Antiviral Activities of Cloned Human Leukocyte Interferons against Herpes Simplex Virus Type 2 Infections of Mice

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(Accepted 10 June 1983)

SUMMARY

Human α-interferons (IFN-αs) made in bacteria were examined for antiviral activity against herpes simplex virus type 2 (HSV-2) infections of mouse L-cells in vitro, and acute cervicovaginal and lethal systemic HSV-2 infections of BALB/c mice. The recombinant DNA-derived hybrid interferon IFN-αAD(Bgl) showed pronounced antiviral activity in vitro, exceeding the activity of either of the parental subtypes IFN-αA and IFN-αD and that of the other hybrids IFN-αAD(Pvu) and IFN-αDA(Bgl). A combination of topical and systemic treatments with IFN-αA and IFN-αAD(Bgl) failed to protect mice from subsequent challenge with an acute cervicovaginal infection of HSV-2. Protection from lethal systemic HSV-2 infection in mice was observed when IFN-αAD(Bgl) and IFN-αAD(Pvu) were administered systemically, whereas IFN-αA failed to confer protection. These results suggest that for protection against infection with HSV-2, the routes of introduction of the virus and of the interferon influence the host response to interferon therapy.

In recent years the incidence of herpesvirus genitalis has increased so dramatically that it is now regarded as one of the most widely spread of venereal diseases. Furthermore, the proposed role of herpesvirus infections in the genesis of carcinoma of the uterine cervix (Fish et al., 1982) has resulted in this virus infection becoming a leading candidate for prospective therapeutic agents.

Several individual molecular species of human leukocyte interferon (IFN-α) have been isolated and expressed in bacteria using recombinant DNA techniques (Goeddel et al., 1980; Nagata et al., 1980; Yelverton et al., 1981). Overall, the known human IFN-αs show 52% homology at the amino acid level and each subtype differs from others in 4 to 29 amino acid positions (Goeddel et al., 1981). Because there is 84% homology at the gene level, with common restriction endonuclease sites, it has been possible to construct genetic hybrid interferons containing portions of different parental molecules (Weck et al., 1981b; Streuli et al., 1981). Such hybrid interferons have been shown to have unique antiviral and antiproliferative activities distinct from those of the parental subtypes (Weck et al., 1981a, b; Streuli et al., 1981; Lee et al., 1982a, b).

We have demonstrated that human cells pretreated with various human leukocyte interferon subtypes derived from bacteria are protected against subsequent challenge with herpes simplex virus type 2 (HSV-2) (Fish et al., 1983). The relative degrees of protection conferred varied with the type and dose of interferon used. The subtypes IFN-αB, IFN-αC and IFN-αF confer least protection against HSV-2 and IFN-αA, IFN-αD and the hybrid IFN-αAD(Bgl) have the greatest effect. The present studies were designed to determine whether in vitro antiviral activity correlated with efficacy in vivo. Although natural human leukocyte interferon preparations do not show notable activity in mouse cells, the hybrid recombinant interferon IFN-αAD(Bgl) is active both in vitro and in vivo (Weck et al., 1982).
Table 1. Antiviral activities of interferon subtypes and hybrids on mouse L-cells challenged with HSV-2*

<table>
<thead>
<tr>
<th>Interferon type</th>
<th>Dose (IU/ml) that reduces virus cytopathic effect by 50%†</th>
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<tbody>
<tr>
<td>Buffy coat</td>
<td>25</td>
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<tr>
<td>IFN-αA</td>
<td>12</td>
</tr>
<tr>
<td>IFN-αD</td>
<td>12</td>
</tr>
<tr>
<td>IFN-αAD(Bgl)</td>
<td>1</td>
</tr>
<tr>
<td>IFN-αAD(Pvu)</td>
<td>3</td>
</tr>
<tr>
<td>IFN-αDA(Bgl)</td>
<td>25</td>
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</tbody>
</table>

* Mouse L-cells were pretreated for 24 h with dilutions of the different IFNs, then challenged with HSV-2. Virus cytopathic effect was spectrophotometrically determined. The mean values of replicate assays are presented above.

† Doses > 10 IU/ml indicate poor activity.

