Detection of herpes simplex virus type 1-encoded RNA by polymerase chain reaction: different pattern of viral RNA detection in latently infected murine trigeminal ganglia following in vitro or in vivo reactivation

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Herpes simplex virus type 1 (HSV-1) establishes latent infection in the sensory ganglia. To investigate the process of reactivation from latency, we used the RNA polymerase chain reaction (RNA-PCR) to detect the expression of several HSV genes. BALB/c mice were inoculated in the anterior ocular chamber with HSV-1 strain KOS and the trigeminal ganglia were examined at least 8 weeks after inoculation. Latency-associated transcripts (LATs) were found in the latently infected ganglia and remained detectable 120 h after explantation. Besides LATs, we detected transcripts for infected cell protein 0 (ICP0) (Vmw110) 24 h after explantation, but RNAs encoding ICP4 (Vmw175), ICP27, thymidine kinase and VP16 (ICP25; Vmw65) remained undetectable for 120 h after explantation. Following in vivo reactivation of HSV-1 by administration of cyclophosphamide and dexamethasone, all viral transcripts including ICP0 RNA became detectable. The RNA-PCR enabled us to detect ICP0 RNA much earlier than has been previously reported in studies using the Northern blot technique and has laid a foundation for further study of viral and cellular transcripts during reactivation. Our results suggest that the process of reactivation of HSV-1 from trigeminal ganglia may be divided into at least two steps: (i) initiation of ICP0 gene transcription and (ii) detectable transcription of the other genes. The second step may be regulated in part by the host immune system, since cyclophosphamide and dexamethasone administration enabled the detection of several viral transcripts.

Although several antiviral agents are available for the effective treatment of herpes simplex virus (HSV) infection, it is still impossible to eliminate latent virus from the host or to prevent reactivation (Hino et al., 1988; Nash & Löhr, 1992). The molecular processes of both lytic and latent infections with HSV-1 have been studied extensively (Roizman & Sears, 1987; Stevens, 1989; Wagner, 1990), but the mechanism of reactivation of the virus from latent infection has not been completely clarified.

The latency-associated transcripts (LATs) originally found in latently infected neural tissues (Rock et al., 1987; Spivack & Fraser, 1987; Stevens et al., 1987) are not considered to be essential for the establishment of latency (Ho & Mocarski, 1989; Sedarati et al., 1989; Gordon et al., 1990). There are reports suggesting a role for the LATs in virus reactivation (Hill et al., 1990; Trousdale et al., 1991). The precise functions of the LATs have yet to be clearly defined (Fraser et al., 1992). The infected cell protein 0 (ICP0) gene is also dispensable for in vitro virus growth, but it seems to be required for efficient virus propagation (Sacks & Schaffer, 1987) and the establishment of and reactivation from latency (Leib et al., 1989). In this report, we were able to detect the desired RNA sequences, by the RNA polymerase chain reaction (RNA-PCR), from a few mouse trigeminal ganglia (TG) during latency and reactivation following intracameral inoculation which, like corneal inoculation, has been used for studying latency (Røedahl & Stevens, 1992).

Vero cells were grown in Eagle’s minimum essential medium (Nissui Pharmaceutical) supplemented with 5% calf serum. HSV-1 strain KOS was used for inoculation. For intracameral inoculation of HSV-1, 6- to 8-week-old female BALB/c mice (Laboratory Animal Center, Kyushu University) were anaesthetized and the virus (2 × 10⁵ p.f.u. in 5 μl) was introduced as described previously (Liu et al., 1993). At a minimum of 8 weeks after inoculation, TG from latently infected mice were collected aseptically. Five to 10 TG were stored as one specimen for RNA isolation immediately or after being incubated in MEM with 2% calf serum without Vero cells for in vitro reactivation by explantation. Some TG were cocultivated with Vero cell monolayers and observed for c.p.e. for 28 days.

In vivo reactivation of HSV-1 was performed essentially as described by Shimeld et al. (1990): latently infected mice were injected intraperitoneally with 250 mg
of cyclophosphamide (Endoxan; Shionogi Pharmaceuticals) per kg in PBS (day -1). Twenty-four hours later, they were injected with 20 mg of dexamethasone phosphate (Decadron; Banyu) per kg via the same route (day 0). The animals were sacrificed on day 0, 1 or 2 and their TG were collected.

