Increased Expression of Endogenous Xenotropic Murine Retrovirus by Treatment with the Tetrapeptides, Tuftsin and Kentsin

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SUMMARY

Enhancement of endogenous xenotropic virus expression has been found upon treatment with tetrapeptides of a high-passage clone of Balb/c (K-Balb) mouse cells transformed with Kirsten sarcoma virus. Tuftsin (Thr-Lys-Pro-Arg) and kentsin (Thr-Pro-Arg-Lys) increased the expression of virus that was infectious for rat, but not mouse, cells in a concentration-dependent fashion. The enhancement of virus expression by the tetrapeptides was proportional to the spontaneous release of virus. The infectivity of the enhanced virus was neutralized by goat anti-RLV gp70 serum. Actinomycin D inhibited the induction of virus, suggesting that enhanced expression required de novo RNA synthesis. The effects observed using K-Balb cells offer an opportunity to study the many biological effects of these peptides in a fibroblast culture system.

The induction of latent virus genomes has been shown with a variety of chemical and physical agents from a myriad of cell types. With type C viruses, the virus genomes are transmitted from one generation to another as part of the host chromosome and expression can occur spontaneously with age or following chemical treatment. Chemicals that block protein synthesis (Aaronson & Dunn, 1974a; Long et al., 1978) and halogenated pyrimidines (Aaronson et al., 1971; Lowy et al., 1971) have been shown to be very efficient inducers of xenotropic virus. Spontaneous production of type C RNA viruses has been reported in mouse and rat cells transferred frequently at high densities for extended periods of time (Aaronson et al., 1969; Aaronson & Todaro, 1968; Klement et al., 1973). It has been known for some time that in rapidly dividing, DNA-synthesizing cells, the replication of mouse leukaemia virus is increased (Nakata & Bader, 1968). In addition, superinduction of endogenous type C viruses has been demonstrated in transformed, tumorigenic subclones derived from the Balb/3T3 mouse (Lieber et al., 1973). Superinducibility appears to be associated with the loss of growth control and the transformed phenotype, presumably because these cells are less able to control normally repressed virus genetic information.

In the present report, we describe the increased expression of an endogenous xenotropic type C virus following exposure of cells to the tetrapeptides, tuftsin (Thr-Lys-Pro-Arg) and a structurally related molecule, kentsin (Thr-Pro-Arg-Lys) (Kent, 1975). Tuftsin is the active component of leukokinin, a cytophilic molecule that carries γ-globulin (Nishioka et al., 1972). Treatment of phagocytic cells, granulocytes, monocytes and macrophages with tuftsin results in phagocytosis and pinocytosis (Najjar & Nishioka, 1970; Nishioka et al., 1973a, b; Najjar, 1974), chemotactic migration (Nishioka et al., 1973b; Najjar, 1974), bacteriocidal activity (Martinez et al., 1977), immunogenic function (Tzehovel et al., 1978; Florentin et al., 1978), and tumoricidal and tumoristatic activity (Florentin et al., 1978; Nishioka, 1979). At present, it is believed that tuftsin-specific receptors, which confer susceptibility, are present only on phagocytic cells (Stabinsky et al., 1978). This report shows that increased expression of type C virus occurs when highly transformed fibroblasts are exposed to these two tetrapeptides.
K-Balb/c 19a cells were cloned from a Kirsten sarcoma virus-transformed Balb/c/3T3 cell line (K-Balb), originally obtained from Dr S. Aaronson (National Cancer Institute, Bethesda, Md., U.S.A.) This clone was chosen for virus induction studies because of the high levels of type C virus expression following treatment with inducers (Aksamit & Long, 1978). This clone (K-Balb 19a) was used in the induction studies after having undergone long-term propagation at high cell densities (> 50 subcultures), and will be referred to here as K-Balb 19a/h. Fischer rat embryo cells (FRE) were obtained from Dr E. Scolnick (National Cancer Institute, Bethesda). The cells were grown in Eagle's minimum essential medium (EMEM) containing 100 international units/ml penicillin, 100 μg/ml streptomycin and 10% foetal calf serum (Flow Laboratories). All cells were free of mycoplasma contamination in tests performed by R. Del Giudice (Frederick Cancer Research Center, Frederick, Md., U.S.A.).

