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Studies on a New Virus (OMV) from *Oncorhynchus masou*—I. Characteristics and Pathogenicity

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During 1978, we isolated a previously undescrived pathogenic virus from ovarian fluids of normal appearing adult landlocked masu salmon (*Oncorhynchus masou*) at the Otobe Salmon Hatchery, Hokkaido, Japan.

Characteristics of the newly recognized virus conform to those of the *Herpesvirus* group, and the agent is provisionally named *Oncorhynchus masou* virus (OMV).

The OMV has proven to be lethal for chum salmon (*O. keta*) by artificial immersion infection. Following immersion, 80 to 150-day-old fry began to die at 11 to 12 days later, and 35 to 60% of them succumbed in the ensuing 50 days. However, no death occurred among 240-day-old chum salmon that were similarly infected.

Marked histopathologic changes were observed in liver sections. These were multiple foci of severe necrosis and syncytia formation.

Further repeated experiments using other species of salmonids revealed that coho salmon (*O. kisutch*), kokanee salmon (*O. nerka*), and rainbow trout (*Salmo gairdneri*) were also susceptible to OMV, although some variations in susceptibility were noted.

From the evidence thus far obtained, OMV is clearly a new pathogen of salmonids.

In Japan during recent years, losses of cultured fish due to infectious diseases have been increasing proportionally with the rapid growth of the aquaculture. Viral diseases of fish, in particular, have received much attention because of their highly damaging and prevalent nature, as well as, an absence of any effective antiviral therapy. A number of new viral diseases have been described in addition to IPN and IHN of salmonids in Europe, Japan and North America, VHS and SVC in Europe, and CCV of catfish in the United States (PILCHER and FRYER, 1980a, b).

To establish disease control measures a survey for fish pathogenic viruses in cultured salmon was conducted in Hokkaido. During the course of this study, in September 1978, we isolated a new herpesvirus from ovarian fluids of landlocked masu salmon (*Oncorhynchus masou*) at the Otobe Salmon Hatchery. The new virus was apparently distinct from known fish herpesviruses: channel catfish virus (CCV) (WOLF and DARLINGTON, 1971), *Herpesvirus salmonis* (WOLF *et al.*, 1978) or nerka virus in Towada Lake, Akita and Aomori Prefecture (NeVTA) (SANO, 1976).

In this report, we describe the characteristics and the pathogenicity of the new virus provisionally named *Oncorhynchus masou* virus (OMV).

Isolation of OMV:

In September 1978, 30 of ovarian fluid samples were randomly collected from 800 brood fish of normal appearing landlocked masu salmon at the Otobe Salmon Hatchery, Hokkaido. The samples were subjected to virus isolation by standard methods (MCDANIEL, 1979) using RTG–2 cells at 15°C.

Cytopathic effect (CPE) which appeared distinct from that of IPN or IHN, were observed in 4 samples. The Otobe Salmon Hatchery has had a history of bacterial kidney disease since 1977 (KIMURA, 1978); however, the adult population does not have a history of significant prespawning mortality, but the annual survival rate of progeny is very low.

In the following year, virus isolation was again carried out twice in the same hatchery. The virus was found in 4 of 60 and 8 of 60 samples.

The same virus was also found in one of 30

ovarian fluid samples taken from 300 fish of same species held in another commercial farm 150 km distant from the Otobe Hatchery and using a different water system.

All of the isolates were serologically identified as being OMV.

Properties of OMV:

At a near-optimal incubation of 15°C, OMV shows distinctive CPE within 5 to 7 days, i. e., rounded cells, followed by marked syncytium formation, and eventual lysis of RTG-2 and other cell lines derived from salmonid tissues (Fig. 1). The cells derived from non-salmonid species such as FHM, SBK, EPC, and BB are refractory. The maximum titer of culture-grown virus is about 10^8 $TCID_{50}$/m*l*, with some variation depending on the cell line used.