For RNA isolation, TG were homogenized in 4 M-guanidinium thiocyanate, 0.5% sodium N-lauroylsarcosine, 100 mM-2-mercaptoethanol, 25 mM-sodium citrate (pH 7.0), and total RNA was isolated as described by Chomczynski & Sacchi (1987). The PCR primers described in Table 1 were prepared with a DNA synthesizer (MilliGen/Biosearch). Primer sequences for thymidine kinase (TK) and LAT/ICP0 genes were obtained from Lynas et al. (1989a, b). The other HSV primers were designed on the basis of the available DNA sequence data (Rixon & Clements, 1982; McGeoch et al., 1988; Perry & McGeoch, 1988). The sequences of the primer pair for detecting mouse β-actin RNA were reported by Shull et al. (1992).

RNA-PCR was performed essentially as described by Spivack et al. (1991). Two μg of total RNA and 5 μg of random hexamers pd(N)₆ (Pharmacia LKB Biotechnology) in reverse transcriptase (RT) buffer (Bethesda Research Laboratories) with 200 nmol dithiothreitol and 20 nmol of each deoxynucleoside triphosphate were heated to 80 °C for 3 min and chilled on ice. After addition of 40 units (U) of RNase inhibitor (Toyobo) and 200 U of Moloney murine leukaemia virus RT (Bethesda Research Laboratories), the reaction mixture (total volume 20 μl) was incubated for 1 h at 37 °C, heated to 94 °C, and chilled on ice. To 10 μl of the first-strand reaction, 1 pmol of each PCR primer and 2.5 U of Taq polymerase (Promega) were added and the volume was increased to 100 μl in 0.9 x PCR buffer (Promega) and 0.1 x RT buffer. Amplification of the ICP4 gene was performed for 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 2 min and extension at 72 °C for 2 min. Amplification of the other genes was performed at 94 °C for 1 min, 55 °C for 2 min and 72 °C for 3 min for 30 cycles. PCR products were resolved by electrophoresis in a 3% NuSieve/1% SeaKem agarose (FMC BioProducts) gel and were visualized under u.v. light after ethidium bromide staining. To detect LATs or ICP0 RNA, 100 pmol of one of the LAT/ICP0-specific primers was used instead of pd(N)₆ to obtain reverse transcription in the desired direction only. In preliminary experiments, different amounts of HSV-1 DNA were used as the template to compare the sensitivity of the primers. The LAT/ICP0 PCR product was detectable from 10 pg of template DNA and the sensitivity of the other primer pairs was equal to or greater than that of LAT/ICP0 (H. Minagawa, S. Tanaka, Y. Toh & R. Mori, unpublished results).

### Table 1. Sequences of PCR primer pairs and the sizes of the resultant PCR products

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Product length (target genes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>GACAGCAAAAAAAATCCCCCTGAG</td>
<td>195 bp</td>
</tr>
<tr>
<td>2</td>
<td>ACGAGGAAAAACAAATAAGGG</td>
<td>(LAT/ICP0)</td>
</tr>
<tr>
<td>TK A†</td>
<td>ATACCGAGCAGATATGGACCCT</td>
<td>110 bp</td>
</tr>
<tr>
<td>TK B</td>
<td>TTATGGCCGTCATAGGGCGG</td>
<td>(TK)</td>
</tr>
<tr>
<td>ICP27</td>
<td>AGCCGCGACCCACACACTGT</td>
<td>(ICP27)</td>
</tr>
<tr>
<td>ICP4</td>
<td>TTTCCCACCAGAACATCGAC</td>
<td>446 bp</td>
</tr>
<tr>
<td>bA-1</td>
<td>GACGGGCTCTCTCTACCA</td>
<td>(ICP4)</td>
</tr>
<tr>
<td>bA-2</td>
<td>CTCTTTGATGTACGAGTTTTCCTTC</td>
<td>(β-actin)</td>
</tr>
</tbody>
</table>

* (Lynas et al., 1989b).
† (Lynas et al., 1989a).
‡ (Shull et al., 1992).

### Table 2. Detection of HSV-1 RNAs in mouse TG after in vitro explantation

<table>
<thead>
<tr>
<th>Time* (h)</th>
<th>LAT</th>
<th>ICP0</th>
<th>ICP4</th>
<th>ICP27</th>
<th>TK</th>
<th>VP16</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>48</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>72</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>96</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>120</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Post-explantation.
† ND, Not determined.

