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SHORT COMMUNICATION

Activation of cellular interferon-responsive genes after infection of human cells with herpes simplex virus type 1

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Previous studies have shown that infection of human fibroblasts with human cytomegalovirus (HCMV) results in activation of cellular interferon-responsive gene expression. We demonstrate here that infection of human fibroblasts with herpes simplex virus type 1 (HSV-1) in the absence of *de novo* protein synthesis also induces the expression of interferon-responsive genes. Five genes tested (encoding ISG54, IFI56, ISG15, 9-27 and MxA) were activated by infection with HSV-1, although the degree of response varied between the individual genes. HSV-1 was a less efficient inducer than HCMV. The effect was a consequence of binding of the virus particle to the cell surface or of the presence of virion components within the infected cell. Induction was mediated by a pathway other than the mechanism through which interferon-α mediates its effects on cellular gene expression.

Interferons are produced by cells as defences to infection by viruses. The antiviral effects of interferons are mediated by cellular interferon-responsive gene products, which inhibit virus replication in a variety of ways (Darnell *et al*., 1994; Stark *et al*., 1998). Interaction of interferons with their receptors stimulates the cellular membrane-bound Janus protein kinases (JAKs) Tyk2 and JAK1, which in turn phosphorylate and activate proteins known as signal transducers and activators of transcription (STATs), resulting in increased levels of many cell mRNAs (Darnell *et al*., 1994; Der *et al*., 1998; Stark *et al*., 1998). Activated STAT1 and STAT2 form heterodimers and migrate to the nucleus, where they bind to p48 (ISGF3γ), forming the complex ISGF3 that recognizes specific target sequences in the promoters of interferon-α-responsive genes. The expression of interferon-responsive gene products can also be provoked by virus infection directly, and in the best studied example, that of infection by paramyxoviruses, the cellular signalling mechanisms differ from those used by the cytokines themselves in that the JAK/STAT pathway is bypassed (Marié *et al*., 1998; Wathelet *et al*., 1998; Guo *et al*., 2000). The spectra of cellular genes induced by interferon-α and by paramyxovirus infection are similar but not identical, with subsets of genes responding to either or both inducers (Wathelet *et al*., 1992).

Cellular interferon-responsive gene expression is induced upon infection of human fibroblasts with human cytomegalovirus (HCMV). The effect was observed even in the absence of *de novo* protein synthesis, indicating that it is mediated by virion components (Zhu *et al*., 1997, 1998; Navarro *et al*., 1998), and binding of glycoprotein B to the cell surface is sufficient to elicit the response (Boyle *et al*., 1999). There are differences in the details of the reports describing induction by infection with HCMV: in one case a number of interferon-responsive genes, including those encoding cellular genes named ISG54, ISG15 and 9-27, were activated (Zhu *et al*., 1997, 1998), whereas in the other ISG54 was switched on but ISG15 and 9-27 were not (Navarro *et al*., 1998). Activation of ISG54 synthesis correlated with the formation of a novel protein complex that bound to sequences in the promoter of this gene, suggesting that, as in the case of paramyxovirus infection, induction by HCMV and by interferon itself does not follow exactly the same signalling pathway (Navarro *et al*., 1998). In the report of Zhu *et al*. (1997), infection with herpes simplex virus type 1 (HSV-1) gave only very small increases in the level of ISG-specific RNAs, leading to the conclusion that HSV-1 does not significantly affect the expression of this set of cellular genes. We have re-examined the expression of interferon-responsive genes in cells infected with HSV-1, by using mutants impaired for immediate early (IE) transcription and by carrying out infection in the presence of cycloheximide to block viral protein synthesis.

Human foetal lung (HFL) fibroblasts, the human fibrosarcoma line 2fTGH, and mutants of 2fTGH named U1A (mutant for Tyk2), U3A (mutant for STAT1) and U4A (mutant for JAK1) were used (Pellegrini *et al*., 1989; McKendry *et al*., 1991; Darnell *et al*., 1994). HCMV was strain AD169 and wild-type HSV-1 was strain 17. The HSV-1 mutants *in*1814, specifying a defective virion transactivator VP16, and *in*1312, which has mutations that inactivate VP16 and the IE transactivator proteins ICP0 and ICP4, have been described

