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Induction of Cellular Transcription Factors in Trigeminal Ganglia of Mice by Corneal Scarification, Herpes Simplex Virus Type 1 Infection, and Explantation of Trigeminal Ganglia

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In a mouse model for herpes simplex virus type 1 (HSV-1) latency in which the virus was inoculated via the eye after corneal scarification, HSV-1 replicated in corneal epithelial cells and infected the nerve cell endings. HSV-1 reached the trigeminal ganglia by fast axonal transport between 2 and 10 days postinfection (p.i.) and established a latent infection in neuronal cells or replicated and spread to nonneuronal cells. By using in situ hybridization, we showed that cellular transcription factors are stimulated by HSV-1 infection in trigeminal ganglia. This stimulation is biphasic, peaking at 1 and 3 to 4 days p.i. The first peak involves c-*jun* and *oct-1* expression in neurons, and the second involves c-*jun*, c-*fos*, and *oct-1* expression in neurons and nonneuronal cells. Corneal scarification, alone or followed by infection with UV-inactivated HSV-1, induced monophasic c-*jun* and *oct-1* expression in some neurons of the trigeminal ganglia, with a peak at 1 day p.i. Corneal infection without prior scarification induced c-*jun*, c-*fos*, and *oct-1* expression in some neuronal and nonneuronal cells of the trigeminal ganglia 2 to 9 days p.i. Explantation of ganglia from latently infected animals resulted in reactivation of the latent virus. Independently of the presence of latent HSV-1 in explanted ganglia, expression of c-*fos*, c-*jun*, and *oct-1* was induced first in nonneuronal cells, peaking 6 to 10 h postexplantation, and then in neuronal cells, with a peak at 24 h after explantation when expression of viral replicative genes was first detectable. Since ocular HSV-1 infection, corneal scarification, and explantation of trigeminal ganglia all resulted in induction of expression of cellular transcription factors in ganglia, these factors may play a critical role in the permissiveness of cells for HSV-1 replication during acute infection, latency, and reactivation.

Following herpes simplex virus type 1 (HSV-1) infection of humans or animals, latent infection is established in neuronal cells of the sensory ganglia that innervate the primary site of infection (1). During latency, viral DNA is detectable in the ganglia, while transcription of viral RNA is limited to the latency-associated transcripts (LATs) (for a review, see reference 43). Most studies on the expression of viral proteins during HSV-1 latency have yielded negative results. In humans and some laboratory animal models, HSV-1 can reactivate spontaneously and produce recurrent disease or asymptomatic reappearance of the virus in the dermatome that corresponds to the HSV-1-carrying ganglion (19, 28).

Reactivation stimuli in vivo range from direct mechanical or pharmacological insults to the neuron or surrounding tissue to systemic changes in immune modulators and neurotransmitters (17, 19). Reactivation can be induced in vitro (19, 23, 37, 43, 47) by explantation of latently infected ganglionic tissue.

Transcription from HSV-1 immediate-early (IE) genes has not been detectable during latency; however, in tissue culture, expression of these genes is a prerequisite for viral replication and is the first transcriptional event during the viral replication cycle (32, 48). It is possible that the wide variety of stimuli that lead to reactivation are similar in the sense that they bring about a reorientation of neuronal gene expression that would include upregulation of transcription factors that facilitate viral IE gene transcription and/or downregulation of inhibiting factors (19, 22, 34, 40). IE viral gene expression in lytic infection is dependent upon the interaction of cellular transcription factors, notably, c-*jun*, c-*fos*, and the octamer-binding protein encoded by *oct-1* (OTF-1), with binding sites in the IE promoters (21, 30). The *oct-1* protein has been shown to mediate the transinducing function of HSV-1 tegument protein Vmw65 during lytic infection and also bind and mediate the function of other not fully characterized proteins (reviewed in references 11 and 25).

Recent studies have revealed that neuronal gene expression can be modulated by neurotransmitters, membrane electrical activity, and neurotrophic growth factors (among them, nerve growth factor [NGF]) and that neurons can react to environmental changes by switching to new programs of gene expression (reviewed in references 25 and 36). The neuronal response appears to be mediated by cellular IE genes (which include c-*fos* and c-*jun*, etc.), whose transcription is activated rapidly and transiently after the cells are stimulated. IE genes encode regulatory proteins that control the pattern of cellular expression of the so-called late-response genes, for instance, that of the NF-L and NF-M genes that encode subunits of neurofilaments (36).

Interestingly, tissue culture experiments with other herpesviruses, including human cytomegalovirus, Epstein-Barr virus, and HSV-2, have shown that these viruses can induce expression of several cellular transcription factors, including c-*fos*. Viral IE proteins (10, 13) and interactions of viral particles with the cell membrane (3) have both been shown to have this effect. HSV-1 IE proteins have been shown to induce heat shock proteins in tissue culture cells (29). These viral functions, along with the long-known host shutoff function of HSV (reviewed in reference 35), show how viruses can modulate the pattern of gene expression of infected cells.

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As the pattern of gene expression of host cells seems to play an important role in the activation of HSV-1 gene expression, in the present study, we examined the expression of some cellular genes which may be important factors in the regulation of viral transcription and thus in the establishment, maintenance, and reactivation stages of latency. Cellular genes selected for study included the NGF gene, as treatment of neurons with NGF has been shown to regulate HSV-1 replication in a tissue culture reactivation model (50). Moreover, the c-*fos* and c-*jun* genes, which may regulate expression of NGF following nerve injury (15), and expression of the IE viral genes in tissue culture were studied. The *oct-1* gene was selected for its role in viral IE gene expression, as discussed above. Transcription from the NF-L gene was also studied, as it represents a potential target of cellular IE genes in neurons (36).

We also studied the expression of three viral genes representing the α or IE (ICP4), βγ or early-late (VP5 major capsid protein), and δ (LATs) kinetic classes of HSV-1 genes (35, 39) as markers of the viral growth cycle.

In our laboratory, the molecular events of the establishment, maintenance, and reactivation stages of HSV-1 latency have been studied in a mouse trigeminal ganglion latency model that involves ocular inoculation with HSV-1 and reactivation of the virus by explantation of ganglia 28 days or later to tissue culture medium. In this model, HSV-1 reaches the ganglia as early as 12 to 24 h postinfection (p.i.) (40) and replicates there between 2 and 10 days p.i. (38). By 28 days p.i., HSV-1 is present in the ganglia only in a nonreplicative, latent state (38, 44).

