Inhibition of Replication of Herpes Simplex Virus in Mouse Macrophages by Interferons

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SUMMARY

The replication of herpes simplex virus (HSV) type 1 in macrophages grown from spleen cells of mouse strains susceptible to HSV infection in vivo was very sensitive to interferon (IFN). Different types of mouse IFN (α, β, γ) exhibited similar antiviral activities. However, treatment of cells with IFN-γ in combination with IFN-α or IFN-β resulted in a synergistic inhibition of virus growth. As shown by assaying HSV DNA polymerase, IFN inhibited expression of the β-genes. Inhibition of enzyme induction correlated well with the reduction of viral yield. Induction of HSV DNA polymerase was delayed by IFN in a dose-dependent manner. These results show that IFN inhibits HSV replication at an early step prior to or during the synthesis of β-proteins.

Mouse macrophages play an important role in non-specific host defence against herpes simplex virus (HSV) (Hirsch et al., 1970; Mogensen, 1979; Morahan et al., 1980) and have been identified as interferon (IFN)-producing cells (Kirchner et al., 1983). Pure cultures of peritoneal or splenic macrophages have been shown to produce IFN after challenge with HSV (Brücher et al., 1984). IFN is also induced in cultures of bone marrow macrophages by viruses and various chemical compounds (Storch & Kirchner, 1982). It has been shown that precultured macrophages of mouse strains susceptible to HSV in vivo produce only low IFN titres after virus infection in vitro and are permissive for HSV replication (Brücher et al., 1984).

Several studies have indicated that the replication of HSV is inhibited by IFN. However, high IFN concentrations were required compared to other virus groups (Lerner & Bailey, 1976; Panet & Falk, 1983). This makes it difficult to analyse the mechanism of IFN action. However, treatment of precultured mouse macrophages with low doses of IFN results in a very effective inhibition of HSV replication, and the effect of IFN on some parameters of replication of HSV in macrophages is described in the present paper.

Macrophages were grown from spleens of 8- to 12-week-old male DBA/2 mice (Zentralinstitut für Versuchstierkunde, Hannover, F.R.G.). After aseptic removal of the spleen, cells were suspended in culture media as described by Klimetzek & Remold (1980) with some modifications. The medium consisted of RPMI 1640 (Gibco, code 041-1875) supplemented with 4% horse serum, 12% foetal bovine serum (FBS), glutamine, gentamicin, and 16% L-cell supernatant (from L-cells grown in DMEM 10% FBS after 7 days of culture). All other reagents were obtained from Seromed (München, F.R.G.). Cells were seeded in Falcon 35 mm Petri dishes (code 3001 F). On day 5, cells were washed to remove non-adherent cells. Subsequently, media were replaced daily. After 6 days of growth, cultures consisted of 100% macrophages as shown by morphology, by phagocytosis of carbon particles, and by esterase staining (Koski et al., 1976).

A pool of HSV type 1 strain WAL was prepared as previously described (Zawatzky et al., 1982) and will subsequently be referred to as HSV. Virus titrations were performed by a plaque assay using RITA cells. Titres in macrophages were determined after freezing and thawing. Mouse IFN-α (2.7 × 10⁶ IU/mg) and mouse IFN-β (4.7 × 10⁷ IU/mg) were purchased from Stratech Scientific Ltd. (London, U.K.). Mouse IFN-α/β (7 × 10⁷ IU/mg) from the late K.
Table 1. Effect of combinations of different IFNs on the replication of HSV after infection at an m.o.i. of 2

<table>
<thead>
<tr>
<th>IFN type</th>
<th>IFN dose (IU/ml)</th>
<th>log₁₀ Reduction of virus titre*</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>200</td>
<td>2.9</td>
</tr>
<tr>
<td>β</td>
<td>200</td>
<td>2.4</td>
</tr>
<tr>
<td>γ</td>
<td>200</td>
<td>2.6</td>
</tr>
<tr>
<td>Mφ</td>
<td>200</td>
<td>2.6</td>
</tr>
<tr>
<td>α + β</td>
<td>100 + 100</td>
<td>2.6</td>
</tr>
<tr>
<td>α + γ</td>
<td>100 + 100</td>
<td>3.6</td>
</tr>
<tr>
<td>β + γ</td>
<td>100 + 100</td>
<td>3.3</td>
</tr>
<tr>
<td>Mφ + γ</td>
<td>150 + 50</td>
<td>3.3</td>
</tr>
<tr>
<td>α + β + γ</td>
<td>60 + 60 + 60</td>
<td>3.4</td>
</tr>
<tr>
<td>Hu-α₂</td>
<td>200</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Virus yield in the absence of IFN treatment was 8 × 10⁶ p.f.u./ml; cells were frozen 24 h after infection.

