Effect of Human Leukocyte Interferon on Vaccinia- and Herpes Virus-Infected Cell Cultures and Monkey Corneas

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Pretreatment of human fibroblast cultures with human leukocyte interferon (HIF, 1,000 IU/ml) resulted in a 24-h delay of virus replication after infection with vaccinia virus and herpes simplex virus type 1 and type 2. Additional HIF treatment 24 h after infection effectively lowered the maximum yield of viral infectivity. Equal results were obtained in simian cells with 3,000 IU of HIF per ml. The spread of two cell-bound herpesviruses, varicella zoster virus and Medical Lake macaque herpesvirus, was inhibited by 2,000 IU of HIF per ml in human fibroblasts and Vero cells, respectively. Varicella zoster virus infectivity was notably reduced by HIF, whereas the latter system showed a low sensitivity. To study the effect of HIF in the infected cornea, keratitis was induced experimentally in both eyes of 12 rhesus monkeys and 12 African green monkeys by inoculation with vaccinia virus and herpes simplex virus, respectively. In each monkey one eye served as a control for the full cycle of disease. In the other eye HIF treatment was initiated prophylactically 15 h before or simultaneusly with the challenge virus infection or 6 to 20 h postinfectionally or therapeutically after onset of the disease, and the treatment was continued for 2 to 7 days. Prophylactic and simultaneous administration equally resulted in inhibition of both vaccinia and herpes keratitis. Postinfectional and therapeutic administration of interferon moderated the course of keratitis slightly and shortened the period of virus shedding.

One of the main implications of the discovery of interferon in 1957 was its possible use for medical treatment of viral infections. However, several difficulties impeded the evaluation and establishment of interferon prophylaxis and therapy in man for many years, particularly the lack of interferon derived from human cells. In the past few years great efforts have been made to produce larger amounts of human interferon. Several groups of investigators were able to induce human interferon in considerable concentration in human fibroblast cultures (2, 13, 23) and leukocyte cultures (9, 20, 24) either by synthetic or by viral inducers. Using appropriate concentration and purification procedures, for example, ultrafiltration and Sephadex gel chromatography (20), it is possible to prepare human leukocyte interferon (HIF), the activity of which might be sufficient to produce demonstrable antiviral effects in man.

However, the activity of human interferon is usually determined in standard systems in cell culture, and the effectiveness of interferon in many important viral infections in man has not yet been studied. In principle there is no doubt about the broad antiviral potential of interferons, but certain differences in the sensitivity of different viruses have been reported (7, 10, 20, 31). Therefore standard interferon tests with vesicular stomatitis virus or Semliki Forest virus as challenge viruses may finally not be of much predictive value for purposes of clinical application. We thus decided to first challenge the protective effect of HIF prepared under standardized conditions (20) in cell cultures, with those viruses which were to be used in in vivo experiments with monkeys and later in clinical trials: vaccinia virus, herpes simplex virus type 1 and type 2 (HSV 1 and HSV 2), varicella zoster virus (VZV), and Medical Lake macaque herpesvirus (MLM virus). The latter is a simian herpesvirus that resembles VZV in its behavior in culture and causes a disease in monkeys which is similar to that caused by VZV in humans (3). This virus was used because no VZV model in animals exists.

An attempt to evaluate HIF treatment by means of experiments with monkeys involves considerable problems in the choice of an adequate viral infection model. It is of importance.
to induce an experimental disease in which the action of HIF can be shown by clear parameters and which corresponds sufficiently to human disease to permit some conclusions on the usefulness of interferon treatment of man. Studies on local interferon treatment are favored by the experimental conditions of virus infections of the eye, especially when keratitis results from the infection. The surveillance and documentation of subtle morphological criteria of the disease as well as virus isolation are easily performable in the case of infections of the monkey cornea. As far as herpes keratitis is concerned there are direct clinical implications, because this disease is a significant problem in human ophthalmology (30).