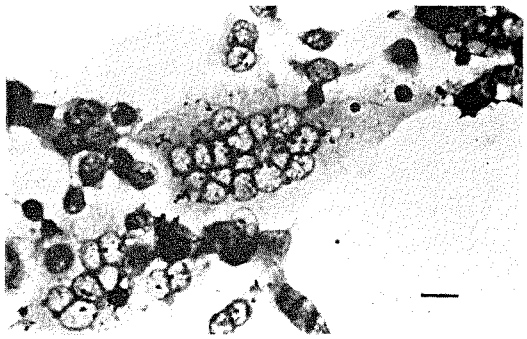

Fig. 1. Cytopathic effect produced by OMV in RTG-2 cells, incubated at 15°C. May-Grünwald Giemsa stain (—— 10 μ).

The OMV is heat-, ether-, and acid (pH 3)-labile, and it is completely inactivated by U.V. irradiation with 3.0×10^3 μw·sec/cm^2. In the presence of 50 μg/m*l* IUdR, replication is inhibited, thus presumptively indicating a DNA virus. Replication of OMV is also apparently inhibited by two newly described anti-herpesvirus agents (KIMURA *et al.*, 1980b) such as phosphonoacetate (SHIPKOWITZ *et al.*, 1973) and acycloguanosine (SHIOTA *et al.*, 1979).

Electron microscopy of infected cells reveals intranuclear hexagonal capsids having a diameter of 115 nm. As aboundance of budding enveloped virions, 200×240 nm in diameter, were also observed on the cell surface and inside cytoplasmic vesicles (Figs. 2 and 3). The calculated number of capsomeres of negatively stained virions is 162 (Fig. 4). The OMV readily passes through a serum-pretreated membrane filters having a mean

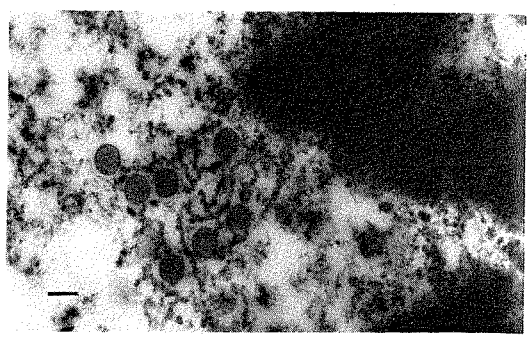

Fig. 2. Electron micrograph of intranuclear hexagonal capsids of OMV in RTG-2 cell (——100 nm).

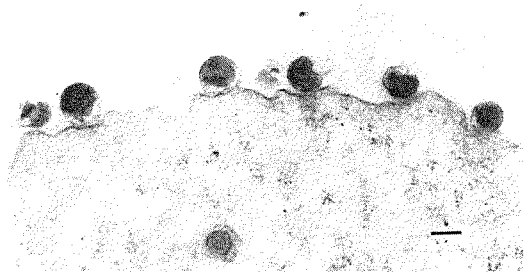

Fig. 3. Electron micrograph of enveloped virions of OMV in the process of budding from the cell membrane of RTG-2 cell (—— 200 nm).

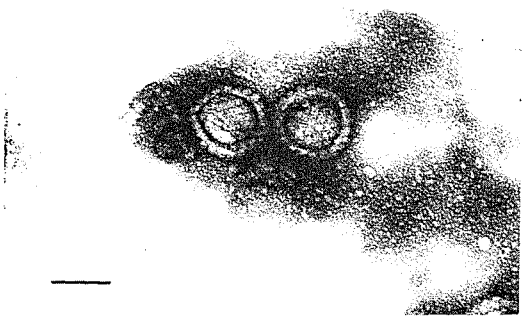

Fig. 4. Electron micrograph of negatively stained (phosphotungstic acid) virions of OMV (—— 100 nm).

pore size of 450 nm, but it is retained on membranes of 220 and 100 nm pore size. These features of OMV conform to the characteristics of the *Herpesvirus* group. Optimal growth temperature of OMV is considered to be 15°C. Viral growth was not observed at 25°C or higher, although replication was recognized at 18°C.

