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# PAMPs and DAMPs stimulate the expression of pro-inflammatory cytokines *in vitro* in a fibroblast cell-line from rainbow trout (*Oncorhynchus mykiss*)

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## Introduction

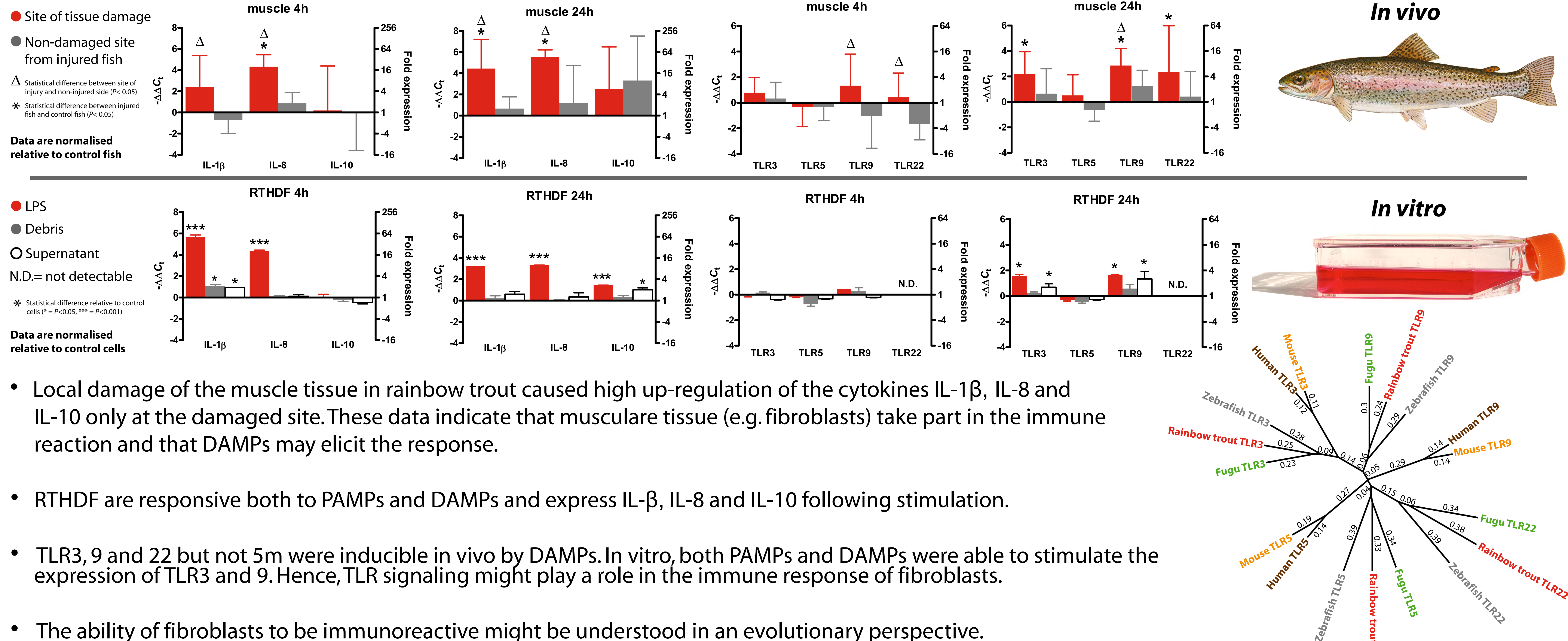
Fibroblasts are traditionally considered as a non-immune cell type. Despite this, experiments indicate that this cell-type may have immune-regulating properties as a result of the presence of pattern-recognition receptors (PRRs) on their surface and their ability to express a range of cytokines following PAMP stimulation. Results obtained from an *in vivo* study in rainbow trout (*Oncorhynchus mykiss*), imply the involvement of fibroblasts in the immune response following a local damage of the musculature. Hence, a fibroblast cell-line (RTHDF; Rainbow Trout Hypodermal Fibroblast) was stimulated *in vitro* with LPS, RTHDF debris and supernatant to examine the ability of this cell-type to react to PAMPs and DAMP stimuli and if this possibly was mediated by downstream TLR signaling.

## Methods

**In vivo** experiment: the muscle tissue of rainbow trouts (n=10) was damaged using 25 sterile needles in a square of 6 x 6 mm. The needles penetrated 5 mm through the skin and into the musculature. Muscle tissue was sampled at the site of injury and at the opposite side of the fish relative to the damage site from 5 fish 4 hours and 24 hours post injury. Tissue from 5 non-injured fish was also sampled as controls at the same time-points. The size of the fish was 9.5 ± 2.6 g and 9.5 ± 0.9 cm.

**In vitro** experiment: triplicate flasks of RTHDF cells were stimulated using either 20 ug/ml of *E. coli* 0111:B4 LPS, sonicated fibroblasts (ratio 1:1 between number of stimulated cells and cells used for sonication) and supernatant from centrifuged sonicate. Cells were harvested 4 hours and 24 hours post stimulation.

Real-time RT-PCR was used to measure the expression of the genes IL-1 $\beta$ , IL-8, IL-10, TLR3, 5, 9 and 22 following RNA isolation and cDNA synthesis from the muscle tissue samples and RTHDF cells.



- Local damage of the muscle tissue in rainbow trout caused high up-regulation of the cytokines IL-1 $\beta$ , IL-8 and IL-10 only at the damaged site. These data indicate that musculare tissue (e.g. fibroblasts) take part in the immune reaction and that DAMPs may elicit the response.
- RTHDF are responsive both to PAMPs and DAMPs and express IL- $\beta$ , IL-8 and IL-10 following stimulation.
- TLR3, 9 and 22 but not 5m were inducible *in vivo* by DAMPs. *In vitro*, both PAMPs and DAMPs were able to stimulate the expression of TLR3 and 9. Hence, TLR signaling might play a role in the immune response of fibroblasts.
- The ability of fibroblasts to be immunoreactive might be understood in an evolutionary perspective.