Isolation of a retrovirus from two fish cell lines developed from chinook salmon (Oncorhynchus tshawytscha) with plasmacytoid leukaemia

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A plasmacytoid leukaemia (PL) of chinook salmon (Oncorhynchus tshawytscha), characterized as a proliferation and infiltration of plasmablasts into the visceral organs and retrobulbar tissue of the eyes, has been causing extensive mortalities of seawater pen-reared fish at numerous culture facilities in western British Columbia since 1988 (Kent et al., 1990). Recently, a retrovirus, salmon leukaemia virus (SLV), was isolated from kidney and eye tumour tissues of chinook salmon with PL and could be transmitted along with the disease to recipient fish by injection of PL+ tissue homogenates (Eaton & Kent, 1992). Retroviruses have previously been associated with various types of proliferative lesions and tumours in several species of fish over the last 25 years (Winqvist et al., 1968; Walker, 1969; Papas et al., 1976; Duncan, 1978; Yamamoto et al., 1983, 1985; Anders et al., 1991). However, until recently, no retroviruses had been reported from fish cell cultures. Frerichs et al. (1991) were the first to demonstrate a piscine retrovirus in cell lines derived from three different species of warmwater fish from Asia.

Attempts to culture SLV in established salmonid and non-salmonid cell lines have been unsuccessful. However recently, two new cell lines were developed from PL+ chinook salmon kidney and eye tumour tissue that are expressing a retrovirus. We report here the evidence for the first retrovirus isolated from cell cultures derived from Pacific salmon.

The two cell lines were developed from about 10 g of kidney tissue (MA-K) or eye tumour tissue (MA-1H) from pen-reared chinook salmon with PL using standard fish cell culture methods (Wolf, 1979) and Leibovitz L-15 medium supplemented with 10% fetal bovine serum. Both the kidney and eye tumour tissues contained stromal cells as well as extensive numbers of the proliferative plasmablast-like cells characteristic of PL.

At about 8 weeks, the primary monolayers, grown at 15 to 20 °C, developed syncytium formation and vacuolation, indicating a cytolytic infection that is similar to those caused by some other retroviruses, such as bovine leukaemia virus (Burny et al., 1978), feline leukaemia virus and caprine arthritis-encephalitis virus (Dahlberg, 1988). The cell lines have since been passaged every 8 to 10 weeks for 18 months and show the same c.p.e. approximately 3 to 4 weeks after each passage (Fig. 1a).

Monolayer cultures showing syncytium formation and vacuolation were processed for electron microscopy in flasks, as previously described (Eaton et al., 1992). Examination of the stained ultrathin sections showed that the cells exhibiting c.p.e. contained enveloped virus particles, morphologically similar to retroviruses. The virus particles were about 110 nm diameter, had a centrally located core, and were observed budding from cellular membranes or found within membrane-lined vesicles in the cells (Fig. 1b).

A reverse transcriptase (RT) assay was performed on extracellular virus-containing pellets from both cell lines. Supernatants from the two cell lines expressing c.p.e. were centrifuged at 100000 g for 1 h; the pellets were resuspended in 0·01 M-Tris-HCl pH 7·5, 1·0 mM-EDTA and 0·01 M-DTT. Purified SLV and a PL+ kidney tissue homogenate were used as controls. The RT assay was conducted at 22 °C using the methods of Eaton & Kent (1992). Briefly, the various samples were analysed for RT
Table 1. Reverse transcriptase activity in two cell cultures derived from chinook salmon with PL, SLV, and normal chinook salmon kidney tissues

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cation added</th>
<th>Activity using a synthetic template (c.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>poly(rA)</td>
</tr>
<tr>
<td>MA-1H</td>
<td>Mn²⁺</td>
<td>18,599</td>
</tr>
<tr>
<td></td>
<td>Mg²⁺</td>
<td>1372</td>
</tr>
<tr>
<td>MA-KE</td>
<td>Mn²⁺</td>
<td>14,567</td>
</tr>
<tr>
<td></td>
<td>Mg²⁺</td>
<td>31,379</td>
</tr>
<tr>
<td>SLV</td>
<td>Mn²⁺</td>
<td>13,977</td>
</tr>
<tr>
<td></td>
<td>Mg²⁺</td>
<td>3,579</td>
</tr>
<tr>
<td>Normal kidney</td>
<td>Mn²⁺</td>
<td>2,023</td>
</tr>
<tr>
<td></td>
<td>Mg²⁺</td>
<td>1,584</td>
</tr>
</tbody>
</table>

Fig. 1. (a) Monolayer cell culture derived from tissue of fish with PL. A cell culture 3 weeks after a routine passage is shown, depicting extensive vacuolation and syncytium (S) formation. (b) Budding (B) or enveloped (P) virus particles found in the MA-1H and MA-KE cell lines showing c.p.e. Bar represents 100 nm.

Fig. 2. (a) Comparison of the level of RT activity in extracts from the two cell cultures MA-1H (●) and MA-KE (■), and the buoyant densities in sucrose of the samples. ▲, Normal kidney tissue. (b) Comparison of the level of RNA labelling with [³H]UTP in the MA-1H (●) and MA-KE (■) cell cultures, and the buoyant densities of the samples. ▲. CHSE-214 uninfected salmonid cell line.

activity by combining a 50 µl sample with a 50 µl reaction mixture [100 mM-Tris–HCl pH 8.3, 50 mM-KCl, 1 mM-MnCl₂ or 20 mM-MgCl₂, 4 mM-DTT, 0.2% NP40, 20 µg/ml poly(rA)-oligo(dT) or poly(dA)-oligo(dT), and 20 µg/ml [³H]TTP] and determining the TCA-precipitable c.p.m. at time 0 (T₀) and at time 60 min (T₆₀). Samples from both cell lines that contained extracellular virus demonstrated RT activity (Table 1) which was Mn²⁺-dependent and directed by the synthetic primer poly(rA)-oligo(dT). All piscine retrovirus-associated RT activity reported to date has also been Mn²⁺-dependent (Frerichs et al., 1991; Martineau et al., 1991; Papas et al., 1976).

