Therapeutic effects of bovine enterovirus infection on rabbits with experimentally induced adult T cell leukaemia

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A bovine enterovirus, MZ-468, showed cytopathic effects on cell line F-647a, which was established by coculture of human T cell lymphotropic virus type 1-transformed MT-2 cells and X-irradiated rabbit lymphocytes. Microcalorimetric assay showed that residual, viable, MZ-468-infected F-647a cells produced less heat than non-infected cells. The therapeutic effects of MZ-468 infection were examined in rabbits in which adult T cell-like leukaemia (ATL) had been induced by inoculation of F-647a cells (1 × 10⁸ cells). Six newborn rabbits were separated into three groups: group A was inoculated with F-647a cells only; group B was treated with MZ-468 at the time of inoculation with cells; group C was treated with the same amount of virus 24 h after the inoculation with cells and then once every 4 days. Both of the animals in group A and one in group C died 10 and 11 days, and 22 days, respectively, after the inoculation with cells. Both rabbits in group B and one in group C survived for more than 4 months. The rabbits that died were examined pathologically; leukaemic infiltrations were found in the lungs of the group B rabbits, and in the lungs, spleens and livers of both group A rabbits. Two identical experiments produced almost the same findings. These results suggest that bovine enterovirus might be used clinically to prolong the life-span of ATL patients.

Anticancer therapy in humans is one of the most difficult medical problems; a great number of chemical and biological agents have been used for this purpose, none of which is completely effective. The problems involved include the non-specificity of the action of the anticancer agents, which is accompanied by severe side-effects. The use of viruses for therapy of malignant tumours is one of the strategies which employ biologically active agents (Casto & Hammon, 1970; Hunter-Craig et al., 1970; Taylor et al., 1971). Our laboratory has tried to identify those viruses which show strong oncolytic effects and minimal side-effects (Shingu, 1976, 1981; Kaneko et al., 1979), and we have found that bovine enteroviruses lytically infect mouse fibroblast cells only when the cells are transformed with simian virus 40 (Shingu, 1976). We have also found that some bovine enteroviruses have an oncolytic effect on solid tumours and prolong the life-span of mice bearing human carcinoma cells (Fujimaru, 1978).

Seto et al. (1988) recently have succeeded in inducing a disease closely resembling an acute type of adult T cell-like leukaemia (ATL) in rabbits by inoculating syngeneic human T cell lymphotropic virus type 1 (HTLV-1)-transformed cells into newborn rabbits. The HTLV-1-transformed T cell line, F-647a, was established from a 2-month-old female (B/J × Chbb:HM) F1 rabbit, which had been infected with the virus neonatally. In brief, peripheral blood lymphocytes were cultured in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS), in the absence of T cell growth factor. The established cell line had the karyotype of a normal female rabbit, and was positive for the HTLV-1 p19 antigen, the pan T cell marker antigen and the Ia antigen, which were detectable using monoclonal antibodies. The F-647a cell line was able to kill newborn rabbits within 7 days when a dose of 1 × 10⁸ cells was inoculated intraperitoneally. In this study we demonstrated that bovine enterovirus has effects against a rabbit model of ATL, a disease caused by HTLV-1 in which patients usually die within about 4 months.

A bovine enterovirus of type 1 subtype 1, MZ-468, was isolated from calf faeces (Tamada, 1977; Urakawa & Shingu, 1987). The virus was propagated in HeLa cells, purified by equilibrium centrifugation in a CsCl gradient and plaque assayed in Wilms tumour cells originally established in this laboratory (Shingu & Chibana, 1981). F-647a cells (Seto et al., 1988) were kindly provided by Professor A Seto, Siga University School of Medicine, Japan, and passaged in RPMI 1640 medium (Nissui) containing 10% FCS (Gibco). MZ-468 (5.0 p.f.u./cell) was inoculated and adsorbed onto suspended F-647a cells (4 × 10⁴) for 1 h at 37 °C in plastic dishes (Falcon), and incubated at 37 °C under 5% CO₂. Virus propagation was examined every 6 to 12 h by
plaque assay in monolayers of Wilms tumour cells. The average virus titre was determined from four or five cultures and MZ-468 replication in F-647a cells was confirmed using a one-step growth curve (Fig. 1).

To determine the oncolytic activity of MZ-468 against F-647a cells in tissue culture, two different techniques were chosen to measure cell activity: measurement of heat production, and the number of cells present after 1, 2 and 3 days of cultivation.

A flow-type microcalorimeter (thermoactive cell analyser ESCO-3000, Denshi Kagaku) was used to measure heat production. Washing medium (1.8 ml), guide medium (1.8 ml) and sample medium (0.6 ml) containing $2 \times 10^6$ suspended cells were added, in that order, to the microcalorimeter. Cell suspension (0.6 ml) was transferred into the microcalorimetric tube which was then introduced into the reaction vessel and heat production was recorded over a period of 15 min. The same medium used for cell culture was used for the measurement of heat production and oxygen consumption. The heat produced by MZ-468-infected (m.o.i. 13) or uninfected F-647a cells was measured after 24, 48 and 72 h of culture at 37 °C. Heat production, i.e. thermal power, was expressed as $\mu$W/$10^6$ cells, and the result presented as a percentage of the rate of heat production.

