Invited Discussion

Interferon and Ocular Viral Disease

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The eye has received considerable attention in the study and evaluation of the effect of interferon and interferon inducers on viral disease. Since this organ is easily visualized, permits easy access to culture material, is frequently involved in viral infections, and can be studied under intense magnification in the in vivo state, it is a logical organ system for such study. Furthermore, most ocular diseases can be treated topically, and the effects of therapy with interferon and/or interferon inducers may be studied without some of the problems encountered in evaluating the influence of systemic therapy on local organ disease.

In 1960, Cantell and Tommila reported on the effects of exogenous interferon on vaccinia keratitis. They noted that the external application of interferon, derived from rabbit kidney cells, to rabbit eyes for 4 days after corneal inoculation of vaccinia virus delayed and suppressed the signs of infection (1). These same investigators also noted that a similar course of treatment did not influence herpes simplex virus infection of rabbit corneas.

Jones et al. treated five cases of human epithelial vaccinia keratitis with topical monkey interferon applied every 1/2 hr during the patient’s waking hours (2). They noted that topical interferon appeared to have a specific antiviral effect on the epithelial stages of the disease, and that if stromal involvement had occurred, these changes were not altered. Rapid healing of the epithelial infection was noted, and it was clear that in no instance was there worsening of the epithelial lesions once interferon was administered.

Tommila and Penttinen attempted to determine why herpes simplex viruses had proved to be relatively insensitive to interferon applied to rabbit eyes in vivo as well as in tissue culture (3). They reasoned that the failure of exogenously applied interferon might be due to the low interferon content of the administered material, as well as to insufficient application of the preparation used. They endeavored to produce a high interferon concentration in the eye by applying the virus that had been utilized in the preparation of interferon in vitro directly to the eye. A study was undertaken, therefore, to determine the effect of ultraviolet irradiated influenza type B,
strain Lee (E 158), on experimental herpes simplex virus infection of rabbit cornea. These investigators found that this inactivated virus was capable of reducing the severity of herpes infection if therapy was initiated the day after viral inoculation. If, however, the same therapy was instituted 4 days after the rabbits had been infected, the inducing virus had no influence on the severity of the ocular infection.

Tommila continued his investigations utilizing irradiated influenza type B virus of the Lee strain as an interferon inducer by employing interferon prepared in primary human amniotic cell culture in the treatment of dendritic keratitis in patients (4). He reasoned that direct interferon application might be effective in patients against herpes simplex, while it is ineffective in rabbits, because of the less stormy course this disease took in humans. Furthermore, it would obviously be easier to assess quantitative administration in patients. Two drops of the interferon preparation were administered every other hour until healing began and then every 3 hr. Control patients were treated with iodine cauterization. It was the author's impression that recovery had started and that corneal ulcers had epithelialized in the interferon-treated group in half the time required by the control group. He further noted that interferon therapy was well tolerated and caused no demonstrable toxic changes in the eye. It was of significance in this investigation that if interferon therapy was discontinued immediately after remission of the dendritic form of keratitis, the disease promptly recurred in several cases.

Following the investigations of Ho, who demonstrated an interferon-like inhibitor in the serum of rabbits that had been inoculated with endotoxin or with killed-cells of *Escherichia coli*, Oh and Gill demonstrated that high titers of interferon-like viral inhibitor could be detected in rabbit serum and aqueous humor if the animals were given intravenous typhoid endotoxin (5, 6). A state of resistance to the toxic corneal effect of Newcastle disease virus was induced if 10 or 100 μg of typhoid endotoxin were given to rabbits intravenously. This favorable influence on corneal resistance correlated well with the titer of an interferon-like viral inhibitor in the aqueous humor. This view was further strengthened by the finding that pretreatment of eyes subjected to Newcastle disease virus by serum or aqueous humor containing the inhibitor suppressed the production of the corneal opacity normally induced by the infecting virus.

