Characterization of immunoglobulins in lumpfish (*Cyclopterus lumpus* L.)

- gene expression analyses of IgM and IgD during ontogenesis and within tissues

Harald S. Lunde[1], Gyri T.Haugland[1], Anita Rønneseth[1], Cecilie Walde[2], Håvard Ø. Eggestøl[1], Thomas Østerhus[1] and Heidrun I.Wergeland[1*]

[1] Department for biologi, University of Bergen, Bergen, Norway
[2] The Norwegian Veterinary Institute, Bergen, Norway

*Heidrun.Wergeland@uib.no

SUMMARY

We have identified IgM and IgD in lumpfish and performed gene expression analyses of soluble IgM (sIgM), total IgM and IgD by use of real-time RT-PCR during ontogenesis. We have collected eggs before and after fertilization, and larvae 18 time points post fertilization (1023 ddg) to investigate the earliest stage of IgM and IgD expression. Further, we have analysed the expression level of IgM and IgD in 18 tissues of young lumpfish (240 g). We have also performed immunohistochemistry and stained various tissues with lumpfish anti-IgM antibody.

INTRODUCTION

The subtypes of immunoglobulins in teleost fishes are IgM, IgD and IgT (called IgZ in zebrafish). IgM is the predominant isotype in serum and specialized in systemic immunity. IgT plays a major role in mucosa immunology and is considered to be the functional equivalent to IgA in mammals and birds. The role of IgD in fish immunology is not yet clarified. In fish, two mutually exclusive B cell lineages excist; IgM or IgT. IgD can be co-expressed with IgM on B cells, but not with IgT. In addition to their role as antigen-producing cell, B-cells in lumpfish (1) and other teleosts (2) play a role in innate immunity by being professional phagocytic cells. In previous studies we have shown that lumpfish produce specific antibodies upon immunization (1), but currently little is known about the development of B cells during ontogenesis in lumpfish or distribution in tissues.

In recent years, production of lumpfish has increased in Europe and Canada due to its use as cleaner-fish for sea-lice control in salmon farming. One of the major challenges is mortality caused by bacterial diseases during production and after transfer to sea cages. Therefore, knowlegde about the development of the immune system is important to determine when effect of vaccines in early stages can be obtained.

MATERIALS AND METHODS

Egg, larvae and fish. Egg and larvae of lumpfish were collected at Fjord Forsk Sogn AS, a commerial breeder in Sogn & Fjordane County in Norway, at different time points; eggs before and after fertilization and 18 samplings of larvae up to 1023 ddg after hatching (Fig. 1). Lumpfish (10 g) was transported to the Industrial Laboratory (ILAB) at Bergen high technology centre and kept in a 500 L tank.

Sample collection. Head kidney leukocytes (HKL), periferal blood leukocytes (PBL) and samples from sixteen tissues of lumpfish (240 g): muscle, liver, gonad, brain, heart, skin, intestine, pyloric caeca, eye, tongue, gill inner, gill outer, skin, thymus, head kidney and spleen were collected. HKL and PBL was isolated by use of Percoll gradient (3).

RNA extraction, cDNA synthesis and real-time PCR. Total RNA extraction (Sigma), DNase treatment (Sigma) and cDNA synthesis (qScript) was performed according to the manufacturer's instructions. The sequences of souble and membrane bound IgM and IgD was identified in de novo assembled transriptome of lumpfish (4). Real-time PCR was performed in a CFX96 (Biorad) using SYBR Green Jumpstart Taq ready mix for quantitative PCR (Sigma) and HPLC purified primers (Merck).

Monoclonal anti-IgM antibody and immunohistochemistry: The anti-IgM Mab was kindly provided by Douglas J. Milne, University of Aberdeen. Immunohistochemistry of head kidney was performed using standard protocol using the chromogen Fast Red TR salt which stain positive cells red.

RESULTS AND DISCUSSIONS

Phylogenetic analysis of Ig's

In the de novo assembled transcriptome of lumpfish head kidney leucocytes soluble IgM, membrane bound IgM and IgD has been identified (Fig. 1). We have not yet identified IgT/IgZ.

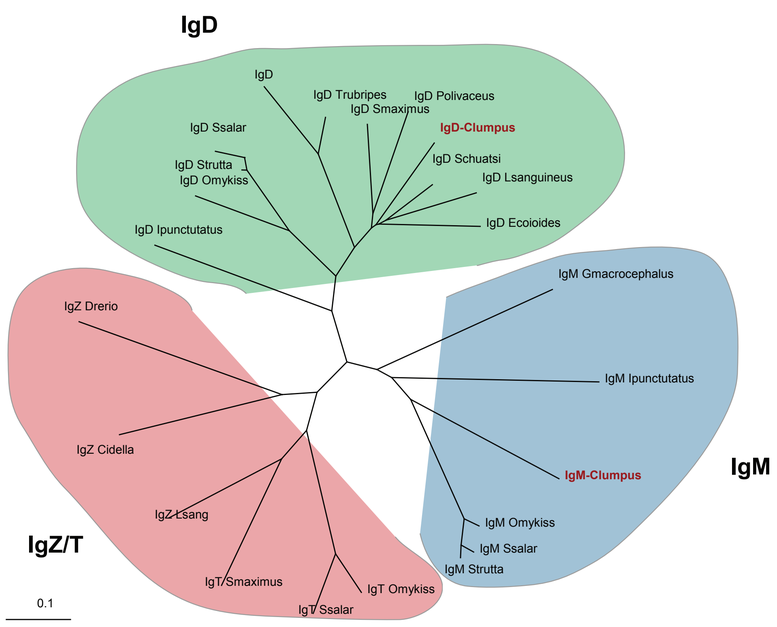

Figure 1. Phylogenetic analyses of IgM, IgT/IgZ and IgD. In lumpfish, we have identified IgM and IgD