Cells were counted in a Bürker chamber. Cell viability was checked by the trypan blue exclusion test during culture and before microcalorimetry.

Infection of F-647a cells in RPMI 1640 by MZ-468 reduced the viability of and production of heat by the residual viable cells, as shown in Table 1. A good correlation existed between the number of cells and the results of the microcalorimetry experiments, indicating that bovine enterovirus can kill F-647a cells.

Table 1. Effect of virus infection on viability of and heat production by viable F-647a cells

<table>
<thead>
<tr>
<th>Time post-infection (h)</th>
<th>Native F-647a</th>
<th>Virus-infected F-647a</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Viable cells ($\times 10^6$)</td>
<td>$\mu$W (%)</td>
</tr>
<tr>
<td>0</td>
<td>4.3</td>
<td>100</td>
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<tr>
<td>24</td>
<td>7.2</td>
<td>100</td>
</tr>
<tr>
<td>48</td>
<td>7.1</td>
<td>100</td>
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* Figures in parenthesis represent the number of viable cells expressed as a percentage of the number of viable, uninfected cells.

Results of this experiment are shown schematically in Fig. 2. The group A animals (receiving no treatment with MZ-468) died 10 and 11 days after inoculation with cells. Pathologically, leukaemic infiltrations were found in the lungs, spleens and livers. In contrast, both group B...
animals (which were simultaneously treated with MZ-468 and the ATL-derived cells) survived for more than 4 months. One of the animals from group C (which was treated 1 day after inoculation with cells) died showing leukaemic infiltration, but the other rabbit survived for longer than 4 months. Two other similar experiments produced almost the same results and pathological findings (data shown in Fig. 3). In this study, bovine enterovirus is effective in the treatment of ATL in experimentally treated animals.

The host range of a virus is generally restricted as to species and this restriction is dependent on the existence of cellular receptors. Bovine enteroviruses do not infect primary cultured human cells, but do infect malignant human cell lines (Shingu, 1976). A possible mechanism for this change in sensitivity may be that the cell membrane changes molecularly during transformation, a phenomenon derived from derepression and amplification of proto-oncogenes, as found for c-erbB-2 (Semba et al., 1985). Thus, expression of cell proto-oncogenes, especially those of the receptor type may produce targets for the adsorption of virus. Another mechanism for a viral oncolytic effect involves unmasking or xenozenicization (Sissons & Oldstone, 1985) of membrane molecules, which might also act as virus receptors. These properties will enable us to utilize the bovine enterovirus as an anticancer biological agent which does not damage normal organs, because only malignant cells will be infected lytically. In agreement with this, many types of enterovirus have been examined experimentally for anticancer activity in vitro and in vivo, and a number have shown remarkable oncolytic effects against solid tumour cells (Imamura & Shingu, 1985). The life-spans of mice transplanted with various human malignant cell lines have been extended by injections of enteroviruses (Ishida, 1984).

We have used a variety of techniques to identify the bovine enterovirus receptor on tumour cells. Receptors for the bovine enterovirus are present on the surface of human adenocarcinoma cell lines, some haematopoietic cell lines of the B cell lineage and activated T cell lines. Anti-HLA-DR monoclonal serum was found to block binding of the oncolytic bovine enterovirus to Raji (B cell lineage, Burkitt's lymphoma origin) or MT-2 cells (T cell lineage, ATL origin), under conditions in which normal mouse serum did not block virus binding. The receptor for the oncolytic bovine enterovirus on the tumour cell is closely associated with the HLA-DR antigen (Shingu, 1989). In this study rabbit models of ATL were used as targets for MZ-468, and a distinct therapeutic effect was observed. This suggests that MZ-468 might be used clinically for treatment of ATL. However, practical administration will necessitate a solution to the potential problems of an anaphylactic reaction and antibody production against the virus after repeated injection. Another problem to be overcome is the identification of a means of virus administration by which it efficiently reaches its targets. Although these problems remain, virus injected intraperitoneally can be effective in the treatment of end-stage ATL patients, in which it reduces the number of ascites cells disseminating from infiltrated organs, such as the liver, lung and spleen. It is possible that this route of administration will prolong the life-span of the ATL patient.

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References


Fig. 3. Twelve newborn rabbits were inoculated with HTLV-1-transformed F-647a cells and divided into groups D, F and H (inoculated with 1 x 10^6 cells), and E, G and I (inoculated with 6 x 10^5 cells). Groups D and E were control, untreated groups; two rabbits, one from each group, died 10 and 11 days after inoculation. Groups F and G were treated with F-647a cells that had adsorbed. Groups H and I were treated with F-647a cells onto which MZ-468 had adsorbed. Groups F, G, H and I were inoculated with MZ-468 or F-647a cells adsorbed with virus 1 day after inoculation with F-647a cells and once daily for 4 days. These rabbits were cured without showing any side-effects over a 90 day period. Solid arrows, inoculation with F-647a cells; open arrows, inoculation with 2.2 x 10 ^6 p.f.u. MZ-468 or 4.4 x 10^5 F-647a cells onto which 2.2 x 10^6 p.f.u. MZ-468 had been adsorbed. The length of the bars indicates the life-span of each animal.


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