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# Herpes simplex virus as an inducer of interferon in human monocyte cultures

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## Summary

Induction of alpha interferon in human monocyte cultures exposed to moderate titres of herpes simplex virus (HSV) is a regular phenomenon, but shows variability in the resulting interferon titres. Thirty recent clinical isolates of HSV (15 type 1 and 15 type 2) were found to be equally effective interferon inducers, irrespective of their origin. Ultraviolet irradiation or thermal inactivation of the virus or blocking of the DNA synthesis by foscarnet did not destroy the capability of the virus to induce interferon. These results show that the interferon response in HSV-exposed human monocytes is relatively independent from the inducing virus strain and does not require replication of HSV.

Herpes simplex virus; Human monocytes; Interferon induction

## Introduction

Herpes simplex virus (HSV) can spread throughout the body in the leukocyte fraction of blood in generalized HSV infections (Graig and Nahmias, 1973). The capability of different leukocyte types to enable the replication of HSV or resist HSV infection in vitro has been reported in several earlier publications (Kirchner

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et al., 1977; Daniels et al., 1978; Plaeger-Marshall and Smith, 1978; Kirchner and Schröder, 1979; Grogan et al., 1981).

We have previously shown that interferon (IFN) induced in vitro in human monocyte cultures in response to HSV infection has a role in the restriction of HSV replication (Linnavuori and Hovi, 1981,1983). Others have reported similar phenomena in animal models (Zawatzky et al., 1982; Brandt Pedersen et al., 1983).

In our experience production of IFN by HSV exposed human monocytes is a regular phenomenon, but considerable variability is observed in the resulting IFN concentrations. The yields usually vary from 10–320 international interferon units (IU)/ml, while individual cultures may produce more than 1000 IU/ml. Difference between parallel cultures is minimal. In this paper we have evaluated to what extent the IFN response depends on the nature of the inducing virus strain and the infectivity of the virus inoculum.

## Materials and Methods

*Monocyte cultures*

Mononuclear leukocytes isolated from human peripheral blood with the Ficoll-Isopaque centrifugation (Böyum 1968; Hovi et al., 1976) were let to adhere onto plastic Petri dishes (Nunclon$^R$, A/S Nunc, Denmark) at 2–3 $\times$ $10^6$ cells/cm$^2$ in a medium consisting of a mixture of RPMI-1640 and Medium 199 (Gibco Biocult, Glasgow, Scotland) (50/50 vol/vol; PR-medium) supplemented with 5% newborn calf serum (NBCS) or foetal calf serum (FCS). After 2–3 h or, in some experiments, an overnight incubation, the nonadherent cells were washed off and the remaining adherent cells collected with a rubber policeman. The cells were seeded onto 24-well flat-bottom tissue culture plates (Linbro, Flow Laboratories Ltd, Scotland) 1.5 $\times$ $10^6$ cells in 1 ml of PR+5% NBCS or FCS per 2 cm$^2$ well. The final adherent cell cultures contained more than 95% of nonspecific esterase (Horwitz et al., 1977) positive cells. The cultures were then kept for 1–2 h at +37°C before HSV infection.

*Viruses*

HSV laboratory strains (HSV-1 strain F(1) and HSV-2 strain G from the American Type Culture Collection) were propagated in Vero cells, a continuous cell line of green monkey kidney origin, using a serum free growth medium and diluted virus inocula [multiplicity of infection (MOI) 0.1 plaque forming units (PFU) per cell]. Recent clinical isolates (15 type 1 and 15 type 2) from various specimens were similarly passaged for 2 cycles in Vero cells. Two temperature sensitive HSV-1 mutants tsLB2(HFEM) (Halliburton et al., 1977) and vhs-1(KOS) (Read and Frenkel, 1983) were likewise propagated in Vero cells at the permissive temperature of +33°C. These mutants and their wild-type parent strains were generous gifts from Dr. I. Halliburton, Leeds, U.K. and Dr. N. Frenkel, Chicago, U.S.A., respectively. Vesicular stomatitis virus (VSV; Indiana strain) was propagated in NBL cells, a continuous bovine cell line, also starting from diluted virus inocula.