Gene expression analyses of IgM and IgD in different tissues

The levels of membranebound and soluble IgM and IgD in PBL, HKL and in different tissues of lumpfish was measured by real-time PCR. The level of IgM was highest in spleen and head kidney, while the level of IgD was highest in PBL and spleen (Fig. 3)

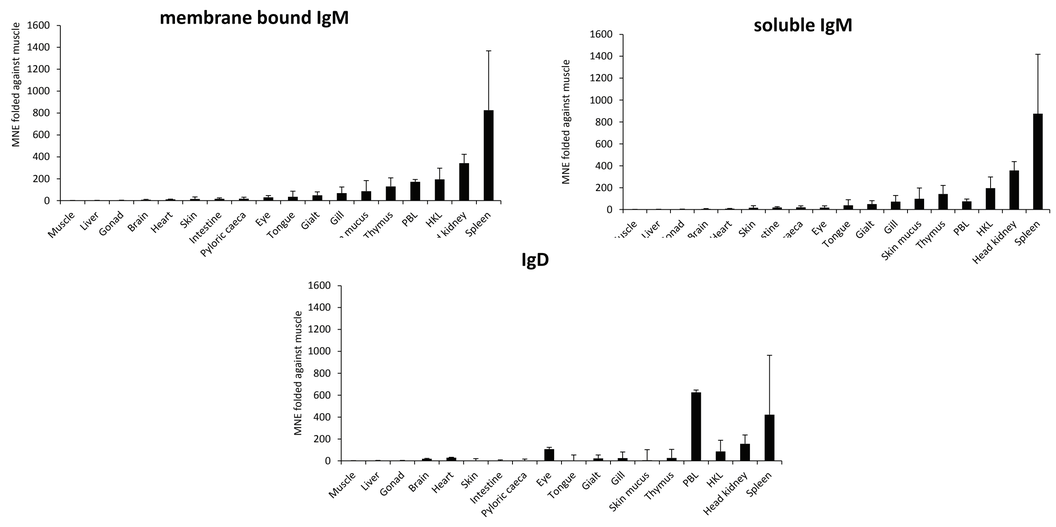

Figure 3. Relative expression of membran-bound and soluble IgM and IgD transcripts in PBL, HKL and 16 different tissues of lumpfish normalized with RPS20 expression. The MNE are folded against muscle.

Gene expression analyses of IgM and IgD during ontogenesis

In order to measure the level of Igs during ontogenesis, SYBR-green assays were made. The controls NTC (non template control), -RT (cDNA synthesis without reverse transcriptase) were confirmed negative and the efficiency of the assays was determined (Fig. 2A, B). RPS20 was selected as endogene reference gene (Fig. 2C). The level of total IgM and soluble IgM was determined in eggs and larvae up to 1023 ddg post hatching (Fig. 2D).

Figure 2. Expression of total IgM (tIgM) and soulble IgM (sIgM) during ontogenesis of lumpfish. Photo of egg and larvae: Anita Rønneseth

Immunohistochemistry of head kidney with anti-IgM

Presence of IgM positive cells in head kidney was visualized by immunohistochemistry staining with monoclonal antibody against IgM (Fig. 4).

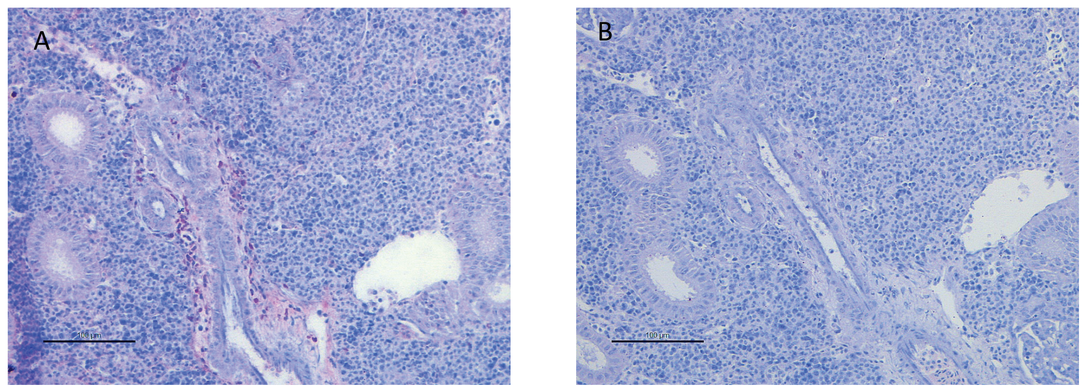

Figure 4. Immunohistochemistry staining of kidney from lumpfish stained with A) monoclonal antibody against IgM and B cells. B) isotype control (negative control). Red cells are positive for IgM. Magnification 200x.

CONCLUSIONS

Knowlegde of development and maturation of B- cells and adaptive immune responses in early life stages is important to identify when immunostimulation can take place and when adaptive and protective immune responses can be obtained.

Aknowlegdement

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