Postexplantation (p.e.) of latently infected ganglia, infectious virus and viral DNA synthesis can be detected first in latently infected tissues at 2 days p.e. (38). Although lytic-cycle transcripts of HSV-1 cannot be detected by Northern (RNA) blot analysis before the appearance of infectious virus, viral proteins have been detected as early as 6 to 21 h p.e. (52).

In this report, we show that following corneal inoculation with HSV-1, expression of cellular transcription factors c-*fos*, c-*jun*, and *oct-1* is induced in trigeminal ganglia. In explanted trigeminal ganglia, independently of the presence of latent virus, expression of the c-*fos*, c-*jun*, and *oct-1* transcription factors was induced first in nonneuronal cells and then in neurons. Thus, we found that during acute replication of HSV-1 in the eyes and trigeminal ganglia of mice following corneal inoculation, cellular transcription factors c-*fos*, c-*jun*, and *oct-1* were induced by the virus, while during explant reactivation these and other factors were induced by the insult of explantation. The changes detected in both cases may have important regulatory roles in determining the permissiveness of the cells involved for HSV-1 replication.

MATERIALS AND METHODS

Virus. HSV-1 strain KOS was propagated in CV-1 cells, concentrated from culture medium, and titrated by plaque assay as previously described (38). The viral stock used in our experiments was 2.5×10^9 PFU/ml. Thymidine kinase-negative HSV-1 mutant *dl*sptk (3.2×10^8 PFU/ml), with a 360-bp deletion in the middle third of the thymidine kinase-encoding sequences, was obtained from D. M. Coen, Harvard University (4). HSV-1 strain McKrae (2×10^7 PFU/ml) was obtained from M. Trousdale (Estelle Doheny Eye Foundation). A UV-inactivated stock of strain McKrae was prepared as described by Notarianni and Preston (29).

Infection of mice. Four- to six-week-old female BALB/c BYJ mice (Jackson Laboratory) were inoculated after ocular scarification with 10^5 PFU of HSV-1 strain KOS, *dl*sptk, or UV-inactivated McKrae per eye (viral strains were diluted in serum-free Dulbecco modified Eagle medium) or an equal volume (10 μl) of serum-free Dulbecco modified Eagle medium (mock infection). For HSV-1 infection by the McKrae strain (10^5 PFU per eye), corneas were not scarified. A control group of mice was anesthetized with Metofane without scarification or infection. At selected times (0, 1, 3, 6, and 24 h and 2, 3, 4, 5, 9, and 28 days p.i.), mice were sacrificed by cervical dislocation, and the trigeminal ganglia were removed aseptically and processed for in situ hybridization.

Explant reactivation. Trigeminal ganglia removed at 28 days p.i. were immediately processed for in situ hybridization or incubated in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum at 37°C for 0.5, 2, 6, 10, 24, or 48 h p.e. At each time point, two to four pairs of ganglia were processed for in situ hybridization.

In situ hybridization. The in situ hybridization protocol used was previously described (9). Four to eight sections were mounted on each slide, one each from the left and right trigeminal ganglia of two to four mice either infected with one of the three HSV-1 strains used or mock infected. For each type of infection and each time point, at least two slides (8 to 16 sections) were examined following hybridization to each probe in repeated experiments. The number of positive neurons or nonneuronal cells in the sections was counted (large numbers were estimated), and the relative strength of hybridization was determined on a scale of 0 to 4. The percentage of cells positive at a given time point was based on the numbers of positive cells in the sections as follows: 0%, no positive cells; 1%, 1 or 2 positive neurons or 1 to 15 positive nonneuronal cells (we used the rough average numbers of 200 neurons and 1,500 nonneuronal cells per section, calculated from 10 representative sections of trigeminal ganglia, as denominators); 2%, 3 or 4 positive neurons or 16 to 30 positive nonneuronal cells; 5%, 5 to 10 positive neurons or 31 to 75 positive nonneuronal cells; 10%, 11 to 20 positive neurons or 76 to 150 nonneuronal cells and so on as multiples of 10%.

Probes for in situ hybridization. Plasmid pRB113 (*Bam*HI-Y; ICP4 probe) and plasmid pRB112, containing *Bam*HI-B, were obtained from B. Roizman, University of Chicago (31). The LAT probe *Sph*I-*Sph*I was isolated from the *Bam*HI B fragment. Plasmid pBR322, containing the *Bam*HI fragment a′ (KOS) (5) and used for a VP5 probe, was obtained from E.K. Wagner and R.H. Costa (University of California, Irvine). Transcription from the c-*jun* gene was studied by using the 1.8-kb *Eco*RI fragment of a plasmid containing the complete rat c-*jun* open reading frame cloned in pGEM4 (33). To detect c-*fos*, the 1.3-kb *Pst*I-*Pst*I fragment of plasmid pfos-1 was used (6). To detect transcription from the *oct-1* gene, the *Bam*HI-*Hin*dIII fragment of the pBSoct-1 plasmid was used, which was obtained from W. Herr (45). The NF-L probe was a gift of R. Lazzarini, Mt. Sinai Hospital, New York, N.Y. *Eco*RI was used to recover an insert from plasmid pHNF5 which was used as a probe to detect transcription from the small neurofilament NF-L subunit gene (27).

NGF expression was studied by a βNGF probe isolated from plasmid phβN8D8 (ATCC 54790) (46).