*Inactivation of HSV infectivity*

Crude suspensions of stock viruses, at 0°C, were exposed, at a distance of 5 cm, to a UV light source (75 W) with a 254+366 nm filter set. Baths of +56°C or boiling water were used for the thermal inactivation experiments. Aliquots were harvested at varying exposure times and used immediately for the experiments.

*Inoculation of cell cultures*

Twenty-five or, in some experiments, 100 µl of HSV dilutions were inoculated into fresh cell cultures. After 1 h adsorption at +37°C the unadsorbed virus was removed by washing the cultures 3 times with HBSS. The growth medium used was PR+5% NBCS or FCS, 1 ml per well. After the indicated infection times the cells were scraped into the medium and the sample suspensions were kept at −70°C until virus quantification by plaque assay. For IFN assays the culture medium was first cleared from detached cells by centrifugation at 1500–2000 rpm for 5–10 min and stored at −20°C.

*Plaque assay*

Plaque assays were performed in Vero cells as described earlier (Linnavuori and Hovi, 1983). Part of the experiments were done by using human normal gamma globulin (Finnish Red Cross Blood Transfusion Centre) (instead of carboxy methyl cellulose) 16% preparation at a dilution of 1:200 or 1:500 when titrating HSV-1 samples and 1:100 with HSV-2 samples (Roizmann and Roane Jr., 1961).

*Biological interferon assay*

Interferon titres were obtained by a microtiter method based on the reduction of cytopathic effect of VSV (Rubinstein et al., 1981). The method was further simplified and adapted to NBL cells in this laboratory as reported in detail elsewhere (Linnavuori, in press). The leukocyte IFN standard (specific activity $1 \times 10^6$ IU/mg protein; kindly provided by Dr. K. Cantell, Helsinki) was used at dilutions of 20, 10, 5 and 2.5 IU/ml. Presence of infectious HSV in the samples did not affect the results. All IFN values are from duplicate parallel titrations and given as international interferon units.

*Characterization of the type of interferon synthesized*

To assay the acid sensitivity of IFN synthesized, specimens from a monocyte batch infected with HSV-1 were divided into 2 parts. The first half was dialyzed overnight at +4°C against a glycine-HCl buffer, pH2, the other half served as a control and was dialyzed against PBS. The pH value was normalized by changing the dialysis tubes into PBS for 2 successive overnight treatments. The acid-treated and control specimens were assayed for IFN as described above.

Antigenic properties of the IFN synthesized in the monocyte cultures were examined by incubating aliquots of culture media with sheep antisera (generous gifts from Dr. K. Cantell) (Mogensen et al., 1975) for 2 h at +37°C prior to the IFN assay. Two different antisera were used. One serum was relatively specific for alpha IFN (neutralization capacity 450 000 IU IFN alpha vs. 3000 IU IFN beta per

TABLE 1

Variability of interferon titers induced in human monocyte cultures by 30 different recently isolated HSV strains.

| Serotype | Multiplicity of infection | IFN (IU/ml) | |
|---|---|---|---|
| HSV-1 | 1–2 | 77±28[a] | ($n$=15) |
| HSV-1 | 0.1–0.2 | 148±29 | ($n$=14) |
| HSV-2 | 1–2 | 104±48 | ($n$=13) |
| HSV-2 | 0.1–0.2 | 65±37 | ($n$=15) |

Specimens were collected at 24 h of infection.
[a] mean ± SD.

ml) while the other neutralized both types equally well (30000 IU/ml).

*Gamma interferon RIA*

The gamma IFN assay was performed with a sandwich type RIA test according to the manufacturer's instructions using a kit prepared at the Finnish Red Cross Blood Transfusion Centre, Helsinki, and obtained through the courtesy of Dr. H.-L. Kauppinen. The standard IFN gamma preparation used in the test was a natural IFN gamma preparation calibrated to the NIH IFN gamma reference standard Gg 23-901-530. All the results are from duplicate measurements.