When stored at −20°C for 17 days, infectivity is reduced by 99.9%, and it completely destroyed within 17 days at temperatures above 15°C, but it was preserved at −80°C for 6 months.

This psychrophilic nature of OMV is a major difference from herpesviruses found in warm-blooded animals and amphibians (HOSAKA *et al.*, 1972).

The general properties of OMV are similar to those of known fish herpesviruses, however, OMV differed in the virion size and in the optimal growth temperature. Furthermore, OMV was found serologically distinct from *Herpesvirus salmonis* which was also isolated from a salmonid.

These findings strongly suggest that OMV is a new virus belonging to the *Herpesvirus* group (KIMURA *et al.*, 1979a).

Pathogenicity of OMV:

One hundred, 80-day-old and fifty, 150-day-old fry of chum salmon (*Oncorhynchus keta*) were immersed for one hour in 10°C water containing 100 $TCID_{50}$/m*l* of culture grown OMV and then held in running water at 10–15°C. As shown in Fig. 5, mortality of younger fry began at 11–12 days post-exposure, and 60% of them succumbed in the ensuing 65 days. On the other hand, mortality of older fry began at 20 days post-exposure, and 35% of them succumbed in the ensuing 120 days. During the same period, the cumulative mortality of control fish of each age group was less than 5%.

The mortality pattern of fifty, 150-day-old fry exposed to experimentally infected fry was similar to that observed as a result of infection by direct immersion. However, there were no deaths among 240-day-old chum salmon fingerlings which were immersed in a suspension of virus and additionally injected intraperitoneally with 200 $TCID_{50}$ (KIMURA *et al.*, 1979b).

Further repeated experiments using other species of salmonids revealed that coho salmon (*Oncor-*

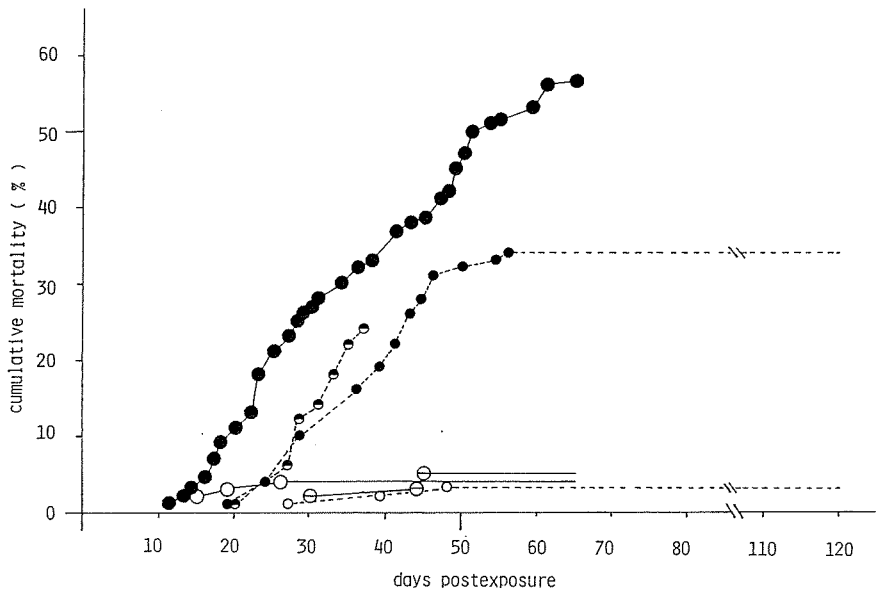

Fig. 5. Comparative response of chum salmon (*Oncorhynchus keta*) fry exposed to OMV. ⊖—⊖: 80-day-old fry control. ○—○: 80-day-old fry exposed to MEM-10. ●—●: 80-day-old fry exposed to OMV; exposing dose, 100 $TCID_{50}$/ml, 60 min. ○---○: 150-day-old fry exposed to MEM-10. ●---●: 150 day-old-fry exposed to OMV; exposing dose, 100 $TCID_{50}$/m*l*, 50 min. ◓---◓: water-born infected 150-day-old fry.