DNA inserts were gel purified and nick translated as described previously (9). The specific activities of the nick-

TABLE 1. In situ hybridization of trigeminal ganglia to viral probes during acute infection

Procedure	Probe	Cell type	Strength[a] of hybridization/% of cells of same type positive at:				
			2 days	3 days	4 days	9 days	28 days
Mock infection, scarification	ICP4	Small	—	—	—	—	—
		Neuron	—	—	—	—	—
	LAT	Small	—	—	—	—	—
		Neuron	—	—	—	—	—
	VP5	Small	—	—	—	—	—
		Neuron	—	—	—	—	—
Infection (McKrae), no scarification	ICP4	Small	1/1	3/1	1/1	1/1	—
		Neuron	1/1	1/5	1/10	1/1	—
	LAT	Small	—	—	1/1	1/1	1/1
		Neuron	1/1	1/1	1/1	2/2	4/10
	VP5	Small	—	1/1	1/1	1/1	—
		Neuron	—	1/5	1/5	1/1	—
Infection (KOS), scarification	ICP4	Small	—	4/5	4/5	1/1	—
		Neuron	2/1	4/5	4/5	1/1	—
	LAT	Small	—	1/1	1/1	1/1	1/1
		Neuron	1/1	1/2	1/2	2/5	3/10
	VP5	Small	—	2/5	2/5	—	—
		Neuron	—	2/5	2/5	—	—
Infection (*dl*SPTK), scarification	ICP4	Small	—	—	—	—	—
		Neuron	—	1/1	—	—	—
	LAT	Small	—	—	1/1	1/1	1/1
		Neuron	1/1	1/1	1/1	2/1	3/2
	VP5	Small	—	—	—	—	—
		Neuron	—	—	—	—	—

[a] 1, weak; 2, moderate; 3, strong; 4, very strong. No hybridization was detected at 0, 1, 3, 6, or 24 h. —, 0/0%.

translated ^{35}S-labeled probes were 1×10^8 to 2×10^8 cpm/μg.

RESULTS

Mice were inoculated with HSV-1 by corneal scarification and placement of 10 μl of a solution containing HSV-1 on the eye as described in Materials and Methods. HSV-1 strains KOS (wild type), *dl*sptk (a *tk* mutant that does not form an acute infection in the peripheral nervous system; 4), and McKrae (a wild-type strain that forms latent infections in the absence of corneal scarification and can be reactivated from latent infections by iontophoresis; 17, 51) were used to infect mice. Some mice were treated by corneal scarification only or mock infected with serum-free Dulbecco modified Eagle medium. At various times p.i. (0 h to 28 days), mice were sacrificed and the trigeminal ganglia were dissected and processed for in situ hybridization. In the absence of infection with HSV-1 or scarification treatment, no hybridization with any of the probes (c-*jun*, c-*fos*, *oct-1*, NGF, ICP4, or LAT) except NF-L was seen (data not shown). The patterns of hybridization seen with tissues derived from animals which were scarified, those which were scarified and mock infected, and those which were scarified and infected with UV-inactivated virus were similar (data presented for scarified and mock-infected mice only; see Tables 1 and 2). See Tables 1 to 4 for summaries of the results of the in situ hybridization experiments.

Detection of viral transcripts in trigeminal ganglia at 0 to 28 days p.i. Following ocular infection of mice with HSV-1 strains KOS and McKrae, transcription from the viral IE ICP4 gene was first detected in the trigeminal ganglia on day 2 (48 h) p.i. in neuronal cells (Table 1). ICP4-positive neurons were detectable until day 9 (216 h) p.i. after infection with strain KOS or McKrae. Perhaps because of cell-to-cell virus spread, ICP4-positive nonneuronal cells were found between days 3 (72 h) (in the case of McKrae, day 2) and 9 p.i. in both infections. At 28 days p.i., no ICP4-positive cells were detected in either infection. However, in ganglia from mice infected with *dl*sptk, the only time when ICP4-positive neurons were seen was day 3 p.i. ICP4-positive nonneuronal cells were not seen at any time point, and the ICP4 probe (*Bam*HI-Y) did not hybridize to ganglional tissues of noninfected animals.

Transcription from early-late viral gene VP5 was detected in both neuronal and nonneuronal cells in the trigeminal ganglia of KOS- or McKrae-infected animals 3 to 4 and 3 to 9 days, respectively, p.i. but never in *dl*sptk-infected animals.

LAT transcription in some neuronal cells of the trigeminal ganglia was detected first on day 2 p.i., independently of the HSV-1 strain used for infection. From days 2 through 28 p.i., both the number of positive neurons and the strength of hybridization increased as a function of time. There was a difference in the number of LAT-positive neurons generated by the KOS and McKrae strains (which replicate in the trigeminal ganglia) and *dl*sptk (which does not). This difference increased as a function of time: at 28 days p.i., about five times more LAT-positive neurons were detected after KOS and McKrae infections than after *dl*sptk infection. A small number of LAT-positive nonneuronal cells were also detected starting at 3 (KOS) or 4 (*dl*sptk and McKrae) days p.i. The LAT probe did not hybridize to noninfected tissues at any time.

Detection of induced cellular transcripts in the trigeminal ganglia at 0 to 28 days p.i. In the trigeminal ganglia of mice

TABLE 2. In situ hybridization of trigeminal ganglia to cellular probes during acute infection

Procedure	Probe	Cell type	Strength[a] of hybridization/% of cells of same type positive at:									
			0 h	1 h	3 h	6 h	24 h	2 days	3 days	4 days	9 days	28 days
Mock infection, scarification	c-*jun*	Small	—	—	—	—	—	—	—	—	—	—
		Neuron	—	2/10	2/10	2/10	2/50	1/50	1/1	—	—	—
	c-*fos*	Small	—	—	—	—	—	—	—	—	—	—
		Neuron	—	—	—	—	—	—	—	—	—	—
	oct-1	Small	—	—	—	—	—	—	—	—	—	—
		Neuron	—	3/10	2/10	2/30	4/50	1/10	1/1	—	—	—
	NF-L	Small	—	—	—	—	—	—	—	—	—	—
		Neuron	2/95	4/30	2/50	2/50	4/95	3/95	3/95	3/95	2/95	2/95
Infection (McKrae), no scarification	c-*jun*	Small	—	—	—	—	—	—	—	2/1	2/1	—
		Neuron	—	—	—	—	—	1/1	2/1	1/1	—	—
	c-*fos*	Small	—	—	—	—	—	—	1/1	1/1	—	—
		Neuron	—	—	—	—	—	1/2	1/1	1/1	—	—
	oct-1	Small	—	—	—	—	—	1/1	1/1	3/1	1/1	—
		Neuron	—	—	—	—	—	3/5	1/1	—	—	—
Infection (KOS), scarification	c-*jun*	Small	—	—	—	—	—	—	—	2/1	1/1	—
		Neuron	—	2/10	2/10	2/10	2/50	1/50	2/5	2/1	—	—
	c-*fos*	Small	—	—	—	—	—	—	—	3/1	—	—
		Neuron	—	—	—	—	—	—	1/1	1/1	—	—
	oct-1	Small	—	—	—	—	—	—	—	3/1	2/1	—
		Neuron	—	3/10	2/10	2/30	4/50	1/10	3/10	3/10	1/1	—
	NF-L	Small	—	—	—	—	—	—	—	—	—	—
		Neuron	2/95	4/30	2/50	2/50	4/95	3/95	3/95	3/95	2/95	2/95
Infection (*dl*SPTK), scarification	c-*jun*	Small	—	—	—	—	—	—	—	—	—	—
		Neuron	—	2/10	2/10	2/10	2/50	1/50	2/1	1/1	—	—
	c-*fos*	Small	—	—	—	—	—	—	—	—	—	—
		Neuron	—	—	—	—	—	—	—	—	—	—
	oct-1	Small	—	—	—	—	—	—	—	—	—	—
		Neuron	—	3/10	2/10	2/30	4/50	1/10	2/10	2/10	1/1	—
	NF-L	Small	—	—	—	—	—	—	—	—	—	—
		Neuron	2/95	4/30	2/50	2/50	4/95	3/95	3/95	3/95	2/95	2/95