In former studies (18, 26) this model was already used for the evaluation of the prophylactic effect of human interferon, and in the early period of interferon research experimental treatment of viral keratitis was performed even in man (15, 28). However the low quality of the interferon used at that time (in one study monkey interferon was administered to men) greatly reduces the value of these experiments.

In this report a series of experiments is presented in which interferon treatment of monkey eyes infected either with vaccinia virus or HSV was started at different times before and after infection.

**MATERIALS AND METHODS**

**Virus-cell systems for experiments in cell cultures.** Vaccinia virus (Lister strain), HSV 1 (strain Mclntyre), HSV 2 (strain MS), and VZV (strain 2591, obtained from F. Behrens, Behringwerke AG, Marburg/Lahn), were passaged in fibroblasts of a diploid cell line derived from human neonatal foreskin (medium: Eagle minimal essential medium with 2% or 5% fetal calf serum). Stocks of vaccinia virus and HSV were prepared by freezing and thawing of infected cultures, sonic treatment, and removal of cell debris by centrifugation at 5,000 x g for 20 min. The stocks were divided into small portions and stored at -70°C.

VZV was passaged by transfer of infected to uninfected cells as described earlier (29). When a maximum of infection was achieved, the cells were trypsinized, pelleted by low-speed centrifugation, and suspended in medium containing 10% fetal calf serum and 10% glycerol. A stock of VZV-infected cells thus obtained was divided into 1-ml portions, slowly frozen in a cryostat, and stored in liquid nitrogen.

MLM virus, obtained from K. McCarthy, Manchester, England, was passaged in Vero cells. The cultivation and stock preparation was performed as described for VZV.

All experiments on the antiviral effect of HIF in cell cultures were performed in these virus-cell systems except for some additional experiments with HSV 1 in Vero cells and in a simian keratocyte line derived from corneal tissue of an adult Cercopithecus aethiops.

**Infectivity titration.** Samples were tested for infectivity of vaccinia virus, HSV 1, and HSV 2 by the end point titration method in microtiter tissue culture trays by using human foreskin fibroblasts. The amount of infectivity in VZV- and MLM virus-infected cultures was estimated by a count of cytopathic effect (CPE) foci formed by passage of the trypsinized and serially diluted cell suspension onto noninfected freshly seeded cells. It is expressed as CPE focus-forming units (FFU).

**Interferon.** For all experiments HIF was used, which was supplied by Behringwerke AG, Marburg. Its standardized preparation has been described in detail elsewhere (20). In short, primary human leukocytes in suspension cultures were infected with Newcastle disease virus for induction of interferon. Twenty hours after infection the supernatant was centrifuged, inactivated at pH 2, concentrated by ultrafiltration, purified by chromatography on Sephaldex gels, and lyophilized. Redissolved in distilled water, the preparations contained 62,500, 100,000, and 150,000 IU of interferon per ml, respectively.

In all experiments with VZV and MLM virus and in most of the monkey experiments, the controls were treated with mock HIF, prepared in the same way as HIF but lacking the viral inducer.

**Standard assay for HIF.** The activity of HIF was controlled according to the micromethod described by Dahl and Degré (4), by using human foreskin fibroblasts and vesicular stomatitis virus as the challenge virus. In each assay HIF standardized by Behringwerke AG, Marburg, was included, which was adapted to the British HIF research standard A 69/19 (27) by J. Hilfenhaus, Behringwerke, Marburg.

**Kinetic experiments with vaccinia virus, HSV 1 and HSV 2.** The experiments were carried out in rubber-stoppered square culture bottles. The monolayers were washed with phosphate-buffered saline prior to infection, overlayed with 5 ml of the diluted stock virus, and incubated for 90 min at 37°C for adsorption of virus. Thereafter the cultures were washed five times with phosphate-buffered saline and again incubated at 37°C with 20 ml of maintenance medium (Eagle minimal essential medium with 2% fetal calf serum). Samples of 0.3 ml were drawn periodically and immediately frozen.

