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

Abstract

Herpesviral hematopoietic necrosis (HVHN) caused by cyprinid herpesvirus 2 (CyHV-2) infection, is a serious lethal disease and cause great economic losses in goldfish *Carassius auratus* and Prussian carp *C. auratus gibelio* aquaculture industries. Since first reported in Japan in 1992, CyHV-2 has now been reported across the world from Asia to 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. In the case of HVHN, reactivation of CyHV-2 in surviving fish, may occur under stressful conditions, resulting in shedding of the virus from the fish into their rearing water. A current study points out that one of the routes of spread for this virus 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.

Firstly, I investigated virus persistence in the goldfish experimentally infected with CyHV-2. The virus DNA in the organs including the spleen, kidney, heart, brain, gills and fin were monitored in fish groups reared constantly at a virus-permissive temperature (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 I also monitored 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. To understand whether virus can establish latent infection, the virus gene transcriptions were detected using survivors which were reared at 25℃ for thirteen months. The two candidates of immediately early and late gene transcriptions were not detected in the survivor. It suggests that 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. In *in vivo* experiment, the virus reactivation was induced by inoculation with immunosuppressant of dexamethasone and cyclosporin A which can mainly depress humoral immunity and cell-mediated immunity of fish, respectively. 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-inoculation. The significant increase of virus DNA in each tissue of the fish in cyclosporine A alone injection group were occurred at 21 days post-inoculation. 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 days post inoculation. This significant difference did not show at 21 days post-inoculation. The ELISA data indicated that the anti-virus antibody titer of cyclosporin A alone injection group

significantly increased at both 10 days and 21 days post inoculation. The virus mRNA expression of only immediately early gene (ORF146) were detected by RT-PCR in some individual of experiment groups at 10 days post-inoculation. The mRNA expression of late gene (ORF78) was detected in one of five fish of dexamethasone only and both dexamethasone and cyclosporin A injection group respectively at 21 days post-inoculation. In addition, the gene expression of MHCII and p47^{phox} which associate to phagocytosis function up-regulated in cyclosporin A group at 21 days post inoculation. These results indicated that virus reactivation can occur in asymptomatic survivor. Although occurrence of virus reactivation may inconsistent among survivors, could be infectious. In addition, anti-virus antibody and phagocytosis function may be important for inhibition of virus reactivation. Cell-mediated immunity may also contribute to resistant virus reactivation and the interaction with humoral immunity could exert a stronger inhibition on reactivation of CyHV-2.

Lastly, inhibition of virus reactivation in persistent cell was investigated. The virus detection was conducted using cells separated from the kidney of survival 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 were the mainly persistent cell of virus. Then the IFN γ induced immune response on inhibition of virus reactivation in leukocytes was investigated. Virus DNA in incubated kidney leukocytes from survival goldfish decreased in recombinant IFN γ (rIFN γ) treated group. Expression of gene p47^{phox} and MHCII up-regulated in rIFN γ treated groups. In *in vivo* experiment, the anti-IFN γ polyclonal antibodies were inoculated in survival 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, I supposed that IFN γ can play the role on inhibition of CyHV-2 reactivation by promoting phagocytosis function or inducing virus-infected monocyte/macrophage to inhibit virus propagation.

In conclusion, asymptomatic 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. It is supposed that the cytokine such as IFNγ which can promote the cell phagocytosis or induce anti-virus effects on infected cell can suppress the virus reactivation.