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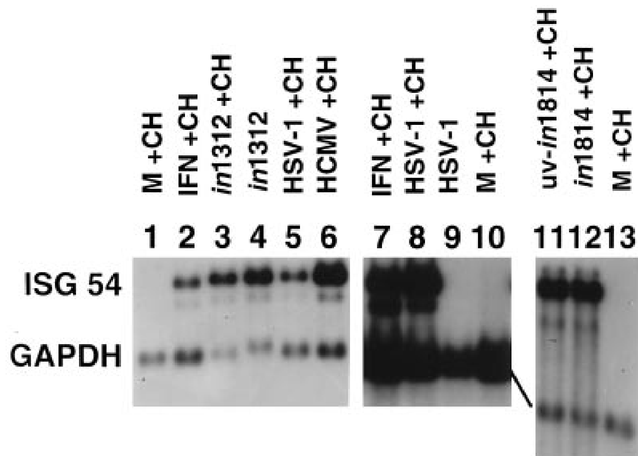

Fig. 1. Induction of ISG54-specific RNA by infection with HSV-1. Monolayers of HFL cells were treated with 50 µg cycloheximide/ml (lane 1) or 10^3 units human lymphoblastoid interferon-α/ml plus cycloheximide (lane 2), or infected with *in*1312 at an effective dose of 5 p.f.u. per cell with (lane 3) or without (lane 4) cycloheximide, infected with wild-type HSV-1 (5 p.f.u. per cell) with cycloheximide (lane 5) or infected with HCMV (1 p.f.u. per cell) with cycloheximide (lane 6). In a separate experiment, HFL cells were infected with 5 p.f.u. of wild-type HSV-1 per cell in the presence (lane 8) or absence (lane 9) of cycloheximide, with control monolayers treated with interferon-α and cycloheximide (lane 7) or cycloheximide (lane 10). In a third experiment, HFL cells were infected with 5 p.f.u. of gradient-purified *in*1814 per cell plus cycloheximide (lane 12) or the same amount of purified *in*1814 after UV-irradiation to reduce the titre by a factor of 5×10^5, plus cycloheximide (lane 11), or mock-infected plus cycloheximide (lane 13). In all cases, polyadenylated RNA was extracted at 6 h after infection at 38·5 °C. RNA blots were hybridized with probes specific for ISG54 and GAPDH.

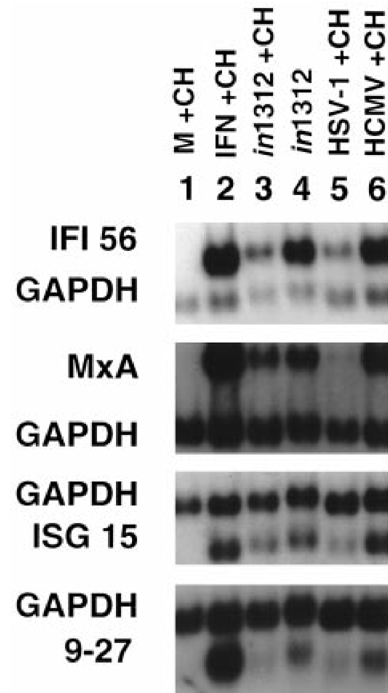

Fig. 2. Induction of interferon-responsive RNAs by infection with HSV-1. HFL monolayers were treated as described in the legend to Fig. 1 (lanes 1–6), and polyadenylated RNA samples were hybridized with probes specific for IFI56, MxA, ISG15 or 9-27, with a GAPDH-specific probe included. A larger MxA-specific RNA showed the same response as the major species depicted (result not shown). The low signal for MxA-specific RNA after infection with wild-type HSV-1 was partially due to reduced recovery of RNA in that sample.

previously (Ace *et al.*, 1989; Preston *et al.*, 1998). Mutant *in*1312 is severely impaired for viral IE gene expression, and cells can survive infection with this virus at relatively high m.o.i. (up to an effective dose of 5 p.f.u. per cell: Preston *et al.*, 1997; Homer *et al.*, 1999). Probes specific for ISG54, ISG15 and 9-27 were prepared by RT–PCR amplification of RNA isolated from HFL cells that had been treated for 6 h with human lymphoblastoid interferon-α (Sigma). The probe for IFI56 was a 270 bp *Bgl*II–*Ssp*I fragment from a cloned copy of the gene (Chebath *et al.*, 1983). A probe for MxA was cleaved from a plasmid containing the cloned cDNA, kindly supplied by F. Weber (Institute of Virology, Glasgow, UK). A probe specific for glyceraldehyde phosphate dehydrogenase (GAPDH) was purchased from Ambion.