When HIF was added to the cultures during the experiment, the medium was not removed subsequently. However, the interferon activity remaining in the medium did not affect the determination of infectivity 33 h after infection and later, as was confirmed by back-titration of a known dose of the challenge virus. If necessary the pH of the medium was corrected by addition of HCl or NaHCO3. At the end of the experiments the whole cultures were frozen. Finally all samples were simultaneously titrated for infectivity.

**Experimental keratitis.** Vaccinia keratitis was induced in rhesus monkeys (Macaca mulatta) and herpes keratitis in African green monkeys (C. aethiops). The challenge virus suspensions were prepared from infected homologous monkey kidney cell cultures as described above for human fibroblast cultures.

After anesthesia by proxymetacain and linear scarification of the corneal epithelium 104 mean tissue
culture infective doses of vaccinia virus (strain WR) and 10^8 mean tissue culture infective doses of HSV 1 (strain Maclntyre), respectively, were inoculated in both eyes of each monkey. The eyes were examined daily by slitlamp control. Corneal lesions were stained with fluorescein and documented by color photographs. For protection from bacterial superinfection, antibiotics (drops containing 50 mg of ampicillin, 50 mg of oxacillin, 2 mg of gentamicin per ml) were applied after each examination.

Virus isolation from infected eyes. Before each ophthalmological examination, samples for virus isolation were taken in the following manner, which avoided causing artificial lesions of the cornea: 0.05 ml of tissue culture maintenance medium (Eagle minimal essential medium with 0.5% albumin) was dropped in each eye, distributed by gently rubbing the eyelid and recovered from the inner angle of the eye by aspiration with a micropipette. Each sample was inoculated into three tubes with human foreskin fibroblasts, which were observed for CPE. After 1 week, blind passages of negative cultures were performed.

Antibody determination. Serum antibodies against vaccinia virus and HSV 1 were estimated in microneutralization tests according to Melnick and Wenner (21) by using human foreskin fibroblasts.

Interferon treatment of eyes. The right eye of each monkey was treated with HIF. The left eye served as control for the development and normal course of the corneal disease. In most of the experiments the left eye was treated analogously to the right one with mock HIF. Some of the earlier experiments were performed without any treatment of the control eyes. HIF was applied in drops containing 10^5 to 1.5 x 10^6 IU per ml. With each administration 1.5 x 10^4 to 2 x 10^4 IU of HIF were dropped into the conjunctival sac. The duration of this treatment was extended to a mean time of 15 min.

For all manipulations mentioned above the monkeys were held in a state of neuroleptics. This was achieved by intramuscular injection of 230 mg of ketamin-hydrochloride per kg, purchased from Parke-Davis Co., Munich, Germany.

RESULTS

Effects of HIF on the multiplication of vaccinia virus and HSV. Human fibroblast cultures infected with a very low multiplicity of vaccinia virus (1/100) show a logarithmic increase of infectivity in the culture medium over a period of 6 days (Fig. 1). When the fibroblasts were pretreated with 1,000 IU of HIF per ml for 15 h, no infectious virus could be detected for 24 h; then a logarithmic increase of infectivity comparable to that in the control occurred. One hundred and forty-four hours after infection the infectivity nearly reached that of the controls. When HIF was added to the culture for another time 24 h after infection, a marked restriction of viral infectivity appearing in the culture resulted (Fig. 1). The CPE in the monolayers was analogous; in the cultures treated twice, before and 24 h after infection, no characteristic CPE was observed, whereas in the controls the CPE appeared 24 to 33 h after infection, and in the cultures only pretreated with HIF it appeared finally 144 h after infection. When the experiment was performed with a multiplicity of infection of 1, the effect of interferon treatment was similar, though the final reduction of virus yield after double treatment was not as pronounced as with low multiplicity.

In the experiments with HSV 1 the kinetics of viral multiplication in the controls show that the increase of infectivity is faster than in the case of vaccinia virus. After the maximum is
reached, a decrease resulting from thermal inactivation is observed. The maximum coincides with the full expression of CPE followed by a total destruction of the monolayer. Interferon treatment had principally the same effect as with vaccinia virus (Fig. 1). The CPE was also found to be corresponding to that in the vaccinia experiment. In the cultures treated twice with HIF only a few groups of CPE cells could be observed, which did not enlarge over the period of 6 days.

