Mol231: Characterization and isolation of Monocytes in Atlantic Salmon (*Salmo Salar* L.)

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Background: Monocytes are a part of the innate immune system which is responsible for detecting exogenous- versus endogenous-matter (1). Monocytes/macrophages are able to kill phagocytosed micro-organisms using a variety of oxygen dependent (Myeloperoxidase, MPO) or independent (Acid Phosphatase, AcP) mechanisms (2). A method to isolate monocytes is yet to be found. Monocytes in mammals contain a membrane protein, CD14. It detects and binds to the lipopolysaccharide (LPS) layer in gram-negative bacteria. To date, CD14 in fish has not been described. Aims: Characterize monocytes functionally, search for CD14 candidates in fish and test various human CD14 reagents for cross-reactivity. If positive, we will have valuable tools to isolate and further characterize monocytes in salmon.

Materials & Methods:

- ❖ Isolation of leukocytes
- ❖ MACS sorting
- ❖ Cytospin preparation
- ❖ Isolation of RNA
- ❖ Phagocytosis
- ❖ Flow cytometry
  - Scatterplot
  - Antibody
- ❖ DNase-treatment and cDNA synthesis
- ❖ Agarose gel electrophoresis
- ❖ SDS-PAGE and Western blot
- ❖ Staining: ColorRapid
  - Acid phosphatase w/wo tartrate
  - Myeloperoxidase
- ❖ Multiple sequence alignment
- ❖ Phylogenetic analysis
- ❖ Domain prediction (Interproscan)

Results:

1. Isolation of leukocytes

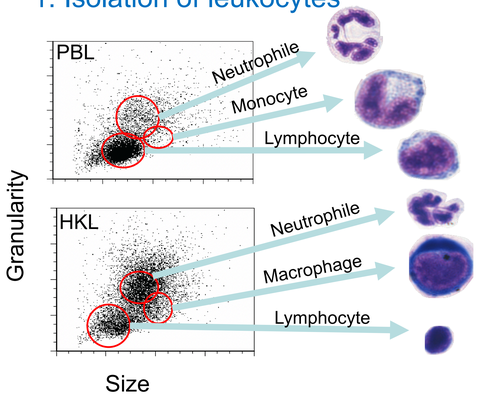

Fig. 1. Scatterplot results from flow cytometry, used to distinguish different lekocytes; neutrophiles, which have high granulerity, the bigger monocytes and the smaller lymphocytes

2. Characterization of leukocytes

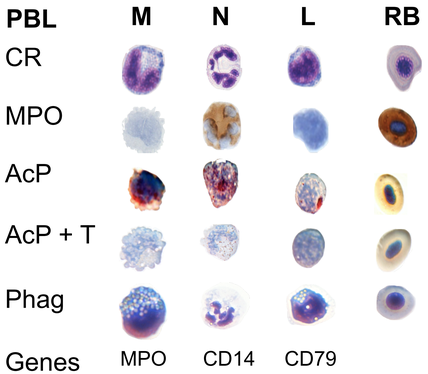

Fig. 2. PBL = periferal blood leukocytes, N = neutrophile, M = monocytes, L = leukocytes, RB = red blood cell, CR = ColorRapid, MPO = myeloperoxidase, AcP = acid phosphatase, T = tartrate, Phag = phagocytosis

3. Phagocytosis

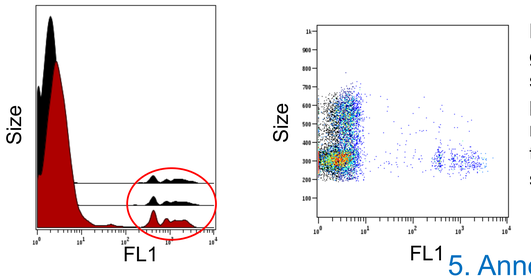

Fig. 3. Phagocytosis of yellow green flourescent beads, showing that lekocytes have phagocytosis of exogenous matter. The right hand side of the histogram and scatterplot shows the phagocytotic cells

4. Percentage of isolated cells from four fish using CD14 plus magnetic beads

1	22.9%
2	24.1%
3	5.15%
4	10.7%

Table 1. Percentage of isolated cells from four fish using CD14 magnetic beads

5. Annotation of TLR2 and CD14 in vertebrates

Fig. 4. TLR2 and CD14 human 3D structures

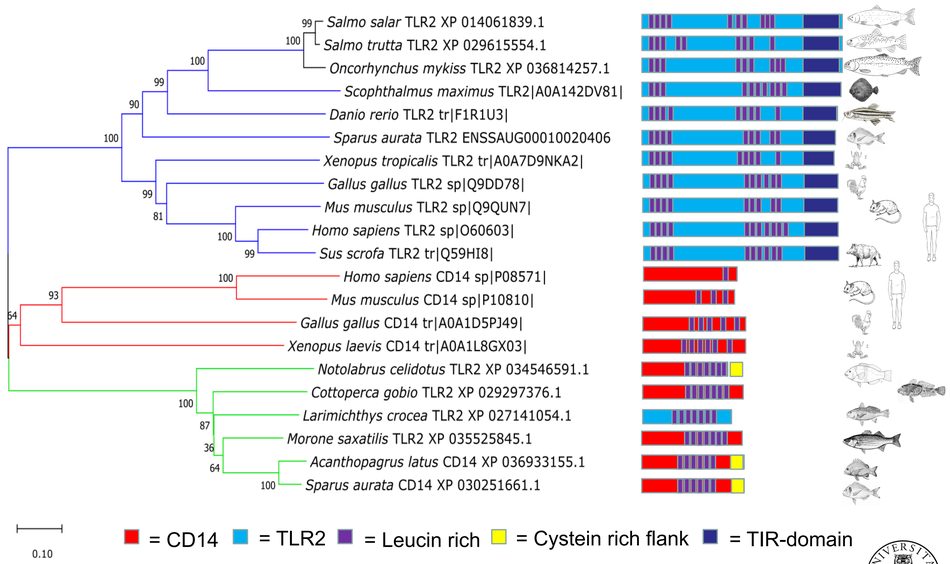

Fig. 5. Domain representation of different vertibrates, with phylogenetic tree. Made via InterProScan and MegaX

- 1. Scatterplot: Differentiation of leukocytes based on granularity and size. Fig. 1
- 2. Cytochemical staining using various methods. Monocytes are positice for AcP and negative for MPO. Fig. 2
- 3. Phagocytosis of fluorescent-beads. Fig. 3
- 4. CD14 mouse anti human microbeads – Miltenyi biotech: Cross checked with Fisher and Köllner . Table 1.
- 5. Fish have CD14 even though it is not very well annotated. Several annotated fish TLR2 shows high similarity to CD14. Fig. 4 and Fig. 5.

Future work:

- Check more human antibodies which might bind to fish monocytes.
- Identify CD14 candidates in salmon.

Acknowledgements:

1. Harald Sæbø Lunde for assistance with the microscope

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