[a] 1, weak; 2, moderate; 3, strong; 4, very strong. —, 0/0%.

which underwent anesthesia but whose eyes were not scarified, transcription from the cellular genes (c-*jun*, c-*fos*, *oct-1*, and NGF) was not detected at any time point (data not shown). However, a neurofilament (NF-L) probe hybridized to about 95% of the neurons at all times with a strength unaffected by time elapsed after anesthesia.

Eye scarification with or without HSV-1 infection led to detection of c-*jun* and *oct-1* expression (as well as increased NF-L expression) in some neurons as early as 1 h after scarification (Table 2; Fig. 1). Scarification-induced c-*jun* and *oct-1* expression peaked at 24 h postscarification (50% of neurons were strongly positive for *oct-1* and c-*jun*). Induction of NF-L was also noted as increased accumulation of grains over a fraction of the neurons. This expression of c-*jun*, *oct-1*, and NF-L was detected at moderately increased levels up to 3 to 4 days after scarification.

In the trigeminal ganglia from mice infected with HSV-1 strain KOS or *dl*sptk, induction of c-*jun*, c-*fos*, and *oct-1* expression in some neurons between days 2 and 9 p.i. was observed. This was in addition to the earlier scarification-induced *oct-1* and c-*jun* stimulation (Table 2). Both the KOS- and *dl*sptk-induced changes peaked on days 3 and 4 p.i., but KOS infection elicited stronger c-*jun* and *oct-1* expression. No c-*fos* induction was seen after *dl*sptk infection. Some c-*jun*, c-*fos*, and *oct-1* expression in nonneuronal cells was found on days 3 to 9 p.i. following KOS infection, but not in *dl*sptk-infected ganglia.

To study further the effects of viral infection on induction of cellular transcription factors in the absence of those induced by scarification, we used HSV-1 strain McKrae, which is capable of reaching the trigeminal ganglia after ocular infection without scarification. Following ocular infection with the McKrae strain (without corneal scarification), no transcription from the c-*jun* and *oct-1* genes was detected until 2 days p.i.; i.e., early (scarification-induced) changes were not observable. Between days 2 and 9 p.i., however, c-*jun*-, c-*fos*-, and *oct-1*-positive neurons (Fig. 2A) and nonneuronal cells (Fig. 2C) were observed in McKrae-infected ganglia but not in the ganglia of animals mock infected with tissue culture medium without scarification (Table 2; Fig. 2B and D).

HSV-1 infection did not induce NGF expression in the trigeminal ganglia (data not shown).

Detection of viral transcripts in trigeminal ganglia at 0 to 48 h p.e. To study induction of genes which may play a role in reactivation, ganglia were explanted from mice mock infected, or latently infected with HSV-1 strain KOS. Table 3 summarizes the data gained from in situ hybridization studies of ganglia explanted for different periods.

Neurons positive with the *Sph*I-*Sph*I probe, which is specific for the LATs, were found at every selected time point in latently infected ganglia but never in noninfected tissues. The strength of hybridization, however, showed a consistent pattern of change p.e. in repeated experiments. It peaked at 0.5 h p.e. and seemed to be downregulated later

