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Interferon Alpha in the Establishment of Latency by Herpes Simplex Virus type 1 Strain tr.

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Abstract

The effect of interferon (IFN) alpha on the establishment and maintenance of neuronal latency and viral reactivation is still not known. Using cell culture methods and sensitive RT-PCR methods, we show that the presence of antiserum to IFN alpha promotes the establishment of HSV-1 tr latent infection. We suggest that IFN alpha is an important tool not only for the control of productive but also latent HSV-1 infection.

Key words: IFN alpha, HSV-1, latent infection

Introduction

All herpesviruses establish latent infections in their natural hosts. Following productive infection of permissive cells at the periphery, herpes simplex virus type 1 (HSV-1) for example, usually colonises neurones of the peripheral nervous system. This virus may, from time to time and by unclear mechanisms, reactivate from latency and cause productive infection at or near the site of initial entry into the host.

During latent HSV-1 infection in sensory neurons, the viral genome is maintained in a non-replicating state and viral gene expression is silenced, with the exception of the viral gene that encodes the latency-associated transcripts (LAT) (Wagner and Bloom, 1997). Reactivation of latent HSV-1 is induced by many different stimuli, including fever, stress and UV irradiation or abrasion to the skin. But the molecular and cellular mechanisms involved in establishing, maintaining, and mediating reactivation from latency are still unclear.

IFN alpha is a secreted protein that plays important roles in host resistance to viral infection. IFN alpha is not an effective inhibitor of HSV-1 replication *in vitro.* However pretreatment with IFN alpha reduces the pathogenesis of HSV-1 infection in mice and human (Harle *et al*., 2001; Noisakran *et al*., 1999).

To determine the effect of IFN alpha on establishment of HSV-1 tr neuronal, latent infection we infected differentiated PC12 cells with HSV-1 tr in presence of mice IFN alpha or antiserum to mice IFN alpha (anti-IFN alpha). The infection was monitored by titration on CV1 cells by a plaque assay. Viral-gene products were tested for the presence in the long-term-infected PC12 cultures, when there was no detectable virus in the culture medium.

Experimental

Materials and Methods

Cells and virus. CV-1 cells, a monkey kidney cell line, obtained from National Bacteriological Laboratory, Department of Virology in Stockholm, were maintained in MEM (Gibco BRL) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Sigma), 100 U/ml penicillin, and 100 μg/ml streptomycin (MEM – 10% FCS).

Rat pheochromocytoma (PC12) cells from American Type Culture Collection (ATCC) were grown in RPMI 1640 (Gibco BRL) supplemented with 10% heat-inactivated horse serum (Sigma) and 5% heat-inactivated fetal calf serum (Sigma) (PC12 medium).

HSV-1 tr, temperature resistant mutant, isolated by Litwińska *et al.*, 1991 from McIntyre strain was prepared in CV-1 cells.

Differentiation of PC12 cells. To differentiate PC12, 2×10^4 cells were seeded on poly-L-ornithine (Sigma)-coated culture tubes with flat bottom. The following day, cells were incubated in PC12 medium containing 100 ng/ml of 2,5S NGF (nerve growth factor)

(Sigma) for one week. Medium was replaced every 3 days. On the 7th day, 20 μM fluorodeoxyuridine (FdUrd) (Sigma) was added to the medium for 2 to 3 days to eliminate undifferentiated PC12 cells. Fresh NGF-supplemented medium was supplied thereafter.

Establishment of long-term HSV-1 infection. Differentiated PC12 cultures were infected with HSV-1 tr at an MOI of 5 (1×10^5 PFU/tube). Following one hour incubation at 37°C, cultures were treated with 1 ml of sodium citrate buffer (pH 3), for 30 s to 1 min to inactivate residual virus. Buffer was removed and tubes were rinsed with PC12 medium once. After low-pH treatment, cultures were incubated at 37ºC with fresh medium containing NGF and suplemented with IFN alpha (1000 IU/tube) or anti-IFN alpha (200 IU/tube). To monitor for the release of HSV-1 progeny the culture medium was collected and titered on CV-1 cells by a plaque assay.

RNA isolation and RT-PCR. Total cellular RNA was isolated from cell culture by using the Trizol reagent (Gibco). RT-PCR amplifications were carried out with SuperScript One-Step RT-PCR with Platinum Taq (Invitrogen) according to the manufacturer's instructions. Primers used in this study are listed in Table I. Amplifications consisted of 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 45 s, and extension at 72°C for 1 min. PCR products were resolved by electrophoresis in 1% agarose gel, visualised by ethidium bromide staining.

