（学位第３号様式）

学 位 論 文 要 旨

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題　目	Analysis on protective immune responses against *Edwardsiella tarda* infection in ginbuna crucian carp, *Carassius auratus langsdorfii* （ギンブナの *Edwardsiella tarda* に対する感染防御機構に関する研究）

Edwardsiella tarda is an intracellular pathogen that causes edwardsiellosis in fish. Cell-mediated immunity (CMI), involving macrophages activated by IFN-γ produced from $CD4^+$ T helper (T_h) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs), plays a major role in protection against intracellular bacterial infection in mammals. However, the principal immune mechanism of protection against *E. tarda* infection remains unclear in fish. In this study, the principal immune system in ginbuna crucian carp, *Carassius auratus langsdorfii*, that acts against *E. tarda* infection was elucidated.

To determine whether CMI and/or humoral immunity contribute to protection against *E. tarda* infection, cell-mediated and humoral immune responses were examined in ginbuna crucian carp infected with *E. tarda*. Bacterial clearance was observed in the kidney and spleen following the up-regulation of CMI-related genes such as *ifng* and *tbx21*, as was an increase in the number of $CD4^+$ and $CD8\alpha^+$ cells, and an increased cytotoxic activity of CTLs, suggesting that CMI contributes to the elimination of *E. tarda*. However, *E. tarda*-specific antibody titers did not increase until after bacterial clearance, indicating that the induction of humoral immunity was too late to provide protection against the infection.

Adoptive transfer of $CD4^+$ and $CD8\alpha^+$ lymphocytes was performed to determine which T cell subset is involved in eliminating *E. tarda* in ginbuna crucian carp. In addition, expression analysis was performed in the tissue leukocytes of recipients at 2 day post-infection. The results of the adoptive transfer of the T-cell subsets showed that the recipients of $CD4^+$ and $CD8\alpha^+$ cells acquired significant resistance to *E. tarda* infection. The expression levels of the genes T-bet and Perforin in recipients of *E. tarda*-sensitized $CD4^+$ cells were higher than in recipients without T cells. Transfer of sensitized $CD8\alpha^+$ cells up-regulated the expression of the genes IFN-γ and perforin. These results indicate that T_h1 cells and CTLs play a crucial role in the protective immunity against *E. tarda*. Moreover, perforin-mediated antigen-specific cell-mediated cytotoxicity may be necessary to eliminate *E. tarda*-infected cells.

Finally, the adaptive immune response in vaccinated fish was examined to determine whether the effect of vaccination differed between live attenuated and formalin-killed vaccines. All fish treated with the live attenuated vaccine survived, but all those treated with the formalin-killed vaccine died after challenge. In addition, the live attenuated vaccine induced strong CMI in *E. tarda* infection. Conversely, vaccination with the formalin-killed vaccine induced humoral immunity and suppressed CMI induction. These results indicate that a live attenuated vaccine that induces strong CMI is effective against *E. tarda* infection. Moreover, a formalin-killed vaccine may allow spreading infection by suppressing CMI. These findings not only provide novel insights into the development of a CMI-inducing vaccine against the intracellular pathogens in fish but also help elucidate the mechanism underlying adaptive immunity in fish.