K-Balb 19a/h cells were plated in complete medium and grown at 37 °C for 24 h before changing to induction medium. The induction medium consisted of EMEM, antibiotics, 10% foetal calf serum, dexamethasone (0.1 μg/ml) to enhance virus production, and the chemical inducer. After incubation in induction medium for a given time, the drug was removed and the cells washed three times with phosphate-buffered saline (PBS), incubated with mitomycin...
Table 1. Neutralization of virus infectivity with anti-RLV gp70*

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<thead>
<tr>
<th>Treatment</th>
<th>Percentage inhibition at dilutions of</th>
<th>Viability (%)</th>
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<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>IdUrd (30 μg/ml)</td>
<td>(524)†</td>
<td></td>
</tr>
<tr>
<td>Tuftsin (100 μg/ml)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Kentsin (100 μg/ml)</td>
<td>(17)</td>
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* Neutralization was determined with varying dilutions of anti-RLV gp70 serum after the induction period, at the time of cell overlay in the focus formation assay. K-Balb 19a/h cells were plated at 3 × 10^5 cells/3.5 cm dish. The focus formation assay was then performed as described. Antiserums were added to the overlaid monolayers for 4 h, which were then washed and complete EMEM was added. Non-specific goat anti-IgG was used as a control in some experiments.
† Numbers in parentheses indicate the mean number of foci per 3.5 cm dish.
‡ NT, Not tested.

C (20 μg/ml) and plated on to FRE monolayers for focus formation (Aaronson & Dunn, 1974a, b). Chemicals were obtained from Calbiochem, San Diego, Calif., U.S.A.

Enhancement of virus expression by tuftsin and kentsin was examined and a dose-response relationship determined (Fig. 1). The maximum enhancement of virus occurred following an 18 h incubation with either tetrapeptide at 100 μg/ml (2 log_{10}); tuftsin was consistently a better inducer at this concentration. When cells were treated with 1000 μg/ml of the tetrapeptides, a lower number of foci was observed. This was not due to cytotoxicity because, at 1000 μg/ml, the relative plating efficiency (RPE) remained between 60 and 70% depending on the tetrapeptide used, and was only 2% below the 100 μg/ml RPE level. As little as 1 h incubation with tetrapeptide was necessary to show enhanced virus expression. Tuftsin-mediated virus enhancement remained comparable with that of cycloheximide for up to 3 h incubation, inducing 64 foci compared with 74 foci for cycloheximide, but was approx. 25% lower after 4 h. The number of foci increased with an increase in the exposure time of K-Balb 19a/h cells to the tetrapeptides.

Most agents that induce virus alter the synthesis of macromolecules. However, tuftsin or kentsin produced only minor effects on the rates of DNA, RNA or protein synthesis in K-Balb cells, suggesting that the basis of virus induction by these compounds is different from that of previously identified inducers, such as cycloheximide and 5-iododeoxyuridine (IdUrd).

To show that a type C virus was associated with the appearance of foci on FRE cells, neutralization experiments were carried out with goat anti-RLV gp70 serum (Versteegen & Oroszlan, 1980). As indicated in Table 1, neutralization abolished transformation of FRE cells. The data show that anti-RLV gp70 serum completely neutralized the focus-forming ability of tetrapeptide-treated K-Balb cells with a 50% endpoint titre of between 1:160 and 1:320. The focus-forming ability of IdUrd-mediated virus induction was neutralized with a 50% endpoint titre of 1:40. Neutralization was not a consequence of toxicity, as determined by the viability studies and appearance of the cells during the experimental procedure.

Induction by both IdUrd and cycloheximide requires de novo cellular RNA synthesis and their activity as inducers can be blocked by treatment with actinomycin D (Aaronson et al., 1974). During activation by both classes of chemicals, virus-specific RNA accumulates in both nucleus and cytoplasm, suggesting that transcriptional derepression may occur (Besmer et al., 1975; Cabradilla et al., 1976). Recently, sensitivity of virus induction to α-amanitin in intact cells was shown at concentrations known to specifically inhibit transcription by RNA polymerase II, thereby providing direct support for transcriptional derepression during induction (Long et al., 1980). It was of interest therefore, to test the effect of inhibiting RNA
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synthesis on tetrapeptide-mediated enhancement of virus. K-Balb 19a/h cells were treated with the tetrapeptides for 18 h in the presence or absence of 1 mM-actinomycin D and then assayed for focus formation; IdUrd and cycloheximide were used as controls. Tetrapeptide-mediated virus induction was inhibited 95% by treatment with actinomycin D, suggesting a requirement for de novo cellular RNA synthesis in tetrapeptide-mediated release of retroviruses from K-Balb cells.