In order to test their antiviral efficacy in mice, individual cloned interferons were selected on the basis of their antiviral activity in vitro on mouse L-cells challenged with HSV-2. Mouse L-cells were seeded in individual wells of 96-well Microtest II tissue culture plates and pretreated with twofold serial dilutions of IFN-αA, IFN-αD, IFN-αAD(Bgl), IFN-αAD(Pvu), IFN-αDA(Bgl) or buffy coat interferon for 24 h. The various interferons derived from bacteria have been specified previously (Weck et al., 1981 a, b) and were kindly provided by Genentech Inc., San Francisco, Ca., U.S.A. The buffy coat interferon was obtained from Dr N. Hill, Wadley Institute for Molecular Medicine, Dallas, Tx., U.S.A. The interferons from bacteria, IFN-αA, IFN-αD, etc., were previously designated LeIF-A, LeIF-D, etc. (Goeddel et al., 1981). The preparations of IFN-αA, IFN-αD, IFN-αAD(Bgl), IFN-αAD(Pvu) and IFN-αDA(Bgl) used were electrophoretically homogeneous and greater than 95% pure. Their specific activities were between 6 x 10^8 and 2.2 x 10^8 IU/mg protein as assayed on bovine kidney (MDBK) cells as described elsewhere (Weck et al., 1981 b). The buffy coat interferon had a specific activity of 1 x 10^6 IU/mg protein. Interferon titres were determined on human amnion (WISH) or MDBK cells challenged with vesicular stomatitis virus (VSV) using a 50% cytopathic endpoint and converted to international units (IU) using an NIH standard (G023-901-527) included in each assay. At the time of virus inoculation, the IFNs were removed and 100 μl of HSV-2 (10^1.6 TCID₅₀/ml; determined on Vero monkey kidney cells) was added to individual wells. Twenty-four h post-infection, the extent of HSV-2 infection was determined by spectrophotometric estimation of virus cytopathic effect (Fish et al., 1983). Briefly, ethanol-fixed cells were stained with crystal violet (0.1% in 2% ethanol) and the inhibition of virus infection was estimated by comparing the absorbances at 570 nm of IFN-treated cells with standards, using a Microplate Reader MR600. For fixed and stained cells, absorbance at 570 nm is directly proportional to the number of cells present. The order of antiviral efficacy in this assay system was as follows: IFN-αAD(Bgl) > IFN-αAD(Pvu) > IFN-αA, IFN-αD > IFN-αDA(Bgl), buffy coat interferon. The respective inhibitory doses in IU/ml that reduce the virus cytopathic effect by 50% are indicated in Table 1. Clearly, IFN-αAD(Bgl) has pronounced antiviral activity, exceeding the activities of either of the parental subtypes and the other hybrids IFN-αAD(Pvu) and IFN-αDA(Bgl). These results support earlier data on the efficacy of IFN-αAD(Bgl) in mouse cells (Weck et al., 1982). In addition, studies from our laboratory have shown that at concentrations up to 5 x 10^2 IU/ml, IFN-αA and IFN-αD have no effect on the proliferation of mouse L-cells, whereas at 5 x 10^2 IU/ml, IFN-αAD(Bgl) inhibits the growth of L-cells by 65% (E. N. Fish, unpublished data). The implications are that whereas IFN-αAD(Bgl) and IFN-αAD(Pvu) cross-react well, IFN-αA and IFN-αD show low activity on mouse cells.

The in vitro studies with HSV-2 have been extended to acute cervicovaginal and lethal systemic infections of BALB/c mice. Cervicovaginal infections were achieved by placing cotton pledgets saturated with a specified dose of virus against the cervix of the animals on 2 consecutive days, allowing 24 h exposure on each occasion (Tobin et al., 1978). Infection at the cervix is detected within 4 to 7 days after virus inoculation by HSV-2-specific immuno-
Fig. 1. Protective effects of IFN-αA and buffy coat IFN against cervicovaginal HSV-2 infection of BALB/c mice. Treatments were cervicovaginal and/or i.p. as indicated in the text. (a) Buffer-treated controls, 10 mice (○); buffy coat IFN, 20 mice given seven treatments at 10⁴ IU/animal between −4 and +96 h (●); IFN-αA, 20 mice given seven treatments at 10⁴ IU/animal between −4 and +96 h (▲). (b) IFN-αA, 20 mice given seven treatments at 10⁵ IU/animal between −4 and +96 h (▲); IFN-αA, 20 mice given six treatments at 10⁵ IU/animal between +20 and +96 h (∆).

fluorescent staining of cervicovaginal smears (Tobin et al., 1978). By day 14 post-inoculation, the acute local phase of the infection has passed and the virus resides in a latent state in the local pelvic sensory ganglia. Spontaneous recurrent exacerbations occur periodically at the cervix (Tobin et al., 1978). Accompanying a severe cervicovaginal infection, a systemic infection may develop which results in dissemination of virus via neurological and haematogenous routes, the virus ascending ultimately to the brain and leading to paralysis and an encephalitic-like death (Fish et al., 1979).