## Results and Discussion

*Induction of interferon by individual clinical isolates*

Monocytes prepared from 6 different buffy coats, all of blood group A, were pooled, and parallel cultures were infected with 15 type-1 and 15 type-2 HSV isolates from various clinical specimens originating from orolabial, cutaneous and genital lesions, at 2 multiplicities (1–2 and 0.1–0.2 PFU/cell). Specimens of culture medium were collected at 24 h after infection and assayed for IFN activity. The amounts of IFN produced varied only moderately (Table 1). Specimens from cultures inoculated with the higher multiplicity were collected and assayed also at 3 days after infection. The variation of interferon levels was even less than at 24 h (not shown). One could argue that putative inter-strain variability in the IFN inducing capacity of HSV may be masked by the use of pooled cells. However, we have tested several individual cultures with 4 independent HSV strains without seeing more variation in the resulting IFN yields (not shown). Slightly higher levels of IFN were obtained with the lower MOI of HSV-1 isolates while for the HSV-2 the reverse appeared to be true. Further dilutions of these type-1 isolates were not tested, but in more than 10 independent experiments the optimal yields of IFN obtained with the F(1) strain varied between multiplicities of 1 and 0.1 PFU/cell (not shown). Uninfected controls remained negative, suggesting that the observed induction of IFN was not due to a mixed lymphocyte reaction by putative lymphocyte contaminants in the pooled monocyte cultures.

*Characterization of the IFN produced by HSV exposed human monocytes*

Previous experiments have suggested that the type of IFN responsible for the restriction of HSV replication in human monocytes is alpha (Linnavuori and Hovi, 1983). By using the classical criteria we now show that the IFN produced by HSV exposed human monocytes is acid-resistant and neutralizable with an antiserum preparation specific for alpha IFN. No gamma IFN could be detected by the RIA test used (Table 2). It thus seems that the IFN activity is due to IFN alpha.

*No requirement for infectivity of the inducing virus*

Earlier work has shown that when using unfractionated human peripheral blood leukocyte cultures both heat-inactivated (Haahr et al., 1976) and UV-inactivated HSV (Green et al., 1981) are able to induce 'type I' (alpha or beta) interferon. We wanted to confirm that this also holds true for HSV induced IFN synthesis in monocytes. Therefore, an HSV-1 stock was inactivated in several different ways. Aliquots of totally or partially inactivated virus corresponding to multiplicities of 1–5 PFU/cell and similar amounts of intact control virus were used to inoculate monocyte cultures and the resulting interferon production at 24 h was assayed. Viral infectivity was destroyed (reduction by >7 logs) by heating the virus at +56°C for 5 min. In fact, viruses heated for as long as 1.5 h did not lose their capability to induce IFN. However, boiling viruses for 5 min destroyed their inducing capacity (not shown).

Ultraviolet irradiation for 50 sec decreased the titer of the HSV 1 stock by 2 logs. Irradiation for 10 min totally destroyed the infectivity. Virus suspensions inactivated with UV were as good inducers of IFN as the untreated control suspensions. As a matter of fact, IFN production was greater when inoculum had been

TABLE 2

Characterization of the interferon produced by herpes simplex virus type-1 exposed human monocytes.

| | IFN (IU/ml) | | |
|---|---|---|---|
| Pretreatment of the specimen or test performed | M0I:1 | M0I:0.1 | C |
| None | 640 | 80 | <5 |
| pH2 dialyse | 640 | 80 | <5 |
| Antiserum against IFNα[a] | <5 | <5 | NT |
| Antiserum against IFN α + β | <5 | <5 | NT |
| Gamma IFN RIA | <15 | <15 | <15 |
| IFN activity (5 days) | 640 | 40 | <5 |
| Gamma IFN RIA (5 days) | <15 | <15 | <15 |

Specimens were collected at 24 h after infection unless otherwise indicated.

