antibody response against IPNV antigen. Serum 22 showed higher neutralizing antibody activity than serum 29, suggesting stronger neutralization of IPNV infection. NAb refers to neutralizing antibody activity.

Conclusion:

IPNV was detected in infected cells using immunostaining, and antibodies produced following vaccination showed neutralizing activity. qPCR assays for antiviral and cytotoxic responses were highly specific and provide an important basis for measuring cytotoxic T cell responses.

References:

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Sønnervik, 2022, *Master's thesis*, UiB. Nerbøvik, 2013, *Master's thesis*, UiB. Runestad, 2024, *Master's thesis*, UiB

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