When the same experiment was carried out on monkey cells (Vero cell line and Cercopithecus keratocytes), 3,000 IU of HIF per ml was required to produce an equal effect. In cultures infected with HSV 2 the same results were obtained as shown for HSV 1 in Fig. 1.

**Effect of HIF on cell-bound herpesviruses (VZV and MLM virus).** As kinetic experiments like those described above are not feasible with cell-bound viruses, the effect of HIF treatment was shown in subsequent cycles of propagation of infected cells. Cultures of human fibroblasts were infected with VZV and cultures of Vero cells with MLM virus by transfer of infected cells. In the experiments shown in Tables 1 and 2, the cells of the cultures originally infected were passaged three and four times, respectively, in this way with intervals of 48 h. In line A, HIF (2,000 IU/ml) was added at the time of infection in all but the last passage. In line B, an additional dose of HIF was added 15 h before passages 2 and 3 (and 4 with MLM virus) to the infected cultures and to the uninfected cultures used as cell donors for these passages and for passage 1, too. In line C the same treatment as in B was carried out with mock HIF. The last passage was performed in A, B, and C without any HIF or mock HIF treatment in order to obtain an indicator for remaining VZV and MLM virus infectivity.

In general neither HIF nor mock HIF had any negative effect on cell growth in the concentration employed in these experiments. The few HIF-treated cultures showing differences of more than 15% of cell count compared with the mock HIF-treated controls were excluded from the experiment.

The appearance of CPE foci was completely suppressed by HIF during passages 1 to 3 both in lines A and B of the experiment with VZV (Table 1). However, the fourth passage, without HIF, revealed that VZV infectivity had persisted and multiplied in spite of HIF treatment during the first three passages. Nevertheless the rate of multiplication was much smaller than in the mock HIF-treated control: 5% with simple treatment (A) and 1.2% with additional pretreatment of the cells before passage (B).

The results of the analogous experiment with

### Table 1. Multiplication of VZV in human fibroblasts

<table>
<thead>
<tr>
<th>Line</th>
<th>FFU/inoculum</th>
<th>Foci/culture</th>
<th>Multiplication factor</th>
<th>% of inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2 × 10³</td>
<td>0</td>
<td>10</td>
<td>5 × 10³</td>
</tr>
<tr>
<td>B</td>
<td>2 × 10³</td>
<td>0</td>
<td>10</td>
<td>1.2 × 10³</td>
</tr>
<tr>
<td>C</td>
<td>1.3 × 10⁴</td>
<td>1.3 × 10⁴</td>
<td>2.2 × 10⁹</td>
<td>100 × 10³</td>
</tr>
</tbody>
</table>

* Line A was treated with HIF (2 × 10³ IU/ml) at the time of inoculation; B was treated with HIF (2 × 10³ IU/ml) 15 h before and at the time of inoculation; and C was treated with mock HIF as in line B.

### Table 2. Multiplication of MLM herpesvirus in Vero cells

<table>
<thead>
<tr>
<th>Line</th>
<th>FFU/inoculum</th>
<th>Foci/culture</th>
<th>Multiplication factor</th>
<th>% of inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2 × 10³</td>
<td>2 × 10³</td>
<td>1 × 10²</td>
<td>10 × 10³</td>
</tr>
<tr>
<td>B</td>
<td>2 × 10³</td>
<td>2 × 10³</td>
<td>1 × 10²</td>
<td>3 × 10³</td>
</tr>
<tr>
<td>C</td>
<td>2 × 10³</td>
<td>4.5 × 10¹</td>
<td>2.8 × 10³</td>
<td>23 × 10³</td>
</tr>
</tbody>
</table>

* See footnote a to Table 1.

