[1] Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei, 430070, China
[2] Guangdong Provincial Water Environment and Aquatic Products Security Engineering Technology Research Center, Guangzhou Key Laboratory of Aquatic Animal Diseases and Waterfowl Breeding, Guangdong Provincial Key Laboratory of Waterfowl HealthyBreeding, College of Animal Sciences and Technology, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong, 510225, China
[3] Hubei Engineering Technology Research Center for Aquatic Animal Diseases Control and Prevention, Wuhan, Hubei, 430070, China

Abstract

Snakehead vesiculovirus (SHVV) is a new rhabdovirus isolated from diseased hybrid snakehead fish (*Channa maculate* ♀ x *Channa argus* ♂) and has caused serious economic losses in snakehead fish culture in China. To better understand the pathogenicity of SHVV, we developed a reverse genetics system for SHVV by using human and fish cells. In detail, human 293T cells were cotransfected with four plasmids encoding the full-length SHVV antigenomic RNA or the supporting proteins including nucleoprotein (N), phosphoprotein (P), and large polymerase (L), followed by the cultivation in Channel catfish ovary (CCO) cells. We also rescued a recombinant SHVV expressing enhanced green fluorescent protein (EGFP), which was inserted into the 3' non-coding region (NCR) of the glycoprotein (G) gene of SHVV. Our study provides a potential tool for unveiling the pathogenicity of SHVV and a template for the rescue of other fish viruses by using both human 293T and fish cells.

keywords: Snakehead vesiculovirus, reverse genetics, EGFP, vaccine

---

Corresponding author.
E-mail addresses: linli@zhku.edu.cn (L. Lin), tujiagang@mail.hzau.edu.cn (J. Tu).

P-038.

Evidence of trained immunity in teleost fish: Conserved features in carp macrophages

Jules Petit [1], *Carmen W.E. Embregts* [1], *Maria Forlenza* [1],
Geert F. Wiegertjes [1,2,#].

[1] Cell Biology and Immunology Group, Wageningen University & Research, Netherlands
[2] Aquaculture and Fisheries Group, Wageningen University & Research, Netherlands

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 β-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 *IL*6 and *TNFα*. Stimulated, trained macrophages showed heightened production of reactive oxygen and nitrogen species as compared to the corresponding stimulated but untrained cells. Measurement of the production of reactive oxygen species proved particularly informative to identify ligands able to train carp macrophages. We discuss the value of our findings for future studies on trained immunity in teleost fish.

keywords: Trained immunity, Cyprinidae, Monocytes/Macrophages, innate immunity,

---

Corresponding author.
E-mail address: geert.wiegertjes@wur.nl (G.F. Wiegertjes).

P-039.

TLR5 activation site in *Edwardsiella tarda* flagellin is important to induce expression of interleukin-1B and NF-κB genes in Japanese flounder, *Paralichthys olivaceus*

Natsuki Morimoto [1], *Tomoya Kono* [2], *Masahiro Sakai* [2],
Jun-ichi Hikima [2,#].

[1] University of Miyazaki, Interdisciplinary Graduate School of Agriculture and Engineering, Japan
[2] University of Miyazaki, Faculty of Agriculture, Department of Biochemistry and Applied Biosciences, Japan

Abstract

Flagellin is the subunit protein that composes bacterial flagella and is recognized by toll-like receptor 5 (TLR5) as a ligand. Flagellin protein (*e.g.*, FliC and FlaA) contains the D1, D2, and D3 domains; the D1 domain is important for recognition by TLR5 for activation of the innate immune system. In teleosts, there are two types of TLR5, the membrane form (TLR5M) and soluble form (TLR5S), the latter of which is not present in mammals. In this study, the potential of flagellin from *Edwardsiella tarda* (EtFliC) to induce inflammation-related genes interleukin (IL)-1β and NF-κB-p65 through TLR5S in Japanese flounder (*Paralichthys olivaceus*) was elucidated. A transient overexpression system was developed in flounder natural embryonic (HINAE) cells using constructs encoding two flagellin genes derived from *E. tarda* (pEtFliC) and *Escherichia coli* (pEcoFliC) and the flounder TLR5S gene (pPoTLR5S). Expression of inflammation-related genes in EtFliC- and PoTLR5S-overexpressing HINAE cells was significantly lower than in EcoFliC- and PoTLR5S-overexpressing cells. To clarify the difference between EtFliC and EcoFliC potency, the amino acid sequence of EtFliC was compared with that of other bacterial flagellin. The 91st arginine residue, known as the mammalian TLR5 activation site, was conserved in the flagellin of *E. coli* and other bacteria but not in EtFliC. To reveal the importance of the 91st arginine residue in FliC, a pEtFliC construct in which the 91st asparagine was mutated to arginine (pEtFliC_N91R) was generated. Expression of the IL-1β and NF-κB-p65 genes in the HINAE cells co-transfected with pEtFliC_N91R and pPoTLR5S was significantly higher than that in cells co-transfected with pEtFliC and pPoTLR5S. The results suggested that the 91st arginine residue of bacterial flagellin is involved in inflammatory response through TLR5S in teleosts. Thus, EtFliC improved by site-directed mutagenesis could be an effective adjuvant against *E. tarda* infection in Japanese flounder.

keywords: *Edwardsiella tarda*; FliC; TLR5S; IL-1β; NF-κB-p65; Japanese flounder (*Paralichthys olivaceus*)

Corresponding author.
E-mail address: jhikima@cc.miyazaki-u.ac.jp (J.-ichi Hikima).