Study on mechanism of persistent infection and reactivation of Cyprinid herpesvirus 2

博士学位論文内容要約

専　攻 Major	応用生命科学	氏　名 Name	WEI CHANG
論文題目 Title	Study on mechanism of persistent infection and reactivation of Cyprinid herpesvirus 2 （キンギョヘルペスウイルスの持続感染と再活性化のメカニズムに関する研究）		

Herpesviral hematopoietic necrosis (HVHN), cause by cyprinid herpesvirus 2 (CyHV-2) infection is a highly pathogenic viral disease resulting in sever mortality in goldfish, *Carassius auratus* (L.) and Prussian carp, *C. auratus gibelio* (Bloch). Since first reported in Japan in 1990s, cause severe mortality in goldfish farms, this disease has been reported in different countries of Asia and Europe. CyHV-2 has been proposed to be a member of the family Alloherpesviridae in the order of Herpesvirales which is characterized by persistent or latent infection. The virus remains dormant and non-infectious for a long period, which can reactivate to become pathogenic with the host subsequently showing clinical signs. A current study points out that one of the routes of spread for CyHV-2 may be through the global trade of apparently healthy infected goldfish. In this study, I investigated the process of establishing a persistent and latent infection of CyHV-2 and virus reactivation both *in vivo* and *in vitro* for the purpose of constitution a cornerstone for developing control strategies of HVHN.

Herpesviruses can enter a latent or persistent state after its host survives acute infection. Among the viruses in the Alloherpesviridae family, channel catfish virus (CCV: Ictalurid herpesvirus 1) has been suggested to have the ability to become latent following an acute infection. Another Alloherpesvirus, cyprinid herpesvirus 3 (CyHV-3), also known as koi herpesvirus (KHV), which is also a member of the genus Cyprinivirus and closely related to CyHV-2, becomes latent in leukocytes and other tissue and can be reactivated from latency by temperature-fluctuation stress on the host fish. In the case of CyHV-2, the virus DNA were detected in apparently healthy goldfish**.** Mortality due to CyHV-2 infection occurs during spring, when the fish mature. Virus DNA can be

detected from their ovarian fluid by PCR. All these evidences indicated that CyHV-2 could establish persistent or latent infection in goldfish. In the first part of this study, the persistence of CyHV-2 in the organs of surviving fish reared at a virus permissive temperature (28°C) and the fish exposed to HT treatment to obtain fish with different virus loads were investigated using experimental infections. In addition, virus mRNAs expression was detected using survivors reared at 25℃ for thirteen months after infection. The study demonstrated that CyHV-2, like other herpesviruses, can cause persistent infections in goldfish that survived an experimental virus infection at a virus-permissive 28°C. Cumulative mortality of the fish group was 89% in 2 weeks, and the spleen and kidney of the survivors showed high detection rate of virus DNA at 81 days after infection. Then we monitored the virus DNA in survivors, which had been treated with non-permissive water temperature (34°C) for 4 days initiated at 24, 48 and 72 h after virus infection and were subsequently reared at 25°C to make the fish with different virus loads. The results showed that DNA-positive rates of the organs were high in severely infected fish (72 h-group) even at 30 days after infection and the spleen and kidney showed commonly positive by PCR in all the groups. The detection results of virus gene transcriptions of survivors which rare at 25℃ for thirteen months showed that the two candidates of immediately early and late gene transcriptions were not detected in the survivor. Taken as a whole, the study suggests that CyHV-2 can set up persistent infection in some organs following a primary infection, and the spleen and kidney have the greatest potential to be sites for persistent infection with CyHV-2 in goldfish. In addition, the virus may establish latency in survivors over the time.

To understand whether the asymptomatic survivors will be able to produce the infectious virions. The reactivation of CyHV-2 was investigated *in vitro* by incubation of the tissues dissected from survival fish in medium and *in vivo* by inoculation the immunosuppressants in survivors. Some