### Footnote

- Percentage of cells transferred in the passage = passage ratio × 100.
- Passage without HIF and mock HIF treatment.
- Foci in passage 5/[FFU/inoculum x cumulated passage ratios].
MLM virus in Vero cells (Table 2) suggest that this system is less sensitive toward HIF. The remaining infectivity of 43% in experiment A shows that a single HIF treatment at the time of infection is not effective in inhibiting the spread of MLM virus in the cultures, whereas the CPE is slightly suppressed. In line B a residual infectivity of 13% indicates a moderate effect of HIF.

Effect of interferon treatment on vaccinia keratitis. Corneal infection with vaccinia virus in rhesus monkeys resulted in keratitis, the first morphological symptoms of which occurred after an incubation period of 2 to 3 days. Once established the disease took a progressive course in most of the animals; within a few days much of the corneal epithelium was destroyed, the stroma showed an increasing reaction of turbidity, the conjunctiva was severely inflamed, and in some monkeys vaccinia pocks of the eyelids occurred. During this normal course of disease, which took 10 to 12 days and was the same in untreated and mock HIF-treated eyes, vaccinia virus was isolated from the eyes. Thereafter the maximum of morphological alterations was followed by a relatively quick restitution of the normal morphology, except that a corneal scar remained in all cases.

In some animals the inoculation with vaccinia virus resulted neither in reproductive infection nor in disease. In these cases as in individual variations of the severity of disease there was no detectable correlation with humoral immunity to vaccinia virus, as determined by serum neutralization tests. (Analogous observations were made with HSV in African green monkeys.) Since in previous experiments no remarkable differences in the disease had been seen between bilaterally infected eyes and no transmission of the disease from one eye to the other had been observed, the untreated or mock HIF-treated left eye was accepted as a control for the HIF-treated right eye (Table 3). The animals in which no keratitis occurred in the control eye were excluded from the experiment.

The clear-cut effect of prophylactic HIF treatment started 15 h before infection (Table 3) had already been found in previous experiments and was reproduced under the standardized conditions of the reported experiments. No signs of disease were observed, nor was virus recovered from the HIF-treated eyes. Simultaneous HIF treatment, i.e., treatment initiated shortly after infection, was equally effective in inhibiting vaccinia keratitis. One of six eyes treated showed suspicious corneal lesions for some days, but no virus could be isolated.

Some problems arose in the group treated therapeutically, i.e., only after onset of the disease, because the morphological alterations expand quickly as soon as the disease becomes manifest. However, the effects of the disease were less severe in the interferon-treated eyes than in the controls (Table 3). The treatment of the single animal of the group was not truly therapeutic, because in retrospect it was concluded that the corneal lesions observed 20 days after infection proved not to be true manifestations of the disease yet. Though interferon was administered only once daily, no scar developed in the treated eye.

Effect of interferon treatment on herpes simplex keratitis. Rhesus monkeys were found to be much more resistant to corneal infection with HSV than with vaccinia virus. Though the African green monkeys finally chosen for our studies were found to be more susceptible to HSV infection, it was decided to perform preliminary experiments in rhesus monkeys. The results of these experiments show that vaccinia virus and herpes simplex virus are not isomorphic, since vaccinia virus keratitis produced by intracorneal inoculation in rhesus monkeys after a period of incubation as long as 5 days in sera from pseudocyes of infected monkeys was not suppressed by HIF treatment (Table 4).

Table 3. Effect of locally applied HIF on the vaccinia keratitis in rhesus monkeys

<table>
<thead>
<tr>
<th>Type of HIF treatment</th>
<th>No. of animals</th>
<th>Scheme of HIF treatment</th>
<th>No. of treated eyes</th>
<th>Vaccinia virus isolated</th>
<th>Keratitis morphologically evident</th>
<th>Residual defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylactic</td>
<td>2</td>
<td>-15 to 44 h, 2 times daily</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Simultaneous</td>
<td>6</td>
<td>1 h to 48 h, 2 times daily</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 h to 48 h, 1 time daily</td>
<td>2</td>
<td>-</td>
<td>/?</td>
<td>-</td>
</tr>
<tr>
<td>Therapeutic</td>
<td>4</td>
<td>20 to 192 h, 1 time daily</td>
<td>1</td>
<td>+</td>
<td>+c</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52 to 192 h, 3 times daily</td>
<td>3</td>
<td>+</td>
<td>+c</td>
<td>Scar</td>
</tr>
<tr>
<td>Controls</td>
<td>12</td>
<td>Corresponding mock-HIF treatment of contralateral eyes</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>Scar</td>
</tr>
</tbody>
</table>