[a] The serum was used at a dilution which had no activity against IFN-beta.

C = uninfected control culture.

NT = not tested.

TABLE 3

Induction of interferon in human monocyte cultures inoculated with HSV-1 inactivated by UV-irradiation.

| UV-irradiation time | IFN (IU/ml) | | Residual infectivity titer of inducing virus (log(PFU/ml)) |
|---|---|---|---|
| | MOI:1 | MOI:0.1 | |
| Untreated HSV | 20 | 40 | 8.7 |
| 50 sec | 40 | 160 | 6.7 |
| 80 sec | 80 | 160 | NT |
| 2 min | 80 | 160 | 5.7 |
| 3 min | 80 | 320 | NT |
| 5 min | 160 | 320 | 3.0 |
| 10 min | 320 | 160 | <1.3[a] |

[a] 1.3 = detection limit of the plaque assay.
Specimens for the IFN assay were collected at 24 h after infection.
NT = not tested.

UV-irradiated (Table 3). This may be related to the fact that supraoptimal inducer doses of infectious untreated virus were used (see Table 1).

These experiments do not, however, exclude the possibility that the mildly denatured virions are entering the cells and causing an abortive infection. Furthermore, since denaturation of the virus preparations may in theory bring about IFN inducing properties that are not present in native virus, the putative requirement for virus multiplication was also examined by preventing viral replication. The antiherpes compound foscarnet (phosphonoformic acid, Astra Pharmaceuticals, Södertälje, Sweden) was added at different concentrations ranging from 50 μM up to 1 mM into the growth medium of cultures inoculated with normal infectious virus. At a concentration of 250 μM foscarnet prevented the replication of the virus. Induction of interferon was not affected by the presence of foscarnet in the culture medium (not shown).

The requirement for replicating HSV for IFN induction was further evaluated by using 2 temperature-sensitive HSV-1 mutants and their parental strains. Replication of both mutants was completely prevented at +39°C while at +33°C the mutants replicated at rates comparable to those of the corresponding wild type parent viruses. Similar IFN levels were seen with all 4 viruses at both temperatures (data not shown). Of course, partial gene expression is allowed under these conditions as well (Halliburton et al., 1977; Read and Frenkel, 1983; Honess and Watson, 1977; Lehtinen et al., 1984). Further studies are needed for identification of the actual inducer molecules in this system.

Interferon induction in different cells and tissues may have a role in the pathogenesis of herpesvirus infections in man. Cunningham and Merigan (1983) suggest that recurrent herpes labialis acts as an in vivo stimulus for the induction of IFN gamma producing cells in peripheral blood. Both IFN-alpha (Overall et al., 1981) and IFN-beta (Torseth et al., 1987) have been demonstrated in the vesicle fluid of lesions of recurrent herpes. The latter article also reports that keratino-

cytes are the main cell type responsible for the production of IFN beta in cutaneous herpes infections. Leukocytes of different categories have been earlier reported to produce IFN when exposed to HSV (Kirchner et al., 1979; Abb et al., 1983; Linnavuori and Hovi, 1983). The capability of inbred mouse strains to respond to HSV by interferon production is an inherited property that has a role in the pathogenesis of experimental herpes disease (Brandt Pedersen et al., 1983). On the other hand, exogenous IFN can effectively induce an antiviral state in macrophages restricting HSV replication (Domke-Optitz et al., 1986; Salo and Ortega, 1986). Elsewhere one of us has shown that the individual variation in the IFN response of cultured human monocytes to a given virus strain is relatively great and the levels of IFN produced are inversely correlated with a history of recurrent mucocutaneous herpes disease and the level of CF-antibodies against HSV (Linnavuori, in press). Since the present experiments show that the inter-strain variation of the capability of HSV to induce IFN in monocytes is not great, it is likely that most of the variation seen between the experiments (see also Green et al., 1981) is cell donor-dependent.

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