Monolayers of 2×10^6 to 1×10^7 HFL cells were infected with *in*1312 (effectively 5 p.f.u. per cell, although the measured titre was lower than this value due to the ICP0 mutation; the number of particles added was equivalent to that when 5 p.f.u. of wild-type HSV-1 was used), wild-type HSV-1 (5 p.f.u. per cell) or HCMV (1 p.f.u. per cell), with 50 µg cycloheximide/ml in some cases, and incubated at 38·5 °C for 6 h. Total cytoplasmic or polyadenylated RNA was isolated, separated on formaldehyde–agarose gels, blotted and hybridized to radiolabelled gene-specific probes (Nicholl & Preston, 1996) (Fig. 1). Monolayers were mock infected with cycloheximide added (lane 1), treated with 10^3 units human lymphoblastoid interferon-α/ml, plus cycloheximide (lane 2), or infected with *in*1312 plus cycloheximide (lane 3), *in*1312 without cycloheximide (lane 4), wild-type HSV-1 with cycloheximide (lane 5) or HCMV with cycloheximide (lane 6). Hybridization with probes specific for ISG54 and GAPDH, a control gene that was not expected to be affected by interferon-α treatment, demonstrated that strong induction of ISG54-specific RNA, comparable to that after addition of interferon-α, was observed after infection with all viruses tested in the presence of cycloheximide, and with *in*1312 in the absence of the inhibitor. In a second set of experiments, HFL cell monolayers were infected with wild-type HSV-1 with or without cycloheximide. Infection in the absence of cycloheximide did not result in increased ISG54 mRNA levels, in agreement with the findings of Zhu *et al.* (1997) (Fig. 1, lane 9), whereas strong induction was observed when the inhibitor was present (Fig. 1, lane 8). Induction of ISG54 occurred after infection with *in*1814 that had been gradient purified by the method of Szilagyi & Cunningham (1991) (Fig. 1, lane 12), suggesting that components of the virus rather than contaminating cellular material were responsible, and the effect was not diminished by heavy UV-irradiation of the *in*1814 preparation to reduce its titre by a factor of 5×10^5 (Notarianni & Preston, 1982), confirming

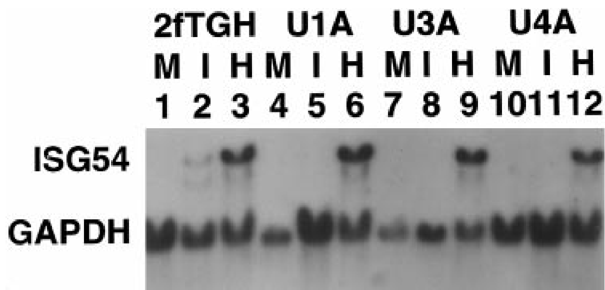

Fig. 3. Induction of ISG54-specific RNA in mutant cell lines. Monolayers of 2fTGH (lanes 1–3), U1A (lanes 4–6), U3A (lanes 7–9) or U4A (lanes 10–12) cells were mock-infected (M), treated with 10^3 units interferon-α/ml (I) or infected with 5 p.f.u. of *in*1814 per cell (H), all in the presence of 50 μg cycloheximide/ml. Cytoplasmic RNA was extracted at 6 h after infection at 38·5 °C, and RNA blots were probed for the presence of ISG54- and GAPDH-specific RNA.

that viral protein synthesis is not required for induction (Fig. 1, lane 11). To determine whether infection stimulated the release of soluble factors (for example, interferon itself), the medium from cells infected with *in*1312 at 38·5 °C for 6 h was centrifuged at 25 000 ***g*** for 1 h to remove virions and applied to fresh HFL monolayers. This procedure did not result in the induction of ISG54 (results not shown).