Oh and Gill suggested that the mechanism by which typhoid endotoxin induced corneal resistance to virus involved at least two steps: the intravenous injection of endotoxin induced an interferon-like viral inhibitor in blood, and endotoxin disrupted the blood aqueous barrier of the eye to permit the escape of the viral inhibitor from the blood into the anterior chamber where it could act on the corneal target cells and modify the effect of the Newcastle disease virus.
Pollikoff et al. extended these previous studies by evaluating the effect of direct corneal or vitreal injections of bacterial endotoxin on corneas scarified with herpes simplex virus (7). Though a single intravitreous dose of purified endotoxin produced ocular inflammation, if this therapy was administered 18 hr prior to corneal infection with herpes simplex virus, the development of herpetic keratitis was significantly reduced. Assays of corneas harvested 48–192 hr after infection showed a 1–1.7 log lower infectivity titer in endotoxin treated eyes than in untreated controls. Similar results were obtained when the endotoxin was injected intracorneally, but subconjunctival administration failed to induce resistance. A marginal but significant therapeutic effect was also achieved when a single dose of endotoxin was administered intravitreally 72 hr postinfection.

These data suggested to the authors that the infectious process might have been inhibited and recovery accelerated, as a result of locally induced interferon-like inhibitor protection of uninfected cells adjacent to or at the margins of foci of corneal infection. These investigators further demonstrated that interferon-like activity was detected in the iris and ciliary body at 3 and 18 hr after injection of endotoxin, whereas the presence of the inhibitor was not demonstrated at any time in aqueous humor, conjunctiva, or cornea. They also noted that intravenous injection of endotoxin prior to corneal infection with herpes simplex failed to prevent the development of herpetic keratitis, despite the induction of a significant level of circulating interferon. It was their conclusion that circulating interferon did not reach the corneal epithelium in order to produce a resistant state, as was suggested by Oh and Gill for the induction of corneal resistance to Newcastle disease virus. They further hypothesized that more local rather than systemic production of interferon was necessary to restrict the progress of herpetic keratitis.

Oh and Yoneda shed more light on this subject by evaluating the induction of ocular resistance to vaccinia virus through the use of intravenous typhoid vaccine (8). These investigators noted that the injection of live vaccinia virus into the anterior chambers of rabbits induced corneal opacities, characteristic microscopic lesions of the corneal endothelium and uveitis. These changes were accompanied by an increase in the amount of virus in both aqueous humor and ocular tissues. However, single intravenous injections of 1 ml of typhoid vaccine, 3, 6, or 10 hr prior to virus infection suppressed both the production of the ocular lesions and virus multiplication. If the inoculum of vaccinia was small, the single intravenous injection of typhoid vaccine could suppress ocular infection up to 7 days. If a large inoculum of virus was injected, the onset of infection was still delayed, though for only 1 day.

Intravenous injection of the typhoid vaccine was followed within 3 hr by the appearance of an interferon-like viral inhibitor in both blood and aqueous humor, and in some cases, the interferon-like substance persisted
in the aqueous humor for as long as 10 hr. These authors concluded that typhoid vaccine induced corneal resistance to vaccinia virus in rabbits through the systemic induction of interferon which acted locally.

After several investigators had demonstrated the potency of double-stranded RNA interferon inducers, Park and Baron carried out studies to evaluate the effect of systemic and local polyinosinic-polycytidylic acid complex (In·Cn) therapy on established herpes simplex keratitis (9). 0.1 ml of In·Cn (1000 μg/ml) administered three times daily was used in topical therapy. Treatment, which was initiated in various rabbit groups as early as 3 hr after infection and as late as 4 days after inoculation, was continued for 6 days in all groups. These investigators found that rabbits that were treated beginning at 3 hr after inoculation and up to 24 hr after inoculation developed minimal keratoconjunctivitis, and that corneal lesions cleared by the 6th day of therapy. Though rabbits in which treatment was initiated 2–3 days after inoculation all developed moderate to severe keratoconjunctivitis as well, their corneal lesions also subsided during the 4–5 days of treatment with In·Cn. No therapeutic effect was observed in rabbits in which treatment was initiated 4 days or longer after viral inoculation.

Studies utilizing In·Cn administered intravenously or directly into the anterior chamber resulted in conclusions similar to those derived from topical therapy. These investigators further observed that after intravenous administration of In·Cn, a prompt and high interferon level resulted, and that partial refractoriness to the continued production of circulating interferon occurred after the 6th daily dose of 1000 μg of In·Cn intravenously.