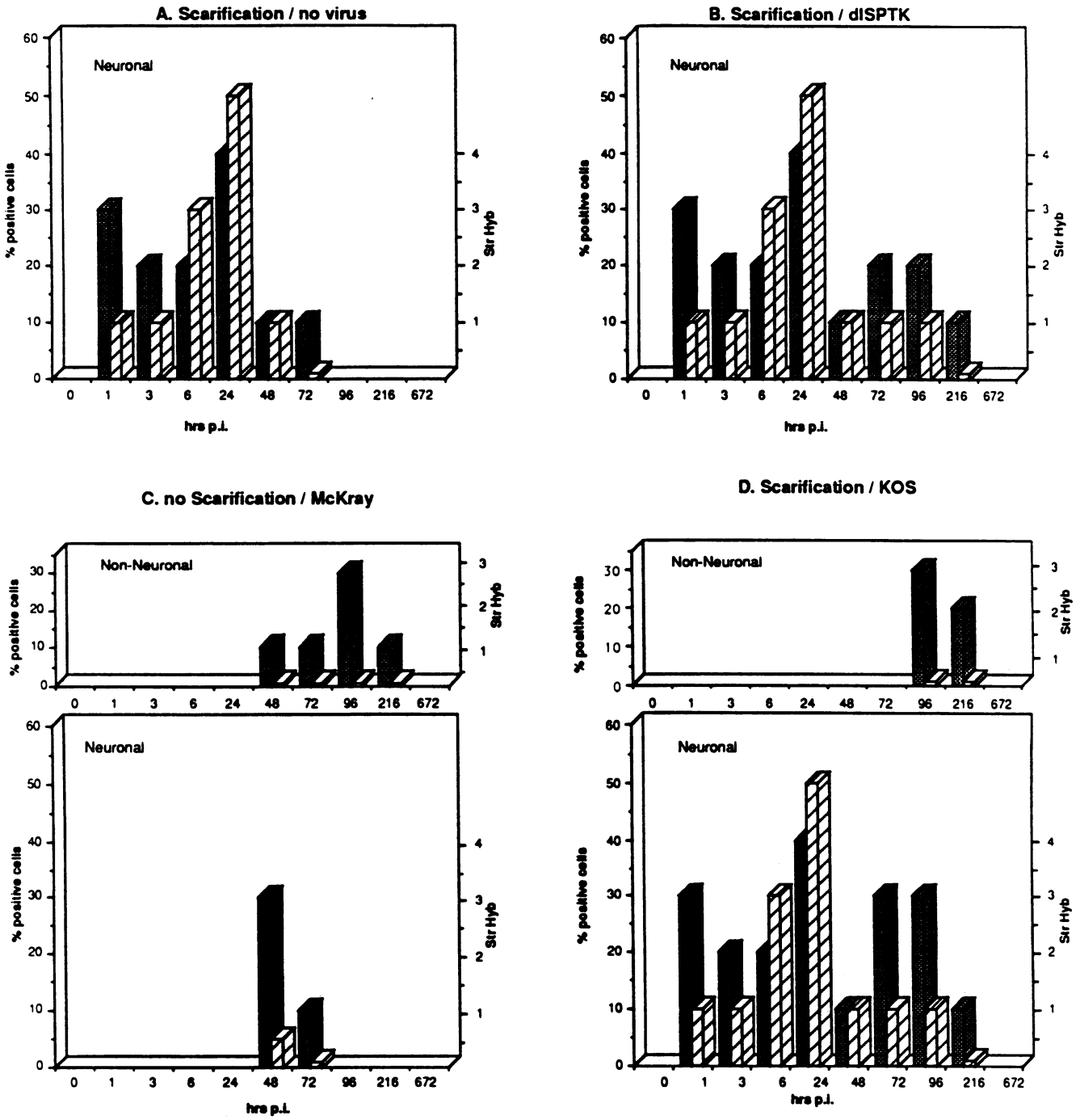

FIG. 1. *oct-1* expression in neuronal and nonneuronal cells of trigeminal ganglia detected by in situ hybridization as a function of time after corneal scarification (A), corneal scarification followed by infection with HSV-1 strain *dl*sptk (B) or KOS (D), and ocular infection with HSV-1 strain McKrae without scarification (C). Filled bars show the detected relative strength of hybridization on a scale of 0 to 4, and striped bars show the percentages of cells of the given types (neuronal and nonneuronal) that were positive. No positive nonneuronal cells were detected following corneal scarification without infection or with *dl*sptk infection (data not shown).

(Table 3; Fig. 3A, B, and C). A few LAT-positive nonneuronal cells were also detected at every time point studied.

The *Bam*HI-Y probe used for detection of ICP4 hybridized to a small number of neurons and satellite cells of latently infected ganglia explanted for 24 to 48 h. This probe did not hybridize to tissues of latently infected ganglia explanted for 0 to 10 h or mock-infected ganglia explanted for 0 to 48 h.

The *Bam*HI-a′ probe, which detects transcription from the major capsid protein VP5 gene, hybridized neither to explanted, previously mock-infected ganglia at 0 to 48 h p.e. nor to explanted, latently infected ganglia at 0 to 10 h p.e.

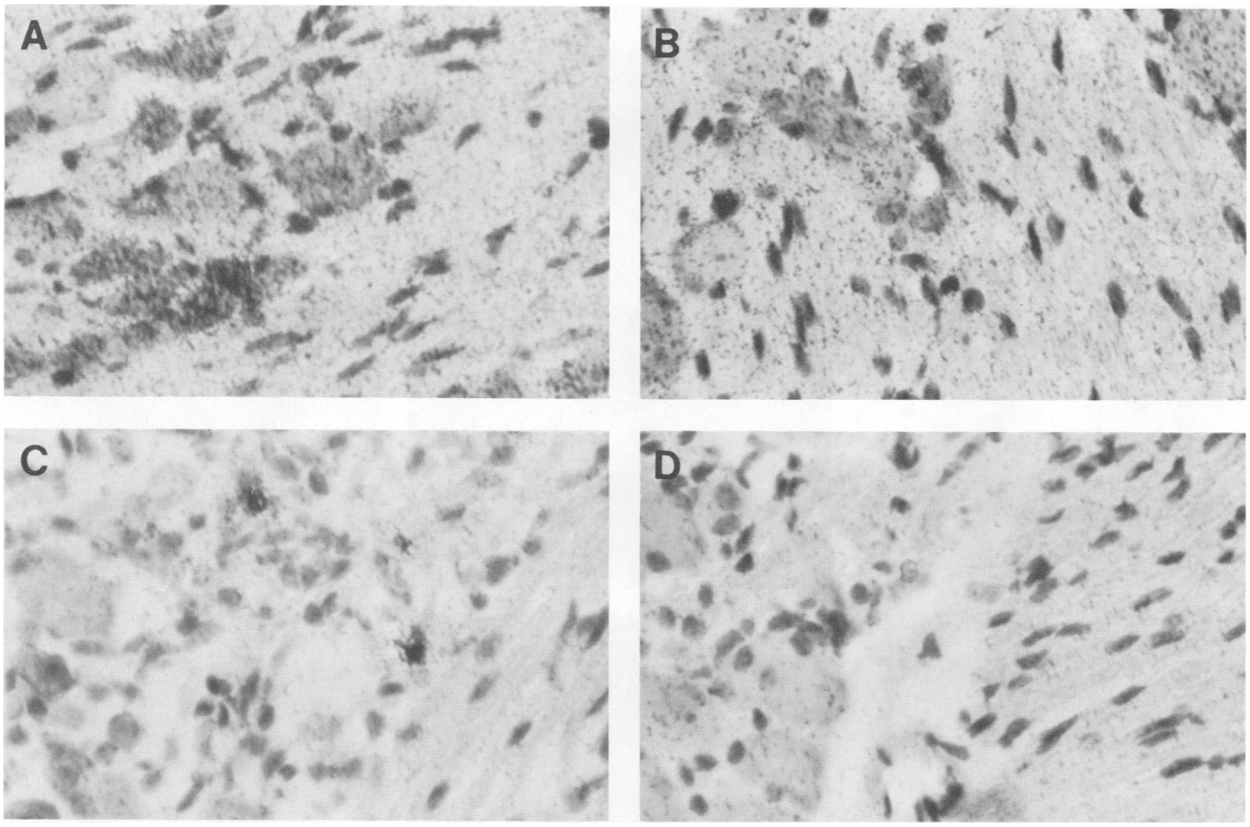

FIG. 2. In situ hybridization for detection of *oct-1* mRNA in the trigeminal ganglia of mice 2 (A) and 4 (C) days after ocular infection with HSV-1 strain McKrae without corneal scarification or 2 (B) and 4 (D) days after ocular mock infection with tissue culture medium without corneal scarification.