* Hours before (negative sign) and after infection.
* In one of two animals there was suspicion of but no evidence for keratitis.
* Smaller degree of morphological alterations compared with the controls.
herpes experiments were more susceptible than the rhesus monkeys, some of these animals also resisted infection. The problem of controls was solved in the same way as in the vaccinia study. However, the normal course of the disease in untreated and in mock HIF-treated eyes was essentially less severe and extended than in the case of vaccinia infection.

After 2 days of incubation the keratitis began with prevailing epithelial lesions, which reached a maximum within 1 or 2 days and healed spontaneously within another 2 days without leaving a scar. The period of virus excretion was found to be correspondingly short. The inhibition of the disease by prophylactic or simultaneous administration of HIF was as marked as in the vaccinia experiment (Table 4). Only in one of two monkeys treated for a very short period (35 h) did a breakthrough of keratitis and virus shedding occur. Two additional animals of the simultaneous group are not included in the table because no certain evidence for keratitis was observed in the control eyes although virus shedding occurred. The treated eyes were free of signs of infection.

Therapeutic experiments in a strict sense are not feasible because of the short course of the disease which does not allow the observation of a “curing” effect of HIF. The postinfectious treatment was relatively effective when started early and intensively (Table 4). This effect was expressed by a shortening of the period of virus shedding (2 days’ difference) and a slight difference in the morphological course of the disease.

Side effects of interferon treatment in the eye. In all monkeys HIF treatment more than once a day resulted in delicate toxic effects on the corneal epithelium reversible within 1 day. In the cell culture experiments (human and simian), similar toxic effects had never been observed. Therefore the question arose whether the effects were related to certain properties of the cornea, regardless of its human or simian nature, or whether the effects were specific for monkey tissue.

In order to obtain an answer to this question, human and simian keratocyte cultures were established and incubated with HIF. In the simian cells a toxic effect resulted which was comparable with that observed in vivo and reversible after medium displacement. The human keratocytes were not affected by HIF. To complete this experiment two of the authors and two additional volunteers checked the effect of HIF on their own corneal epithelium; 3 drops of HIF administered thrice daily over a period of 5 days exerted no toxic effect, as was proved by slitlamp control.

**DISCUSSION**

The results obtained in infected cell cultures show that a human leukocyte interferon preparation available for larger standardized experiments and clinical trials inhibits vaccinia virus, HSV 1 and 2, and two cell-bound herpesviruses, VZV and MLM, in cell cultures.

In the multicycle replication systems used with all viruses, the inhibiting effect was enlarged when interferon was given repeatedly, i.e., before and during viral multiplication. In agreement with reports by other authors (8), our results do not support the conclusion drawn from earlier investigations (11, 12, 14) that HSV

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**Table 4. Effect of locally applied HIF on the herpes simplex keratitis in African green monkeys**

<table>
<thead>
<tr>
<th>Type of HIF treatment</th>
<th>No. of animals</th>
<th>Scheme of HIF treatment</th>
<th>No. of treated eyes</th>
<th>HSV isolated</th>
<th>Keratitis morphologically evident</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylactic</td>
<td>6</td>
<td>- 15 to 72 h, 1 time daily</td>
<td>4</td>
<td>-/-</td>
<td>-/ -</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 15 to 20 h, 2 times daily</td>
<td>2</td>
<td>-/+b</td>
<td>-/+</td>
</tr>
<tr>
<td>Simultaneous</td>
<td>2</td>
<td>- 1/2 to 90 h, 2 times daily</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Postinfectious</td>
<td>4</td>
<td>20 to 72 h, 1 time daily</td>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 to 96 h, 2 times daily</td>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Controls</td>
<td>12</td>
<td>Corresponding mock-HIF treatment or no treatment of contralateral eyes</td>
<td>12</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Hours before (negative sign) and after infection.
* One animal of two was positive.
* Smaller degree of morphological alterations and shorter period of virus shedding compared with the controls.