Paucker's laboratory was a gift of Dr R. M. Friedman to Dr H. Jacobsen. Mouse IFN-γ (3.6 × 10⁶ IU/mg) purified according to Gribaudo et al. (1984) was kindly donated by Dr S. Landolfo (Institute of Microbiology, University of Turin, Italy). Macrophage IFN (IFN-Mφ; 1 × 10⁷ IU/mg) was induced in bone marrow macrophages by 10-carboxymethyl-9-acridanone as described by Storch & Kirchner (1982). It was partially purified using the procedure of G. Brehm et al. (unpublished). Recombinant human IFN-α₂ (1 × 10⁷ IU/mg) was given by Dr S. Hiemstra (Essex Pharma, München, F.R.G.). Antiserum against mouse IFN-α/β (5 × 10⁴ IU/ml) was purchased from Stratech Scientific Ltd. Interferon titrations were performed as described by Zawatzky et al. (1982) using L-cells and vesicular stomatitis virus (VSV).

Cells were used for experiments at about 90% confluency. At this time, cell number per dish was about 1 × 10⁷. Cells were washed and incubated with IFN for 18 h. Prior to infection macrophages were washed three times to remove IFN and infected with 0.2 ml virus at a multiplicity of infection (m.o.i.) as indicated in each experiment. After virus adsorption for 1 h, cells were washed again, supplemented with 2.0 ml of fresh medium and incubated for various times. Infected cells were stored at −70°C until assay of virus yield. All experiments were carried out with duplicate cell cultures.

For assaying DNA polymerase activity, cell monolayers were washed twice with cold phosphate-buffered saline and once with cold extraction buffer (10 mM-Tris-HCl pH 8.0, 2 mM-2-mercaptoethanol, 20% glycerol). Cells were incubated for 5 min at 4°C in 0.4 ml extraction buffer, scraped off with a rubber policeman and stored at −70°C. After thawing, post-mitochondrial supernatants were prepared by centrifugation at 12000 g for 15 min at 4°C. The supernatants were kept at −70°C. Protein concentrations were determined according to the Bio-Rad protein assay (Bradford, 1976).

Enzyme activities were determined as described by Panet & Falk (1983) and Purifoy & Benyesh-Melnick (1975). The incorporation of [3H]TTP (sp. act. 50 Ci/mmole) into TCA-insoluble material was measured with activated DNA as template primer. The assay mixture contained KCl (0-2 M) to suppress cellular DNA polymerases. Residual cellular enzyme activities are included in the figures. All enzyme assays were performed in triplicate.

Macrophages grown from spleens of DBA/2 mice were permissive for HSV and HSV replication exhibited a high sensitivity to IFN. Different types of mouse IFN (α, β, γ) reduced virus titres by more than 2 log₁₀, whereas HSV replication was not affected by Hu-IFN-α₂ (Table 1). After stimulation with HSV, macrophage cultures produced IFN-α/β (Kirchner et al., 1983). Compared to other IFNs, endogenous macrophage IFN (IFN-Mφ) induced by 10-carboxymethyl-9-acridanone in bone marrow-derived macrophage cultures and partially purified (G. Brehm et al., unpublished results) did not differ in its activity against HSV.

Inhibition was shown to be synergistic with combinations of IFN-γ and IFN-α, IFN-γ and IFN-β, or IFN-γ and IFN-Mφ. For example, log₁₀ reduction of virus titre was 3.3 for a mixture of 100
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IU/ml IFN-β and 100 IU/ml IFN-γ. As calculated from dose–response experiments an inhibition of 3.3 log₁₀ would have been expected with 2000 IU IFN-β per ml (data not shown). Thus, potentiation of antiviral activity in the mixtures of IFN-γ and IFN-β was 10-fold.

The effect of different doses of IFN on HSV replication in mouse macrophages is shown in Fig. 1. Even after infection at an m.o.i. of 4 a 1.5 log₁₀ reduction in virus titre was observed with IFN doses as low as 10 IU/ml. The degree of protection was strictly dose-dependent. To study the effect of IFN on early parameters during the viral replication cycle we have analysed the induction of HSV DNA polymerase. Production of this β-protein precedes the synthesis of viral DNA. An inhibition of viral enzyme activity was observed even with low IFN doses. After treatment of macrophages with high IFN concentrations HSV DNA polymerase activity measured 6 h after infection was reduced to cellular background levels. The reduction of DNA polymerase correlates well with the reduction of yield of infectious virions.