However, this probe hybridized to a few neurons and satellite cells of explanted, latently infected ganglia at 24 and 48 h p.e.

Detection of cellular transcripts in trigeminal ganglia at 0 to 48 h p.e. No difference was found between the patterns of hybridization of mock-infected and latently infected tissues to probes for transcripts from cellular genes at up to 48 h p.e. (Table 4 and Fig. 4). The NF-L probe hybridized to the neuronal cells at all of the times studied. The strength of hybridization was the strongest at 0.5 and 24 h p.e. and seemed to decline at intermediate times (Fig. 3D, E, and F).

The c-*fos*, c-*jun*, and *oct-1* probes showed similar patterns of hybridization between the neuronal and the nonneuronal cells. At 0 and 0.5 h p.e., no positive cells were seen (Fig. 3G and J). With some variation in the time course, these probes hybridized to supporting cells at 2 to 48 h p.e. The highest number of positive cells and the strongest hybridization were seen at 6 and 10 h p.e. (Fig. 4H and K). At 2 h p.e., c-*fos*- and

TABLE 3. In situ hybridization of explant-reactivated trigeminal ganglia to viral probes

Procedure	Probe	Cell type	Strength[a] of hybridization/% of cells of same type positive at:						
			0 h	0.5 h	2 h	6 h	10 h	24 h	48 h
Mock infection, scarification	ICP4	Small	—	—	—	—	—	—	—
		Neuron	—	—	—	—	—	—	—
	LAT	Small	—	—	—	—	—	—	—
		Neuron	—	—	—	—	—	—	—
	VP5	Small	—	—	—	—	—	—	—
		Neuron	—	—	—	—	—	—	—
Infection (KOS), scarification	ICP4	Small	—	—	—	—	—	1/1	2/1
		Neuron	—	—	—	—	—	1/1	1/1
	LAT	Small	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		Neuron	3/10	4/10	3/10	2/10	2/10	2/10	3/10
	VP5	Small	—	—	—	—	—	1/1	2/1
		Neuron	—	—	—	—	—	2/1	2/1

[a] 1, weak; 2, moderate; 3, strong; 4, very strong. —, 0/0%.

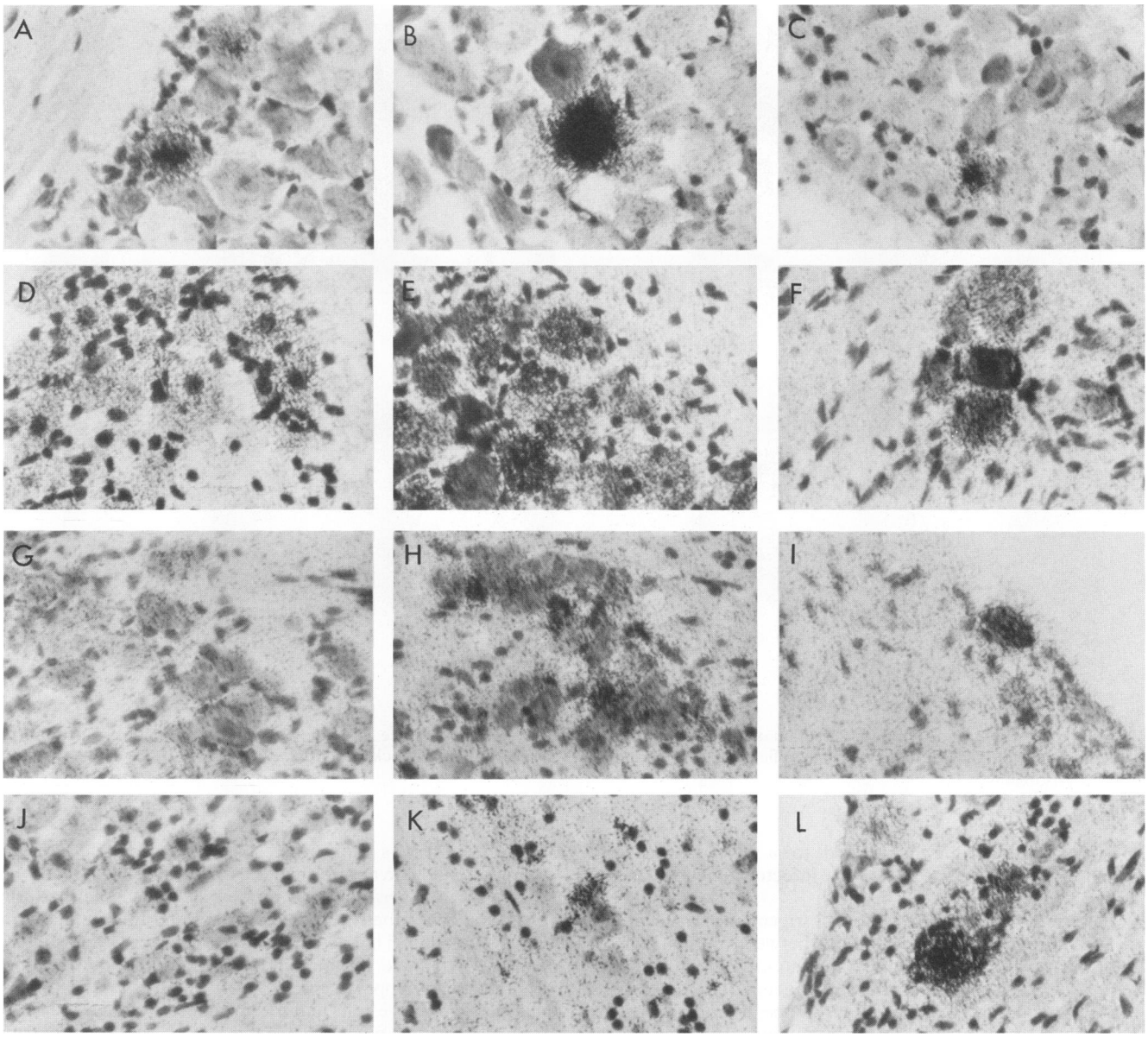

FIG. 3. In situ hybridization for detection of cell- or HSV-1-specific RNA in explanted latently infected trigeminal ganglia of mice. *Sph*I-*Sph*I probe specific for the LATs at 0 (A), 0.5 (B), and 24 (C) h p.e. The probe that detects transcription from the gene of the small neurofilament subunit (NF-L) at 0 (D), 0.5 (E), and 24 (F) h p.e. Detection of c-*jun* RNA at 0 (G), 10 (H), and 24 (I) h p.e. Detection of *oct-1* RNA at 0 (J), 10 (K), and 24 (L) h p.e.