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has a low sensitivity toward interferon, especially when compared with vaccinia virus.

As the main purpose of the experiments in cell cultures was to establish a background for experiments in monkeys and trials in man, the results obtained have to be examined for information which could be useful in preparing investigations in monkeys and in man. In the case of vaccinia virus and HSV, a large number of the cells in infected monolayer cultures are protected by HIF from virus multiplication and cell death. If the conditions given in our experiments are accepted as a "model" for infections in vivo, one can conclude that even therapeutic application of HIF in cases of vaccinia virus or HSV infections could be of some value. In view of the fact that there is an additional effect of immune response in vivo, the chance of HIF action may be considered even more optimistically. On critical examination, the experiments with VZV and MLM virus present more problems than those with vaccinia virus and HSV, especially in regard to their predictive value for in vivo experiments. The relatively good effect of HIF on VZV multiplication in vitro corresponds to reports of in vitro experiments (1) and of prophylactic and therapeutic administration of HIF in varicella and zoster disease in man (5, 16).

Unfortunately no VZV model in monkeys exists. Therefore we used MLM virus as another cell-bound virus of the herpes group. MLM virus was markedly more resistant than VZV toward HIF in cell cultures, perhaps due to the species difference (human interferon/monkey cells). Despite this we hope that experiments with MLM and monkeys will contribute further information on the usefulness of HIF in vivo especially in respect to the group of herpesviruses.

The results obtained in monkeys show that local treatment with HIF is efficient in providing protection from experimental viral keratitis. Kaufman et al. (18) and Sugar et al. (26) had already reported similar results using the same challenge viruses with owl monkeys treated with a HIF preparation of comparable activity. These experiments, however, provided limited evidence compared with the data presented here, because (i) treatment of all monkeys was intensively prophylactic, starting from 32 h (two eyes 24 h) before infection, and (ii) both eyes of each animal were treated with HIF, so that there was no individual control of the disease. Particularly our experiments with simultaneously started HIF treatment show that interferon could be of some value in the treatment of human viral keratitis. In the case of an accidental inoculation with vaccinia virus treated immediately with HIF, the situation would be similar to that in our simultaneous group. Therefore one can expect that the treatment would be at least as effective as in the monkey. Widespread interest in HIF can be expected only if therapeutic administration is shown to be useful. For this purpose our models have not proved to be satisfactory: the vaccinia model because of the severe progressive course of the keratitis and the herpes model because of its quick spontaneous healing. In addition there might have been two other adverse factors in our therapeutic and postinfectional experiments which are not present in man. (i) The reversible toxic effect of HIF observed only in monkeys and in simian keratocyte cultures may have been of negative influence on the healing process and thus indirectly adverse to the specific action of HIF. An explanation of this effect is not available from our experiments. However, a specific inhibitory effect on cell regeneration such as that reported for other interferons (6, 19) seems not to be involved. (ii) There was loss of effectiveness of HIF in a monkey species, which can be estimated to be about two-thirds on the basis of the cell culture experiments.

In view of these considerations the evident prophylactic and slight therapeutic effects encourage the initiation of trials with HIF in human ophthalmology. Especially the most pressing problem, the inhibition of recurrent corneal herpes, can only be included when human trials begin, because no nonhuman primate model for herpes recurrence exists to date (26). First results of systemic administration of HIF (5, 16, 25), in which no severe side effects but in some cases a good antiviral effect was seen, and reports of effective topical HIF treatment in other sites, especially the respiratory tract, (17, 22) are additional encouraging reasons for controlled trials in man.

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LITERATURE CITED