Fractionation of the cell culture samples through a 10 to 60% sucrose gradient showed that the peak RT activity occurred at a density of about 1.16 to 1.18 g/ml (Fig. 2a). A sample from the RT⁺ fractions was examined by negative stain electron microscopy and was found to contain virus particles of retroviral morphology similar to those seen in the cell culture thin sections.

A [³H]UTP uptake assay was conducted to determine the levels of RNA labelling in the two cell lines. Monolayer cultures of the two cell lines (15 ml cultures)
Fig. 3. PAGE analysis of virus purified from the MA-1H and MA-KE cell lines using standard procedures and 12% gels. The M_r values of the viral polypeptides are shown, determined by comparison with M_r markers run in the same gel. Lane 1, MA-1H virus isolate; lane 2, MA-KE virus isolate; lane 3, negative control from uninfected CHSE-214 cell supernatant.

were incubated for 24 h with 20 µCi/ml [3H]UTP. The cell-free supernatant was centrifuged for 1 h at 100000 g, the pellet was resuspended in 0.01 M-Tris–HCl pH 7.5 and 1·0 mM-EDTA, then centrifuged for 18 h at 100000 g through a 10 to 60% sucrose gradient. The gradient was fractionated into 0·3 ml samples and the buoyant density and TCA-precipitable c.p.m. of each sample were determined. Supernatant from uninfected chinook salmon CHSE-214 cells was used as a negative control. Further evidence for a retrovirus was indicated by the elevated RNA labelling with [3H]UTP that occurred in the MA-1H and the MA-KE cell lines, which was associated with a density of about 1·16 g/ml in sucrose (Fig. 2b).

Virus was purified from both the MA-1H and the MA-KE cell cultures by collecting the supernatant from monolayers showing c.p.e. and clarified by centrifugation for 15 min at 10000 g. The supernatant was then centrifuged at 100000 g for 60 min, the pellet resuspended in TNE buffer (0·01 M-Tris–HCl pH 7·5, 0·001 M-EDTA, 0·1 M-NaCl) and centrifuged through a 10 to 60% sucrose gradient, which was then fractionated into 0·3 ml samples. Each fraction was analysed for RT activity and buoyant density. The RT⁺ fractions found at the densities of 1·16 to 1·18 g/ml were pooled, mixed with standard SDS–PAGE sample buffer, and analysed by PAGE using the procedures of Laemmli (1970) and 12% gels. Major polypeptides with approximate M_r values of 120K, 82K, 65K, 43K, 36K and a probable core protein of 24K or 27K were associated with the purified virus particles (Fig. 3). This electrophoretic pattern is similar to that found associated with SLV (Eaton & Kent, 1992). Supernatant from the uninfected cell line, CHSE-214, was similarly processed and the polypeptides were analysed by PAGE as a negative control. A faint protein band appeared at about 31K when the supernatant from uninfected cells was examined by PAGE (Fig. 3).

The results reported here demonstrate that both cell lines derived from chinook salmon with PL contain poly(rA)-oligo(dT)-directed, Mn⁺⁺-dependent RT activity. In addition, this activity is associated with elevated RNA replication and virus particles of retroviral morphology and density which show a protein banding pattern similar to other retroviruses (Dahlberg, 1988). Thus, this is the first report of the presence of a retrovirus in a cell line derived from Pacific salmon.

The role of this virus in the aetiology of PL in chinook salmon remains to be determined. Attempts to transmit SLV into salmonid and non-salmonid cell lines have been unsuccessful. In addition, attempts to transmit the MA-1H- and MA-KE-associated viruses into salmonid and non-salmonid cell lines or into salmon have so far been unsuccessful; however, we are continuing such experiments. Work is also being conducted to compare the cell culture isolates with SLV. If the cell culture isolates and SLV represent the same virus, it would provide an in vitro system to complement the chinook salmon/SLV in vivo system, currently used in our laboratories, to study PL and perhaps other B cell type neoplasias of lower vertebrates.

The taxonomic status of the various piscine retroviruses is unclear. Viruses of ‘C-type retrovirus-like morphology’ have been observed in several different types of fish, and recently in cell lines derived from different species of warmwater fish. In addition, retrovirus pol gene-like sequences have been found in rainbow trout (O. mykiss), brook trout (Salvelinus fontinalis) and Atlantic salmon (Salmo salar) (Stuart et al., 1992). However, the relatedness of these virus-like sequences and the various fish retroviruses to other established retrovirus groups, or to one another, has not been determined.

There is some evidence to suggest that the piscine retroviruses belong in their own group: the dermal sarcoma retrovirus from walleye pike (WDSV) was found recently to have molecular characteristics of the spumavirus, oncovirus and lentivirus groups (Martineau et al., 1992). Molecular examination of SLV, the cell culture isolates described in this paper, and other fish retroviruses is required to determine whether they are
similar to WDSV, to confirm that these fish viruses represent a new group of retroviruses.

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References


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