organs dissected from the four of five survivors at 5 months after virus infection, where the virus DNA was negative by PCR initially, turned positive after being incubated *in vitro* in medium for 5 days. The spleen, kidney and heart showed highest virus detection rates. By inoculation of the homogenate of the PCR-positive kidney tissue after being incubated *in vitro*, one of three fish died due to the virus infection and virus DNA was detected in other two fish in the same group after 10 days post-inoculation. In *in vivo* experiment, I performed the detection of virus DNA copies and virus mRNA expression, anti-virus antibodies titer test, and immune-related genes expression analysis in the surviving goldfish injected with immunosuppressants of dexamethasone and cyclosporin A which can mainly depress humoral immunity and cell-mediated immunity of fish, respectively. As a type of cortisol, dexamethasone can reduce the formation of plasma cell and inhibit the accumulation of inflammatory cells, including macrophages and leukocytes. Cyclosporin A decreases the production of inflammatory cytokines by T-lymphocytes and inhibits function of Th1 cell. The results demonstrated that CyHV-2 can reactivate when the immunity of carrier decreases. Taqman qPCR analysis on the tissues collected from the experimental fish revealed significant increase of the virus DNA were detected in the dexamethasone alone and both dexamethasone and cyclosporin A injection group at 10 days and 21 days post-injection (dpi). The significant increase of virus DNA in each tissue of the fish in cyclosporine A alone injection group were occurred at 21 dpi. In addition, the virus DNA load of the spleen, kidney, brain, gills and fin in both dexamethasone and cyclosporin A injection group were significantly higher than that in dexamethasone alone injection group at 10 dpi. Although the infectious of reactivated virus was not confirm by infection experiment, the transcripts of virus ORF146 (immediate-early gene) and ORF78 (late gene) were detected in some individuals of experimental groups instead of the asymptomatic survivors without injection. It suggested that the small number of virus particles

may be produced in the survivors. The production of virus may stimulate the immune responses of survivors. The ELISA data indicated that the anti-virus antibody titer of cyclosporin A alone injection group significantly increased at both 10 days and 21 dpi. In addition, the gene expression of MHCII and p47^{phox} which associate to phagocytosis function up-regulated in cyclosporin A group at 21 dpi. These results indicated that virus reactivation can occur in asymptomatic survivors. Although occurrence of virus reactivation may inconsistent among survivors, could be infectious. The anti-virus antibody is the primer factor that can inhibit the reactivation of virus. The phagocytosis function also essential to suppress virus reactivation. Although, the function of cytotoxic T cells on inhibition of virus reactivation is vulnerable compare with humoral immunity, works with anti-virus antibody may contribute to control virus reactivation more effectively.

In the third part of the study, the inhibition of virus reactivation in persistent cell was investigated. Firstly, the virus detection was conducted using cells separated from the kidney of surviving fish to identify the virus harboring cell. The virus genome DNA was quantified by qPCR in each cell population of lymphocytes, monocytes and neutrophils separated by 51% percoll. Result showed that monocytes are the mainly persistent cell of virus. Then, the function of IFN γ induced cell-mediated immune response to inhibit virus reactivation were investigated using the kidney leukocytes of survivors. Virus DNA in incubated kidney leukocytes from surviving goldfish decreased in recombinant IFN γ (rIFN γ) treated group, in which the expression of gene p47^{phox} and MHCII up regulated. In *in vivo* experiment, the anti-IFN γ polyclonal antibodies were inoculated in surviving fish to block the function of IFN γ. The virus DNA did not increase in surviving fish after injection with anti-IFN γ polyclonal antibodies. However, the significant increase of anti-virus antibody titer was detected in experimental group. According to the results,

we suppose that IFN γ contribute to remove the virus in the virus persistent cells due to activation of persistent cells and/or monocytes.

In conclusion, our studies demonstrated that symptomatic healthy surviving fish are the potential source of the infection. The overt infection of CyHV-2 mostly occurs in spring due to decrease of immunity in asymptomatic surviving fish during sexual maturation resulting in reactivation of persistent virus. Inhibition of virus reactivation could be achieved by promoting the production of anti-virus antibody and enhancing the phagocytosis function. The cytokine such as IFN γ which can promote the cell phagocytosis or induce anti-virus effects of infected cell can suppress the virus reactivation.

Our study suggested that CyHV-2 may establish latency in asymptomatic surviving goldfish. However, all the study limited to the tissues level. The mechanism of latency is mysterious and complex, genes expressed during latency have been found to play important roles during latency establishment, maintenance, and reactivation. In the case of CyHV-2, the virus genome DNA are mainly detected in monocytes of the survivor. The latency-associated genes remain unknown. In the future study, the transcripts of virus latency-associated genes should be investigated. The results can provide the evidence to understand the virus strategies of immune evasion and to study mechanism of reactivation. In addition, the undifferentiated monocytes can differentiate into macrophages or dendritic cells. Weather the CyHV-2 reactivation are the differentiation-dependent is a good topic to reveal the mechanism of virus reactivation. Establishment of virus reactivation model system using monocytes from survivor could contribute to investigate the mechanism of reactivation *in vitro*.