Results

The rapidity of establishment of long-term infected with HSV-1 tr PC12 culture. PC12 cells were seeded onto poly-L-ornithine coated tubes and differentiated with 100 ng of NGF/ml as described in Materials and Methods. Differentiated cultures were then infected at an MOI of 5 with HSV-1 tr and were maintained continuously in NGF-supplemented medium with addition of IFN alpha or anti-IFN alpha. To monitor of virus growth, medium was collected and assayed for infectious particles on a CV1 monolayer by a plaque assay. In each case – with IFN alpha, with anti-IFN alpha and without any addition four tubes were examined. The production of progeny virus peaked and dropped between 1 and 2 day after infection in each variant. Following the initial period of virus production there was a complete lack of detectable virus in the culture medium. Cultures surviving infection with HSV-1 tr are at this point, designed as long-term or quiescently infected cultures. At first, long-term infected cultures were established in the variant with anti-IFN alpha, next, in the variant without addition of IFN alpha or anti-IFN alpha, and at last in the variant with IFN alpha (Fig. 1). The significant difference in rapidity of long-term infected culture establishment was analysed by one way analysis of variance (ANOVA). We found out that there is a statistically significant relationship between culture variants ($p = 0{,}0006$). Mean time of long-term infected culture establishment for variant with IFN alpha, without addition and with anti-IFN alpha were respectively 8.5, 7.5 and 6.3. The last one was significantly different from the first one and the second one (t-Student test).

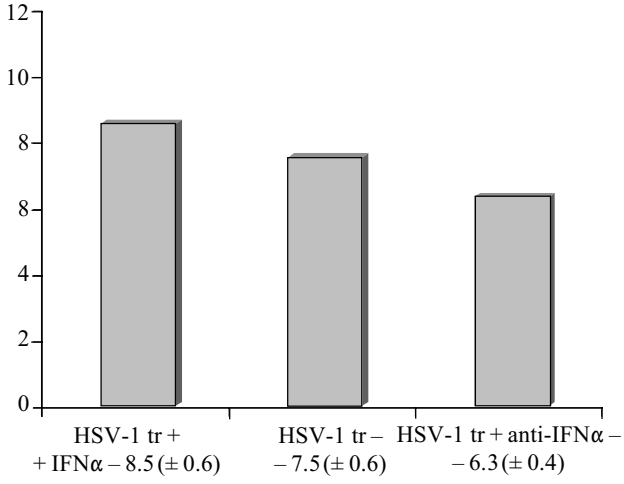

Fig. 1. Influence of IFN alpha and anti-IFN alpha on virus elimination from culture medium.
* – significant difference

HSV gene expression in long-term-infected cells. The hallmark of HSV latency is limited, if any, viral gene expression (Stevens *et al*., 1987). The LAT appear to be the only viral-gene products which accumulate

Table I
Primer sequences used in this study

Primer	Sequences	Product size (bp)	References
Rat GAPDH	Sense: 5' AAC CCT TCA TTG ACC TCA ACT 3' Antisense: 5' CTT CTC CAT GGT GGT GAA GAC 3'	622	Ramakrishnan *et al.*, 1994
HSV ICP27	Sense: 5' TTT CTC CAG TGC TAC CTG AAG G 3' Antisense: 5' TCA ACT CGC AGA CAC GAC TCG 3'	283	Devi-Rao *et al.*, 1994
HSV TK	Sense: 5' ATG GCT TCG TAC CCC TGC CAT 3' Antisense 5' GGT ATC GCG CGG CCG GGT A 3'	531	Tal-Singer *et al.*, 1997
HSV LAT	Sense: 5' GAC AGC AAA AAT CCC CTG AG 3' Antisense: 5' ACG AGG GAA AAC AAT AAG GG 3'	195	Halford *et al.*, 1996