Subsequent studies by Baron et al. indicated that therapy of established rabbit herpetic keratoconjunctivitis was far more effective if the viral inoculum was low, and that in all instances, the conjunctival response was significantly greater than the corneal response to either topical or intravenous In·Cn.1

A preliminary study on the treatment of human herpetic keratoconjunctivitis with topical In·Cn has been reported by Guerra et al. (10). These investigators carried out a double-blind clinical trial in 33 patients suffering from clinical herpes simplex keratitis. 21 patients received topical In·Cn, 1000 μg/ml, every 10 min during the 1st hr, and one drop hourly thereafter. 12 other patients were treated with IDU in the standard clinical dose (0.1%) administered as a single, hourly drop. Therapy with either method was terminated when virus isolation became negative. 17 patients treated with In·Cn and five patients treated with IDU responded promptly.

Further investigations on rabbit herpetic keratoconjunctivitis indicated that topical or systemic administration of In·Cn was significantly more

1 Baron, S., J. Park, W. Schachter, M. Weissenbacher, and M. A. Galin. 1969. Personal communication.
Invited Discussion

Effective in the prevention of lesions than in the cure of an established infection (11). These investigators found that 1000 or 100 μg of intravenously administered In·Cn given 3 hr prior to corneal herpes virus inoculation and daily thereafter provided excellent protection from the development of herpetic keratoconjunctivitis, whereas rabbits receiving 10 or 1 μg similarly administered had limited to poor protection. The level of circulating interferon correlated well with the level of protection. The degree of protection induced by In·Cn against herpes simplex virus was greater than that reported by other investigators who utilized systemic interferon or endotoxin. Studies by Schachter et al. further indicated that topical In·Cn was as effective as IDU in the prophylaxis of herpetic keratoconjunctivitis in rabbits.2

Kaufman et al. have recently reviewed the therapeutic effect of In·Cn in experimental herpes simplex keratitis of rabbits' eyes (12). These investigators noted that topical or systemic treatment of acute corneal herpetic lesions from two virus strains with In·Cn was far less effective than with IDU. They further found that topical or systemic treatment of acute lesions with In·Cn did not decrease the death rate from encephalitis and did not significantly reduce the rate of recurrence of keratitis. The continued topical use of In·Cn provided some protection to the cornea for 6–8 wk, but subsequently, the cornea appeared more susceptible to recurrences of disease even though administration of In·Cn was continued.

Further substantiation of the observation that the ocular prophylactic effect of interferon induction exceeds its therapeutic effect was found by Pollikoff et al. during their investigation of vesicular stomatitis virus on rabbit eyes (13). These investigators injected a single dose of 38 μg of statalon into the vitreous 18 hr prior to scarification of the cornea with VSV, and they found a significant reduction in keratitis as compared to controls. The same amount of drug injected 2 hr after virus scarification, however, was ineffective. They also evaluated the efficacy of 52 μg of In·Cn in a single intravitreal dose 2 hr after VSV scarification of the cornea. It was found that this interferon inducer did not prevent, but significantly reduced, the development of keratitis. Similar findings were obtained even when the drug was injected 6 hr postscarification. Significant levels of interferon were detected in suspensions of the cornea, iris, and ciliary body 18 hr after In·Cn injection. Other routes of administration, such as intravenous or subconjunctival injection directly into the cornea, were equally effective. Intracameral injection, however, was ineffective.

Schachter et al. reconfirmed the observation that herpes simplex virus is relatively resistant to interferon by evaluating the effect of In·Cn on herpes

2 Schachter, N., J. Park, M. A. Galin, S. Baron, A. Billiau, and M. Weissenbacher. Comparison of antiviral action of interferon, interferon inducers and IDU against herpes simplex and other viruses. Submitted for publication.
simplex virus and VSV in vitro. These investigators evaluated in rabbit kidney cells four pools of exogenous interferon obtained from rabbit serum by intravenous interferon induction utilizing Sindbis virus, polyinosinic acid-polycytidylic acid, or In·Cn. All of the samples were less active against herpes simplex virus than against VSV, regardless of the method of interferon induction.