The interferons were applied topically at the cervix or intraperitoneally (i.p.) on six or seven occasions between −4 and +96 h relative to infection and at total doses from 6 × 10⁴ to 7 × 10⁵ IU of interferon per animal per overall treatment. For the cervicovaginal infections, treatments were topical unless a virus pledget was in place, in which case interferons were administered i.p. for 2 days following infection. The survival time of mice was obtained from records prepared twice daily for at least 25 days following infection. For convenience of presentation, deaths occurring in any one day are shown at one time only in mortality curves (Fig. 1 and 2). Significant differences in the dependence of survival on group was investigated by a Chi-squared test. Time effects were accounted for by plotting changes in deviances over time, illustrating the amount of variation in the data accounted for by the group factor. Specific group comparisons were made by dealing with only two groups at a time. Similar statistical methods were used to account for dependence of infection on the group factor. With a cervicovaginal virus inoculum of 50 μl of HSV-2 (at 10³·5 TCID₅₀/ml), at least 90% positive infections occurred with only 30% of the mice surviving at 30 days post-infection (Fig. 1). Both buffy coat interferon at 10⁴ IU/treatment (serum treatments between −4 and +96 h) and IFN-αA subtype at 10⁵ IU/treatment (seven treatments between −4 and +96 h) delayed slightly the onset of acute infection with HSV-2, but did not ameliorate the outcome of infection to any significant extent (P > 0·05) compared with that for control mice (Fig. 1). The incidence of survival was enhanced
by these interferon treatments from 30% (control group) to 50%. IFN-αA at $10^4$ IU/treatment (seven treatments between −4 and +96 h), and at $10^5$ IU/treatment (six treatments between +20 and +96 h) had no effect on the course or outcome of HSV-2 infection. With a lower dose of virus (50 μl of HSV-2 containing $10^3$ TCID$_{50}$/ml) which caused no deaths, IFN-αAD(Bgl) at $10^5$ IU/treatment (seven treatments between −4 and +96 h) reduced the percentage of animals infected from 90% (controls) to 80%, and slightly delayed the onset of infection (data not shown).

Greater efficacy was observed against lethal systemic infection of mice with HSV-2. For this series of experiments, mice received a virus inoculum i.p. of 300 μl of HSV-2 containing $10^4$ TCID$_{50}$/ml. We compared the efficacy of IFN-αA, IFN-αAD(Bgl) and IFN-αAD(Pvu) at $10^5$ IU/treatment administered i.p. on seven occasions between −4 and +96 h relative to infection. The results in Fig. 2 show that IFN-αAD(Bgl) is highly protective ($P < 0.001$) and IFN-αAD(Pvu) also shows some protection ($P < 0.002$). Protection is expressed as survival following a lethal challenge with virus. IFN-αA does not protect the mice from a lethal HSV-2 infection. These results indicate a correlation between in vivo potency and cell culture assay for these interferons against this systemic infection of mice with HSV-2. The poor antiviral activity in vitro of IFN-αDA(Bgl) suggests, as for earlier data (Streuli et al., 1981; Fish et al., 1983; Hannigan et al., 1983), that neither the N- or C-terminal portion of IFN-α alone determines cell receptor binding.

Studies with the IFNs derived from bacteria described here against encephalomyocarditis (EMC) virus infection of BALB/c and BDF mice also have shown a correlation between cell culture and in vivo antiviral effects (Weck et al., 1982). In the EMC virus studies, IFN-αAD(Bgl) was also the most effective of the interferons examined. Studies of HSV-1 infection of the rabbit eye have shown that both IFN-αA and IFN-αD are effective despite very low activity in rabbit cell cultures (Smolin et al., 1982; Grabner et al., 1983). In the rabbit eye model, topical treatment was effective whereas systemic treatment was ineffective (Smolin et al., 1982). Thus, the mechanism of action is effective locally. However, there is a difference between the HSV-1 studies in the rabbit eye and the present systemic HSV-2 studies in mice: treatments as late as 2 days after infection were as effective as treatments commencing before infection of the rabbit eye. Whether direct or indirect mechanisms are involved, the present results are promising with regard to treatment of HSV-2 infections. It remains possible that infection of the cervicovaginal epithelium does not respond to interferon treatment. Such differences in tissue sensitivities to interferon are apparent in the case of other virus infections in mice (Dandoy et al., 1982). Experiments with nude and immunocompetent mice have revealed that the route of inoculation with virus also has an effect on the outcome and host susceptibility to infection (Kapoor et al., 1982). The inference is that different mechanisms of protection, either cell-mediated or humoral, are preferentially induced according to the site of virus inoculation and that the potential of
exogenously administered IFN-α to restrict virus replication may vary accordingly. In the case of HSV-1, the importance of interferons as antiviral agents against lethal infections is indicated by observations showing that resistance to disease is associated with interferon induction by the virus infection (Zawatzky et al., 1982). Extrapolation to the present studies with HSV-2 indicates that the effect of exogenously administered IFN-α may vary according to the site of virus inoculation. More extensive studies involving cervicovaginal infections with HSV-2 are required to clarify this further.

The work described here was supported in part by a National Cancer Institute grant made available to E. Fish. We thank Drs B. R. G. Williams and S. Read for their support and helpful discussions.

REFERENCES


(Received 8 March 1983)