hynchus kisutch), kokanee salmon (*Oncorhynchus nerka*) and rainbow trout (*Salmo gairdneri*) were also susceptible to OMV, although some variations in susceptibility were noted. Furthermore, in chum salmon, it was found that 3-month-old fry were most susceptible while those older than 7-months were resistant (YOSHIMIZU *et al.*, 1980).

Affected fish were anorexic and some showed exophthalmia or petechiation of body surface especially under the jaw. Agonal or abnormal swiming behavior was not observed.

The liver of infected salmon fry was mottled with white spots and in advanced cases, the colour was pearly white. In some cases the spleen was swollen, but no obvious changes were found in kidneys. The digestive tract was devoid of food.

Fig. 6. Multiple foci of severe necrosis in a liver section from moribund chum salmon fry infected with OMV. Methylen blue stain.

As shown in Fig. 6, marked histopathologic changes were observed in liver sections. These were multiple foci of severe necrosis and syncytia formation. Partial necrosis was present in the some spleen sections, and cardiac muscle was edematous. Pancreatic and kidney tissues were essentially normal, however, very young fish, such as 30-day-old, showed evidence of systemic infection with necrosis in each vital organ (YOSHIMIZU *et al.*, 1980).

On the basis of the above observations, the liver is considered to be the primary target organ for OMV infection just as with *Herpesvirus salmonis* (WOLF *et al.*, 1975).

Electron microscopy of infected liver tissue revealed both empty capsids and nucleocapsids in nuclei of infected cells, but enveloped particles were scarce (Fig. 7). Fibrillar strands reported for *Herpesvirus salmonis* (WOLF *et al.*, 1978) could not be found.

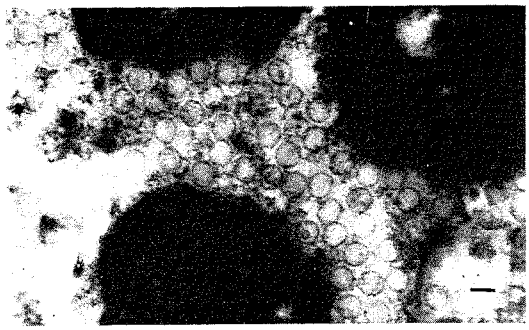

Fig. 7. Electron micrograph showing large numbers of empty capsids and nucleocapsids in cell nuclei of a hepatocyte from a moribund chum salmon fry infected with OMV (——100 nm).

Table 1. Titration of OMV artificially infected chum salmon (*Oncorhynchus keta*) fry

age of fish	fish No.	$TCID_{50}$/g.	$TCID_{50}$/fish
80-day-old	27.	$10^{6.35}$	$10^{6.05}$
	29.	$10^{5.80}$	$10^{5.80}$
	30.	$10^{6.60}$	$10^{6.35}$
150-day-old	6.	$10^{6.05}$	$10^{6.18}$
	7.	$10^{5.05}$	$10^{5.39}$
	110.	$10^{6.05}$	$10^{6.16}$

As shown in Table 1, following the experimental infection, OMV was reisolated from most fish that died, and the virus titer was about 10^6 $TCID_{50}$/g of body weight.

From the evidence thus far obtained, OMV is clearly a new pathogen of salmonids. Furthermore, among the survivors of experimental infection, more than 60% developed epidermal tumors, especially on the head (KIMURA *et al.*, 1980a). It appears, therefore, that OMV is not only virulent, it is also oncogenic. Details of our study of oncogenicity will be given in a later report (KIMURA *et al.*, 1981).

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