In vivo prophylaxis experiments by these investigators compared the sensitivity of rabbit cornea infected with herpes simplex virus to topical administration of pyran copolymer, In·Cn, interferon itself, and IDU. It was found that In·Cn and IDU were the only agents that provided significant protection against herpes simplex keratoconjunctivitis. Eyes treated with pyran copolymer or interferon all developed severe epithelial and stromal keratitis with varying degrees of purulent conjunctivitis. The studies of this group indicate that pyran copolymer is only effective systemically, since the production of interferon by this agent probably is limited to macrophages and the reticuloendothelial system. Their studies also confirm the observation that cells that produce interferon are more resistant to virus than cells receiving interferon from an exogenous source (14). This latter observation has clearly been demonstrated as well in vaccinia virus infection of the rabbit eye and in patients suffering from vaccinial keratitis.

Weissenbacher et al. have studied what areas of the rabbit anterior segment are capable of interferon production after In·Cn stimulation in vivo and in vitro. They demonstrated the presence of interferon in aqueous, vitreous, and serum after systemic or intraocular administration of In·Cn. Intravenous injection with \( \frac{1}{10}, 1, \) or 5 \( \mu \)g of In·Cn induced measurable serum and aqueous levels of interferon. Interferon was not detected in vitreous after intravenous administration of \( \frac{1}{10} \) \( \mu \)g In·Cn but was present with higher doses. Peak interferon levels were found in all media 2\( \frac{1}{2} \) hr after intravenous dosage regardless of the level of drug administered. Local injection of In·Cn into the eye did not result in as high a level of intraocular interferon as when the equivalent dose was given intravenously. The local injection of In·Cn, however, prolonged the duration of interferon within the eye.

The wide range of studies alluded to in this report clearly indicate that ocular prophylaxis to a variety of viruses may be achieved by systemic or topical administration of interferon inducers. Further, the therapeutic efficacy of interferon inducers is significantly less than prophylaxis achieved in a similar way. Furthermore, it is probably correct that, at least for herpes simplex virus, the conjunctival response appears to exceed the corneal response once established infection has occurred. It also appears that thera-

\[1\text{Weissenbacher, M., N. Schachter, M. A. Galin, and S. Baron. Intraocular production of interferon. Submitted for publication.}\]
Invited Discussion

pharmacologic efficacy is significantly subject to the concentration of infective inoculum. Since topical administration of certain interferon inducers, particularly double-stranded RNA, is simple to carry out and evaluate, clinical trials utilizing these agents are probably in order.

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Discussion from the Floor

Dr. James G. Gallagher (University of Vermont): Dr. Baron, I'd like to comment on relative sensitivities of different viruses to interferon, a subject to which you referred briefly. I am convinced that there are indeed definite differences in sensitivity to interferon among animal viruses. In order to reduce the multiplicity of factors which govern host defenses in the intact animal we have examined adenovirus sensitivity to interferon in vitro
systems, using both plaque-reduction and yield-inhibition techniques. We have looked at the sensitivities of approximately one-third of the human adenovirus serotypes (types 1–5, 7, 8, 11, 12, and 18). The group tested contains viruses with marked differences in biochemical and biological properties (including both tumorigenic and nontumorigenic viruses), yet each exhibits much less sensitivity to interferon than do members of most other animal virus groups. In comparison with the Indiana strain of vesicular stomatitis virus (VSV), we find that approximately 125 times as much human interferon is required to reduce adenovirus plaque formation by 50% than is required to reduce VSV plaque counts by the same amount. In addition, we have found vaccinia virus to be less sensitive than VSV to human interferon, and this observation has recently been confirmed.

We have also looked at adenovirus sensitivity to human interferon by quantitative methods, i.e. examining inhibition of adenovirus yields from various human diploid cell strains treated with various doses of interferon. Each of the representative adenoviruses tested (types 2, 7, and 12) was markedly less sensitive than was VSV. At least 100-fold more human interferon was required to reduce adenovirus yields by one-half log₁₀ unit or more than was required to effect similar reductions of yields of VSV. In examining the quantitative effect of rabbit interferon on adenovirus multiplication in cultured rabbit heart fibroblasts, we again found a definite but relatively limited sensitivity to the inhibitor.

**Dr. Baron:** From your data it would seem that adenoviruses, even at low multiplicities of infection, are indeed more resistant than, as shown in the studies I referred to (Catalano and Baron, 1969; and Easton et al., 1969, personal communications), the herpes virus group and the cytomegalovirus group which become highly sensitive to interferon with low infective doses in vivo and in vitro. I think in the adenovirus studies it would be important to control the effect of decay of antiviral resistance since it usually takes a long time for adenovirus replication. I'm sure you controlled this aspect.