### Table 3. Detection of HSV-1 RNAs in mouse TG after in vivo administration of cyclophosphamide and dexamethasone

<table>
<thead>
<tr>
<th>Time* (days)</th>
<th>LAT</th>
<th>ICP0</th>
<th>ICP4</th>
<th>ICP27</th>
<th>TK</th>
<th>VP16</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Uninfected)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
</tr>
</tbody>
</table>

* After dexamethasone injection.
† ND, Not determined.

As shown in Table 2, ICP0 RNA was first detected 24 h after explantation. LATs remained detectable throughout the examination period. RNAs encoding ICP4, ICP27, TK and virion polypeptide 16 (VP16) were undetectable in two separate sets of experiments.

In contrast, as shown in Table 3, not only ICP0 RNA and LATs but also ICP4, ICP27, TK and VP16
transcripts were detected by RNA-PCR in TG within 48 h following in vivo treatment with cyclophosphamide and dexamethasone. There were no apparent signs of local acute infection. HSV-I c.p.e. was detectable within 4 days in culture in three of the five cocultivated TG from cyclophosphamide-injected mice.

The RNA-PCR has been widely used in related fields (Schochetman et al., 1988; Lynas et al., 1989a; Sprecher & Becker, 1992). By using the RNA-PCR instead of Northern blot analysis, we were able to decrease the number of pooled TG needed, and also detect ICP0 RNA 24 h post-explantation, earlier than Spivack & Fraser (1988).

We detected LATs in all the TG samples taken during latency and reactivation. Besides LATs, only ICP0 RNA became detectable after explantation, although infectious virus was detectable by cocultivation of similarly treated TG. Following the report of Spivack & Fraser (1988), we did not cocultivate TG before RNA extraction since there was no means of distinguishing viral RNA derived from TG and that from lytically infected Vero cells. In TG from mice following corneal inoculation, Valyi-Nagy et al. (1991) detected weak signals for ICP4 mRNA by in situ hybridization 24 h post-explantation. The different results may be due to the different inoculation routes.

Following in vivo administration of cyclophosphamide and dexamethasone, the virus transcripts other than LATs or ICP0 were detectable within 48 h. Our results suggest that the process of in vivo reactivation of HSV-1 from TG may be divided into at least two steps: (i) initiation of ICP0 gene transcription and (ii) accumulation of the other virus transcripts. Since infectious virus could be recovered by explantation in which only step (i) occurred, massive accumulation of viral mRNA is unnecessary for the initial virus spread from the latently infected cells to the surrounding susceptible cells. Elshiekh et al. (1991) compared the time course of accumulation of HSV immediate early mRNA and found that the accumulation of ICP0 mRNA did not require infected cell protein synthesis but that of ICP22/47, ICP27 and ICP4 mRNAs required 0-5 to 1 h of protein synthesis. Ecob-Prince et al. (1993) recently studied HSV-1 reactivation by neurectomy and found that ICP0 mRNA was detectable by in situ hybridization 2 to 4 days after neurectomy without detectable viral antigen expression. Our repeated failure to detect RNAs encoding ICP4, ICP27, TK or VP16 during the in vitro explantation is in line with their observations, and could be explained by the requirement of protein synthesis for ICP4 and ICP27 mRNA accumulation (Elshiekh et al., 1991).

Immunosuppressive and antimetabolic treatment with cyclophosphamide and dexamethasone accelerated the transcription of HSV-1-encoded genes other than that of ICP0 by an unknown mechanism. Our results warrant further studies on the immunological aspects of reactivation. Simmons & Tscharke (1992) reported a role of CD8-positive T lymphocytes other than target cell killing in the control of neural infection. Admitting the limitations in using mouse models without spontaneous virus shedding (Stanberry, 1986) for studying the reactivation of HSV, use of mice has the advantage of the availability of well defined inbred strains (Shimeld et al., 1990).

We thank Professor Y. Fukumaki, the Research Laboratory for Genetic Information of Kyushu University, for access to their facilities, Dr Y. Liu for intracameral virus inoculation and Dr Mary Louise Robbins for reviewing the manuscript. This work was performed through Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

References


Short communication


(Received 3 August 1993; Accepted 1 October 1993)