c-*jun*-positive nonneuronal cells were found mostly in the proximity of the site where nerves were cut during removal of the ganglia. At later times, no special pattern of localization of positive cells was observed. The c-*jun* and *oct-1* probes also hybridized to some neurons at 10 to 48 h p.e. (Fig. 3I and L). c-*fos*-positive neurons were detected only at 24 to 48 h p.e.

Positive signals with the β-NGF probe were seen only at 24 and 48 h p.e. and were over nonneuronal cells. Except for NF-L, the probes used for detection of cellular transcripts hybridized to only a portion of the cells at a given time point. This suggests that expression of these genes was transient and that the whole neuronal and nonneuronal cell population was not completely synchronized in expression.

DISCUSSION

In this study, we examined the expression of cellular transcription factors that may play a role in the HSV-1 infectious cycle during both acute infection and reactivation.

Acute infection. We have shown that following corneal inoculation of mice with HSV-1 strain KOS, expression of cellular transcription factors c-*jun*, c-*fos*, and *oct-1* was induced in some neurons and satellite cells of the trigeminal ganglia at 2 to 9 days p.i. This suggests that these cellular transcription factors play an important role in replication of the virus in vivo, as has previously been shown in vitro (21, 30). With HSV-1 strain McKrae (which does not require corneal scarification to replicate efficiently in the eye and

TABLE 4. In situ hybridization of explant-reactivated trigeminal ganglia to cellular probes

Procedure	Probe	Cell type	Strength[a] of hybridization/% of cells of same type positive at:						
			0 h	0.5 h	2 h	6 h	10 h	24 h	48 h
Mock infection, scarification	c-*jun*	Small	—	—	1/5	2/10	2/10	1/5	—
		Neuron	—	—	—	—	1/1	3/20	2/5
	c-*fos*	Small	—	—	1/10	2/10	2/20	1/5	1/1
		Neuron	—	—	—	—	—	2/5	1/1
	oct-1	Small	—	—	—	3/20	2/10	1/5	1/5
		Neuron	—	—	—	—	1/1	3/60	2/10
	NGF	Small	—	—	—	—	—	1/1	1/1
		Neuron	—	—	—	—	—	—	—
	NF-L	Small	—	—	—	—	—	—	—
		Neuron	2/95	4/95	2/95	1/50	1/50	3/95	1/50
Infection (KOS), scarification	c-*jun*	Small	—	—	1/5	2/10	2/10	1/5	—
		Neuron	—	—	—	—	1/1	3/20	2/5
	c-*fos*	Small	—	—	1/10	2/10	2/20	1/5	1/1
		Neuron	—	—	—	—	—	2/5	1/1
	oct-1	Small	—	—	—	3/20	2/10	1/5	1/5
		Neuron	—	—	—	—	1/1	3/60	2/10
	NGF	Small	—	—	—	—	—	1/1	1/1
		Neuron	—	—	—	—	—	—	—
	NF-L	Small	—	—	—	—	—	—	—
		Neuron	2/95	4/95	2/95	1/50	1/50	3/95	1/50

[a] 1, weak; 2, moderate; 3, strong; 4, very strong. —, 0/0%.

form a latent infection in the trigeminal ganglia), we were able to dissociate this viral induction from an earlier, but partially coinciding, stimulation of *oct-1* and c-*jun* expression caused by ocular scarification in trigeminal ganglion neurons.

Although stress to neurons appears to induce cellular transcription factors at early times p.i., HSV-1 also induces these factors at later times. HSV-1 virion components have been shown to induce cellular transcription factors in tissue culture cells (3). However, since UV-inactivated virus did not induce a late peak of cellular transcription factors, the events in neurons are probably not directly comparable. Nevertheless, *dl*sptk, a *tk* mutant strain of HSV-1 that apparently does not replicate in the trigeminal ganglia but does replicate to a low level in the eye (4, 24), did induce a low level c-*jun* and *oct-1* expression in neurons. These data suggest that viral replication in the eye is sufficient to provide the stimulus for induction of cellular transcription factors in the neurons of the ganglia, perhaps by a mechanism similar to scarification, i.e., tissue damage. However, viral replication in the ganglia seems to be required for induction of cellular transcription factors in the satellite cells of the ganglia (Table 2). The mechanism of this induction is unclear; perhaps host defense factors are involved. HSV-1 infection has been found to induce major histocompatibility complex antigen expression on rat microglia and to induce a local immune response involving lymphocytic infiltration of the infected site in the peripheral and central nervous systems (2, 49). Alternatively, one or more HSV-1 IE gene products could be responsible for the observed induction of cellular transcription factors in the ganglia, as the induction was seen only in cell types in which IE viral gene expression has occurred (in neurons after *dl*sptk, KOS, and McKrae infection and in satellite cells after KOS and McKrae infection). Obviously, further studies are needed to define the mechanism of induction of c-*fos*, c-*jun*, and *oct-1* expression during acute infection of the trigeminal ganglia.