The kinetics of viral replication was analysed in IFN-treated macrophages. As expected, infection at a multiplicity of 4 resulted in high virus titres and cell death after the first round of replication (Fig. 2a). Compared to control cells only few infectious virions were produced in IFN-treated cells. Inhibition was similar with 20 and 500 IU IFN per ml 9 h after infection. However, some hours later reduction of virus yield was more pronounced with higher IFN doses. There was a marked effect of IFN on the induction of HSV β-protein synthesis (Fig. 2b). In untreated macrophages the maximum of viral DNA polymerase activity was reached 7 to 9 h after infection and an increase of enzyme activity was first observed 3 h after infection. IFN decreased the maximum of enzyme activity and delayed enzyme induction in a dose-dependent manner. With 20 IU IFN-α/β per ml, the first increase of DNA polymerase was measured 5 h after infection. After pretreatment of cells with 500 IU IFN-α/β per ml, viral enzyme was first detectable 7 h after infection.

So far, little is known about the molecular events leading to the inhibition of herpesviruses in IFN-treated cells. We have studied IFN-mediated inhibition of HSV replication in splenic mouse macrophages. One reason for selecting these cells was the sensitivity of the macrophage–HSV system to IFN. Compared to other systems (Panet & Falk, 1983; Rasmussen et al., 1984) inhibition of HSV in mouse macrophages by IFN was much more effective. Previously, most experiments concerning IFN-mediated inhibition of HSV replication were done in permanent
cell lines (Lerner & Bailey, 1976; Fish et al., 1983). Treatment of macrophages with a mixture of IFN-γ and other IFN types resulted in a more than additive inhibition of virus growth. Similar findings have been made for inhibition of Mengovirus in L-cells (Fleischmann et al., 1979) or cell growth inhibition and inhibition of HSV in human melanoma cells (Czarniecki et al., 1984). The phenomenon of potentiation may indicate that IFN-γ and IFN-α/β activate cells by different mechanisms. It has been shown that an additional set of polypeptides is induced by treatment of cells with IFN-γ compared to IFN-α or IFN-β (Weil et al., 1983).

Analysis of cell supernatants after virus adsorption showed that more than 99% of infectious virus particles were adsorbed to the cell membrane in IFN-treated as well as in control cells (data not shown). Thus, viral adsorption was unaffected by IFN. However, induction of the HSV β-protein DNA polymerase was inhibited by IFN. Additionally, there was a delay in expression of β-genes. This suggests that IFN might act on α-protein synthesis, because sufficient amounts of HSV immediate early α-proteins are required to trigger the synthesis of β-proteins (Honess & Roizman, 1975).

Our results indicate that IFN inhibits HSV replication at an early step. Similar findings have been made with mouse L-cells (Panet & Falk, 1983) and human HeLa cells (Gloger & Panet, 1984). Contrary to these findings Muñoz & Carrasco (1984) reported no inhibition of HSV protein synthesis by IFN in HeLa cells which led them to suggest that IFN acts via the production of defective virions during the first virus cycle. Because of its high sensitivity to IFN the macrophage–HSV system is useful to analyse further the step in viral replication cycle that is blocked by IFN. This might be during penetration of HSV through the cell membrane as shown for VSV (Whitaker-Dowling et al., 1983) or during the synthesis of α-proteins as indicated by Gloger & Panet (1984).

Despite the fact that exogenous IFN was removed at the time of infection, there was no increase of virus yield in IFN-treated macrophages up to 3 days after infection. This continued restriction of viral growth is due to the production of endogenous IFN. Macrophages start producing IFN 9 h after infection and IFN titres of 100 IU/ml are still measurable 72 h after infection. After the addition of an antiserum against IFN-α/β, virus yield increases 2 to 3 log units (data not shown). Thus, the antiserum is active in vitro on splenic macrophages cultured for 1 week. In contrast, Belardelli et al. (1984) have shown with fresh peritoneal macrophages that anti-IFN globulin acts only in vivo.
IFN is known to activate macrophage functions (Bielefeldt Ohmann et al., 1984; Blasi et al., 1984). The efficient antiviral activity could be due to different mechanisms switched on by IFN. One might be unspecific phagocytosis; another method might be the (2'–5')oligoadenylate system and the protein kinase. The latter mechanisms have been shown to be involved in IFN-mediated inhibition of lytic RNA viruses (for review, see Lengyel, 1982). Recent studies with human cells indicate that the (2'–5')oligoadenylate system is not involved in IFN-mediated inhibition of HSV (Cayley et al., 1984). We have initiated studies to test this system in mouse macrophages. So far we have shown an IFN-dependent induction of (2'–5')oligoadenylate synthetase and the protein kinase (data not shown). Further work is needed to characterize the mechanism activated by IFN in macrophages that is responsible for the inhibition of HSV.

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**REFERENCES**


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