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## Evidence of trained immunity in fish macrophages

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### Abstract

Trained immunity is a form of innate immune memory best described in mice and humans. Trained immunity is defined as a heightened response to a secondary infection that can be exerted toward both homologous and heterologous microorganisms. Typical criteria of trained immunity include: 1) induction upon primary infections or immunizations and subsequent protection against a secondary infection, in a T- and B-lymphocyte independent manner, 2) a response that is less specific than an adaptive immune response but that still confers increased resistance upon reinfection of the host and, 3) the involvement of innate cell types such as NK cells and macrophages involved in improved pathogen recognition and an increased inflammatory response. Clear evidence of the evolutionary conservation of trained immunity in teleost fish is lacking. Given the evolutionary position of teleosts as early vertebrates with a fully developed immune system, we hypothesize that teleost myeloid cells show features of trained immunity common to those observed in mammalian macrophages. These would at least include the ability of fish macrophages to mount heightened responses to a secondary stimulus in a non-specific manner.

We established an *in vitro* model to study trained immunity in fish by adapting a well-described culture system of head kidney-derived macrophages of common carp. A soluble NOD-specific ligand and a soluble  $\beta$ -glucan were used to train carp macrophages, after which cells were rested for six days prior to exposure to a secondary stimulus. Unstimulated trained macrophages displayed evidence of metabolic reprogramming, as well as heightened phagocytosis and increased expression of the inflammatory cytokines *il6* and *tnfa*. Stimulated, trained macrophages showed heightened production of reactive oxygen and nitrogen species as compared to the corresponding stimulated but untrained cells. Thereby, trained immunity is a form of innate immune memory that appears conserved in macrophages of common carp. Measurement of the production of reactive oxygen species proved particularly informative to identify ligands able to train carp macrophages.