**Dr. Gallagher:** The time required for adenovirus plaque formation is, indeed, considerably greater than that required for VSV plaque formation. To appreciably reduce the time differential and possible decay of the interferon-induced antiviral effect, we examined the effect of interferon on single-cycle growth of adenoviruses and VSV. All of the yield-inhibition experiments described above were performed during one-step growth with adsorbed multiplicities sufficient to insure that virtually all of the cells were infected.

**Dr. Baron:** It is true, however, that the growth cycle of adenovirus is

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1 Gallagher, J. G., and N. Khoobyarian. 1969. Adenovirus susceptibility to interferon: Sensitivity of Types 2, 7, and 12 to human interferon. *Proc. Soc. Exp. Biol. Med.* 130: 137.
2 Stewart, W. E., W. D. Scott, and S. E. Sulkin. 1969. Relative sensitivities of viruses to different species of interferon. *J. Virol.* 4: 147.
almost twice as long as that of interferon-sensitive viruses like VSV, even in the single-dose cycle.

Dr. Morton Klein (Temple University School of Medicine): We have infected mice intranasally with a minimal infectious dose of influenza type A PR8. The mice were treated with cytoxan at concentrations that completely inhibited antibody formation but not interferon production. The lungs of the mice were assayed for virus up to 15 days after infection. Virus was still present in high titer \((10^5\) infectious doses) after 15 days in mice in which antibody was inhibited by cytoxan, although it was no longer detected in the lungs of the untreated mice. The concentration of interferon was the same in the lungs of both treated and control animals. There was little evidence of lobar consolidation at these minimal infectious doses due to persistence of virus in cytoxan-treated mice. The data suggest that persistence of virus can occur in the absence of antibody, but interferon did not inhibit the prolonged persistence of virus. It is of interest that prolonged persistence of virus was in itself not sufficient to give rise to delayed lobar consolidation.

Dr. George Miroff (Union College): Dr. Levy, have you tried this against spontaneous tumor?

Dr. Levy: We have tried it against the spontaneous Bitner tumor—this is the only spontaneous tumor we tried it against.

Dr. Miroff: What was the result?

Dr. Levy: There was strong protection, depending on when you gave the compound. If you give the compound early in the course of the animal's life, before it really develops the tumor, particularly during the course of pregnancy and lactation, the animal appears not to develop the tumor later in life. If you give the compound after they have developed tumor, the tumor appears to decrease in size. We haven't really yet done much about the determination, in the latter case, of increased survival time.

Dr. Miroff: In the C-57 reticular cell glaucoma, what is the normal regression rate, the spontaneous regression rate of that tumor?

Dr. Levy: Zero, or as close to zero as you can get. Certainly well less than 1%.

Dr. Fred T. Valentine (New York University School of Medicine): Dr. Levy, in your graft versus host studies, have you looked for possible carry-over of the poly I:C with the cells into the recipient animal, and have you considered the possibility that the observed effects could be due to the influx of different cell types into the spleens of donors treated with poly I:C?

Dr. Levy: We thought of this possibility, but we haven't—the answer, strictly speaking, is no. But we put the compound in and then wait 2 days before transferring the spleens from the donor to the host. In other tests where we've incubated the compound with cells, we know that the cell has hydrolyzed the compound long before that time has come. So I think the
likelihood of there being intact poly I:poly C is extremely small. In addition to which, of course, the amount of cells that we transfer in comparison with the amount that is present all together would make an extremely small amount of material that might be transferred.

Dr. Norbert P. Rapoza (G. D. Searle & Co., Skokie, Ill.): Have you done comparable studies with endotoxin?

Dr. Levy: I haven’t; other people have. There are similarities between the action of this material and endotoxin. The amount of antitumor effect, the amount of graft vs. host effect, the amount of cellular alterations, and the timing course appear to be quite different. People who have worked with endotoxin tell me that the pyrogenic capacity and other actions of poly I:poly C bear certain resemblances to those of endotoxin, but they differ in many regards.

From the floor: Have you looked at the adenovirus model?

Dr. Miles A. Galin: No, we haven’t looked at adenovirus in rabbits as of this date. We anticipate looking particularly at adenovirus type 8 because of epidemic keratoconjunctivitis in humans.