Two pseudotypes of endogenous type C viruses have been identified in K-Balb cells (Aaronson & Stephenson, 1973) and there is evidence for partial expression of a third virus (Stephenson et al., 1975). Experiments were carried out to determine the host-range of virus produced in response to tetrapeptide treatment. Tuftsin-treated K-Balb cells produced transformed foci on FRE only, and not on Balb/c mouse, NIH Swiss mouse, mink, human or cat cells. Transformed foci arising from FRE cells were cloned and serially cultured, and focus formation assays were performed using filtered supernates on to the various indicator lines. Foci appeared on the FRE, mink, human and cat cells, but not on the Balb/c or NIH Swiss mouse cells. Thus both the virus released from K-Balb cells following tetrapeptide treatment and the K-Balb virus introduced into the FRE cells had a xenotropic host-range. The kentsin-induced virus behaved in a similar fashion.

During the course of these experiments, we noted that virus release from K-Balb 19a cells was not always susceptible to tuftsin and kentsin treatment, especially when cells had been propagated for less than 50 subculture generations. On several occasions, we also noted a very low level of spontaneous virion release from the K-Balb 19a/h cells. The relationship between enhancement of retrovirus expression by tuftsin and kentsin and spontaneous release over an extended time period is illustrated in Fig. 1 (inset). The level of tetrapeptide-mediated expression appeared to correlate closely with the level of spontaneous release of virus. The work described here was performed on a high-passage clone of K-Balb 19a/h. Other clones of K-Balb cells from which virus can be activated at high frequency following treatment with IdUrd or cycloheximide, responded less well to treatment with these tetrapeptides, as did low-passaged K-Balb 19a cells. This suggests that the increased expression of virus by K-Balb 19a/h cells after treatment with tetrapeptides may be the property of an unusual variant selected in vitro, and not necessarily a general property of Balb/c mouse cells. To determine if the increased virus expression produced by the two tetrapeptides was additive, combinations of tuftsin and kentsin were used to enhance virus. Enhancement was non-additive and the combinations were inhibitory. Tuftsin did not act synergistically with IdUrd to enhance virus production, indicating a difference in mechanism to that of dexamethasone (Dunn et al., 1975).

Tuftsin and kentsin appear able to trigger expression of endogenous type C virus from a highly transformed clone of K-Balb cells selected by continuous passage in culture at high cell densities. The increased expression of virus appears to be specific for the peptide sequence since Gly-Leu-Gly-Leu, Leu-Tyr-Leu, Thr-Lys-Phe, Lys-Lys-Lys-Lys, Try-Gly-Gly-Phe-Met did not increase virus expression (data not shown). The mechanism by which these two tetrapeptides alter virus expression is unclear, but could take place through peptide interaction with cell membrane receptors similar to that reported for polypeptide growth factors (Pickart & Thaler, 1973; Pickart et al., 1973; Todaro & DeLarco, 1978), or an interaction of specific amino acid sequences with nucleic acid, since it has been shown that peptides such as Gly-His-Gly and Lys-Trp-Lys bind to single-stranded DNA regions (Fritzsche, 1972; Hélene & Dimicoli, 1972; Toulmé & Hélene, 1977). A preliminary report noted that tuftsin increased the level of both virion-associated reverse transcriptase and of budding virus from MuLV-shedding mouse cells (Luftig et al., 1977). Since the p12 Rauscher leukaemia virion protein has the same sequence at the amino-terminal end as tuftsin (Luftig et al., 1977; Oroszlan et al., 1978), it may have a regulatory function in virus expression for the
p12 protein. The present system provides a means to compare the mechanism of tuftsin action on phagocytic and fibroblastic cells, as well as a way of examining how small mol. wt. peptides control cell growth and modify gene expression.

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**REFERENCES**


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