Reactivation. In explanted trigeminal ganglia, increased

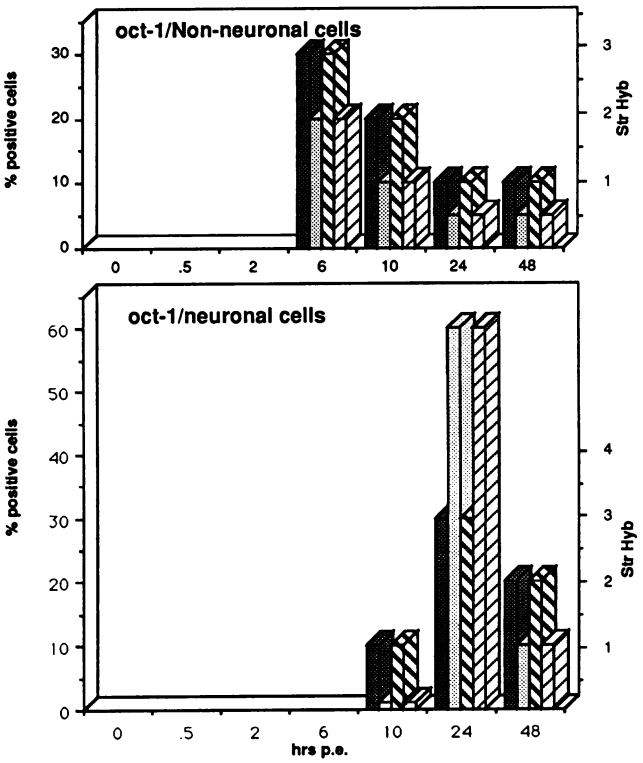

FIG. 4. *oct-1* expression in neuronal and nonneuronal cells in explanted trigeminal ganglia as a function of time p.e. Mice were previously infected (28 days) with HSV-1 (KOS) or mock infected. The relative strength of hybridization on a scale of 0 to 4 over cells from mock-infected or HSV-1-infected mice is shown along with the percentage of positive cells at the given time point. ▩, strength of hybridization with mock infection; ▨, percentage of positive cells with mock infection; ◹, strength of hybridization with HSV-1 infection; ◪, percentage of positive cells with HSV-1 infection.

expression of c-*jun*, c-*fos*, and *oct-1* was observed first in nonneuronal cells with a peak at 6 to 10 h p.e. and then in neurons with a peak at 24 h p.e. This effect was independent of the presence of latent HSV-1.

Increased NGF gene expression in some nonneuronal cells of the trigeminal ganglia at 24 to 48 h p.e. was also noted. In latently infected animals, LAT-expressing neurons were detected throughout the 2-day period p.e. Expression of the viral IE ICP4 gene and the early-late VP5 gene was first detected at 24 h p.e. Interestingly, some of the first cells showing ICP4 and VP5 expression were nonneuronal cells, raising the possibility that HSV-1 may also reactivate from latently infected nonneuronal cells. That nonneuronal cells may harbor latent HSV has previously been shown by Deatly et al. (8) and others.

Relative to 0 and 2 h p.e., an increased density of hybridization was detected with the LAT probe at 0.5 h p.e., suggesting increased transcription of the LATs at that time. The LATs are not required for establishment or maintenance of HSV-1 latency but may facilitate reactivation (18, 26, 41). As LAT transcription seemed to be downregulated after 0.5 h p.e., our data suggest that the LATs can exert their function very early p.e.

As explantation-induced expression of c-*jun*, c-*fos*, and *oct-1* in the ganglia was seen at a time (2 to 48 h p.e.) when viral proteins are first detectable in the explanted tissues (6 to 24 h p.e.) (52) and at the time when viral gene expression, other than that of the LATs, was first detected (24 h p.e.), it seems possible that the induction of c-*jun*, c-*fos*, and *oct-1* expression caused by the trauma of explantation has a role in the reactivation process. Explantation-induced c-*fos*, c-*jun*, and *oct-1* expression in the neurons was rather widespread (Table 4, explanted mock-infected tissues), while expression of replicative viral genes was limited to a few cells. From this distribution, it seems possible that the cell factor expression induces the viral genes. Furthermore, our findings show that viral gene expression in a few reactivating cells does not lead to activation of cell factors over a large number of cells, although we cannot exclude the possibility that induction by virus occurs in a very limited number of cells.

In in vitro HSV-1 reactivation models, NGF deprivation has been shown to induce viral replication (50). In vivo, high NGF levels in sympathetic and sensory ganglia are thought to be due to accumulation from the periphery by axonal transport, rather than local synthesis, and are thought to be essential for maintenance of specialized properties of sensory neurons (7, 16). In sciatic nerve organ cultures, increased NGF synthesis has been observed in nonneuronal cells in response to nerve transection and is mediated by c-*fos* (15). Our observed changes in c-*fos* and NGF expression in the tissues of explanted ganglia are very similar to the results of these studies.

It is possible that nonneuronal cells in explanted trigeminal ganglia respond to nerve transection by a series of events that lead to increased NGF expression in them. NGF synthesized by nonneural cells may take over the role of NGF supplied previously by the periphery. However, during the lag period between nerve transection and the availability of NGF from nonneuronal cells, a new program of gene expression may be initiated in neurons, which includes upregulation of transcription factors which facilitate HSV-1 IE gene expression and viral reactivation.

The complexity of the neuronal response to explantation is demonstrated by our results concerning neurofilament gene expression. We detected increased RNA transcription from the neurofilament subunit gene NF-L at 0.5 and 24 h p.e. It is possible that the first increase represents an immediate, direct neuronal response to explantation, while the late peak is a result of an NGF-mediated nonneuronal response. By 2 days p.e., NF-L expression seemed to be downregulated, a finding similar to the reported downregulation of NF expression 3 days after nerve transection in vivo (12, 20).

The observed upregulation of the *oct-1* transcription factor in the cells of trigeminal ganglia could be of particular importance for HSV-1 reactivation in explanted ganglia, as *oct-1* plays a central role in the regulation of HSV-1 IE gene expression (for a review, see reference 11). We have previously shown that HSV-1 transinducing protein Vmw65 is not required for viral reactivation from explanted trigeminal ganglia and suggested that prior to virus reactivation, *oct-1* binds and mediates the effects of cellular Vmw65 analogs activated by reactivation stimuli (40). We did not detect *oct-1* expression in the trigeminal ganglia up to 2 h p.e., an observation consistent with studies on adult rat sensory ganglia (14). The increased expression of *oct-1* in trigeminal ganglia at the approximate time of the first detectable events of HSV-1 reactivation (52) suggests that the level of *oct-1* itself is an important factor in HSV-1 reactivation. In fact, the level of *oct-1* may be one of the determinant factors of neuronal permissiveness for HSV-1 replication in vivo (11).

Although our findings in the mouse model system represent only initial observations of the very complex gene regulational background of HSV-1 gene expression, they do help us to a new, perhaps still simplistic, level of understanding of the mechanism of HSV-1 replication, latency, and reactivation.

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