To investigate the generality of the cellular response to HSV-1, probes specific for other interferon-responsive genes were hybridized to the RNA preparations analysed in Fig. 1 (Fig. 2). Induction of IFI56, MxA, ISG15 and 9-27 (the last of these to a low but detectable extent) was observed after infection with *in*1312, HSV-1 and HCMV. The response of these genes to infection was lower than observed after interferon treatment, in contrast to that of ISG54 which was induced more strongly by infection. Taking results from a number of experiments, HCMV was routinely found to be a stronger inducer than HSV-1 at the multiplicities used, but the greater response to *in*1312 without cycloheximide than in its presence (lanes 3 and 4) was not always observed to the extent shown in Fig. 2. It is possible that secondary activation of the IFI56, ISG15 and 9-27 genes contributed to the magnitude of the effect seen without cycloheximide.

To determine whether the induction by HSV-1 utilizes the JAK/STAT pathway, mutant cell lines deficient in TyK2, STAT1 or JAK1 were infected with *in*1814 in the presence of cycloheximide and tested for the presence of ISG54-specific RNA (Fig. 3). The parent cell line, 2fTGH, showed activation of expression of the ISG54 gene after treatment with interferon-α or infection with *in*1814 (Fig. 3, lanes 1–3). The three mutant cell lines did not respond to interferon-α, as expected, but nonetheless contained significant amounts of ISG54-specific transcripts at 6 h after infection with *in*1814 (Fig. 3, lanes 4–12). This result demonstrates that the pathway activated in response to infection with HSV-1 does not require the JAK/STAT components that mediate gene activation by interferon-α. In addition, the possible involvement of interferon itself in the induction by HSV-1 is eliminated because the mutant cell lines are unresponsive to the cytokine.

The experiments described here show that HSV-1, like HCMV, induces the expression of interferon-responsive genes in human fibroblasts. The effect does not require viral protein synthesis, and is presumably due to binding of the HSV-1 particle to the cell surface or to the presence of viral structural proteins or RNA within the infected cell. It is possible that cellular proteins packaged by the virus participate in induction. By analogy with the finding that HCMV glycoprotein B can turn on the expression of interferon-responsive genes (Boyle *et al*., 1999), it may be that interaction of HSV-1 glycoproteins with a cellular receptor is sufficient to trigger signal transduction pathways. Activation by VP16 through interaction with Oct-1 and HCF can be eliminated as a contributing factor since *in*1814 and *in*1312 specify a nonfunctional form of the viral protein. Our finding that HCMV induces both ISG15 and 9-27 supports the data of Zhu *et al*. (1997) rather than those of Navarro *et al*. (1998), although it should be noted that the there are methodological differences in that the latter study used ribonuclease protection rather than RNA blots to detect transcripts.

The observation that ISG54-specific transcripts were not detected after normal infection with HSV-1, as opposed to the situation when cycloheximide was present from the time of infection, suggests that, as is the case for other viruses, HSV-1 encodes functions that inhibit the interferon response. It is likely that the relevant virus-specified gene products have a role in combating the interferon-mediated host defence to HSV-1, which has an important bearing on virus pathogenesis (Leib *et al*., 1999).

Paramyxovirus infection activates transcription from the promoter of ISG15, but not that of 9-27, through a novel signalling pathway involving phosphorylation and recruitment of the cell factors IRF-3 and IRF-7 (Wathelet *et al*., 1998; Guo *et al*., 2000). The mechanism used by paramyxoviruses is unlikely to account entirely for the effects of HSV-1 or HCMV, since these herpesviruses do induce 9-27, albeit at least 10-fold relatively less strongly than they induce ISG15. The results may therefore highlight further variation in the ways in which cells respond to infection by different viruses.

The experiments described here report a novel feature of infection with HSV-1. It has been shown previously that HSV-1 induces the transcription of certain cellular genes, in some cases as a very early event in infection (Notarianni & Preston, 1982; Kemp *et al*., 1986 *a*, *b*; Preston, 1990; Hobbs & DeLuca, 1999). The most relevant report demonstrated the presence of a new protein of M_r approximately 56 000 after treatment of human cells with an HSV-1 mutant blocked at an early stage of infection (Preston, 1990), and it is tempting to speculate that this represented one or more members of the ISG54 family.

Our results additionally show that noncytotoxic prototype HSV-1 vectors, or amplicons, that are severely impaired for viral gene expression may nonetheless alter cellular gene

expression. It is not yet clear whether these changes affect cell physiology in any significant way, or if they have an influence on the cellular response to infection by HSV-1 or other viruses.

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