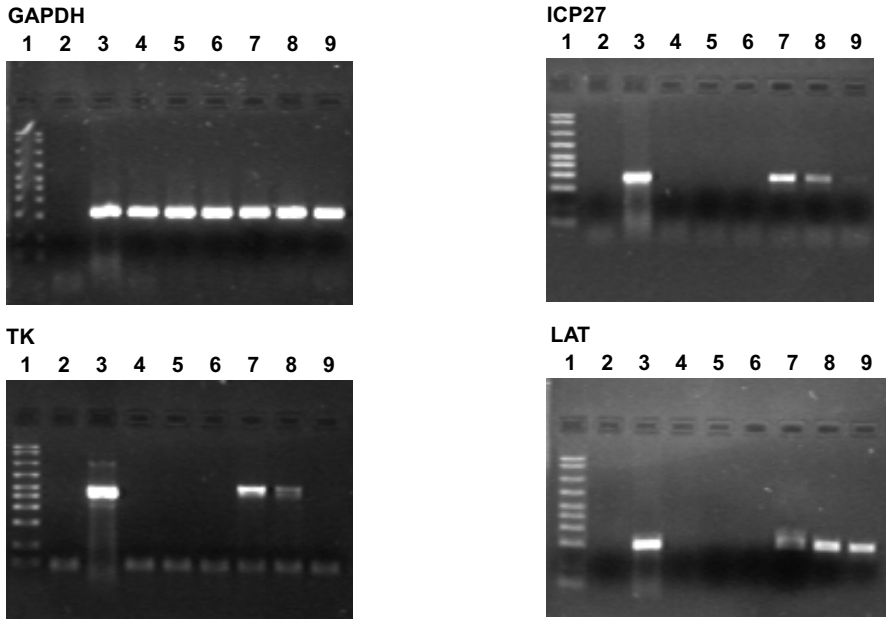

Fig. 2. HSV-1 tr gene expression in long-term-infected PC12 cells.
Line 1 – Molecular ruler (BioRad), line 2 – negative nucleid acid amplification control, line 3 – positive nucleid acid amplification control, line 4 – non-infected PC12 culture, line 5 – non-infected PC12 culture with addition of IFN alpha, line 6 – non-infected PC12 culture with addition of anti-IFN alpha, line 7 – PC12 infected with HSV-1 tr, line 8 – PC12 infected with HSV-1 tr with addition of IFN alpha, line 9 – PC12 infected with HSV-1 tr with addition of anti-IFN alpha

abundantly in neurons derived from latently infected humans and animals (Fraser *et al*., 1992), although low levels of non-LAT viral gene expression have been observed by using extremely sensitive methods (Kramer and Coen, 1995; Kramer *et al*., 1998). It was therefore of interest to determine how addition of IFN alpha or anti-IFN alpha can influence on the presence of viral-gene products in long-term-infected PC12 cell cultures. RNA was isolated from long-term-infected PC12 cultures two weeks after infection, at the time when no virus was detected in the culture medium. The expression of three key viral transcripts (alpha 27, TK, and LAT) representing the different kinetic classes was examined. Figure 2 shows the results of RT-PCR analysis of RNA isolated from the long-term-infected cultures. LAT was detected in all tubes containing long-term-infected cells. ICP27 and TK (transcripts connected with active phase of replication) were not detected in tubes with addition of anti-IFN alpha contrary to the tubes with addition of IFN alpha and without additions of IFN alpha or anti-IFN alpha. The presence of GAPDH RNA, which is a constitutively produced cellular (host) transcript, indicates that there was a correct isolation of RNA in each case. The lack of products of viral transcripts – TK and ICP27 in the tube with anti-IFN alpha confirm that anti-IFN alpha promotes the establishment of HSV-1 tr long-term infection in differentiated PC12 cultures.

Discussion

We studied how interferon alpha affects the establishment of latency by HSV-1. Temperature resistant (tr) HSV-1 mutant from the collection of Department of Virology, National Institute of Higiene was used. The results presented in this report imply that antiserum to IFN alpha promotes the establishment of HSV-1 tr long-term infection in differentiated PC12 cultures. The immediate-early gene product – ICP27 and the early gene product – TK were not detected by RT-PCR only in tubes containing long-term-infected cultures with addition of antiserum to IFN alpha.

Other investigators also detected ICP27 and TK gene products in long-term infected cultures by Southern blot hybrydization to RT-PCR products (Su *et al.*, 1990). There is a hypothesis that it can be "smouldering" infections. It is possible that, occasionally, abortive viral replication occurs in subpopulations. Perhaps abortive reactivations occur during *in vivo* latent infection (Kramer *et al.*, 1998).

Interferons (IFNs) have the ability to protect hosts from HSV infection. Exogenous IFNs reduce the progression of HSV-induced disease (Pinto *et al.*, 1990) and endogenously produced IFNs play an important role in host defences against HSV infection (Hendricks *et al.,* 1991; Lausch *et al.*, 1991; Su *et al.*,

1990). In cultured cells, a significant reduction in virus titre by IFNs was observed (Chatterjee *et al.*, 1984; Domke-Opitz *et al.*, 1986; Oberman and Panet, 1988). For alphaherpesviruses such as HSV-1, establishment of latency correlates with the ability to initiate acute phase replication (Halford and Schaffer, 2000). A detailed analysis demonstrated that the virus efficiently establishes a stable level of latency once acute-phase replication occurs above a threshold level (Halford and Schaffer, 2000; Sawtell, 1997; Sawtell, 1998). IFNs inhibit HSV-1 replication and probably cause longer phase of establishment.

In our studies, the absence of gene products for ICP27 and TK was correlated with faster establishment of latency in tubes with antiserum to IFN alpha than in tubes with IFN alpha and any other additions. So, we suggest that IFN alpha is an important tool not only for the control of productive but also latent HSV-1 infection.

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