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The effect of indometacin, prostaglandin E_2 and interferon on the multiplication of herpes simplex virus type 1 in human lymphoid cells

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Summary

The control of latency and reactivation of herpesvirus (HSV) infection is poorly understood. The activation of latent HSV is usually associated with a local or systemic rise in prostaglandins (PGs). It is possible that PGs may act indirectly by suppressing the inhibitory effect of interferon (IFN) on HSV replication. IFN has also been shown to decrease the number of herpetic recurrences and to speed up the healing of lesions. We investigated the effect of indometacin (IND: a non-steroid anti-inflammatory drug which inhibits PGE_2), PGE_2, IFN-α and various combinations thereof on HSV-1 replication in established human lymphoid cells: Raji and Raji-HSV (a persistently infected subpopulation of Raji cells which continuously produces HSV-1 particles). We found that, in contrast to exogenous PGE_2, IND suppressed HSV-1 replication in both cell lines. Attempts to overcome the inhibitory effect of IND by addition of PGE_2 were unsuccessful. IFN also inhibited HSV-1 replication when a low multiplicity of infection was used. Moreover, the inhibition of HSV-1 multiplication was more marked in cultures treated with IFN in the presence of IND. PGE_2 did not decrease or reverse the protective effect of IFN. Our results also suggest that the effects of PGE_2, IND and IFN on HSV-1 replication depend on the multiplicity of infection. Further, the present observations together with previously published data would indicate that the inhibitory effect of IND on HSV-1 replication is independent of cell type or origin, while the enhancing effect of PGE_2 on virus growth may depend on these factors.

Indometacin; Prostaglandin E_2; Interferon α; Multiplication of HSV-1

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Introduction

Inflammation is an important feature of the damaging effects of HSV infection, especially in herpetic keratitis. Treatment of tissue inflammatory reactions due to HSV has been a problem for a long time. Corticosteroid medication, although effective (Simkin, 1964), is contraindicated in viral infection, since it may not only potentiate viral replication and the susceptibility to HSV infection, but also mask the inflammatory signs and symptoms of the latter (Kaufman and Maloney, 1962; Kaufman et al., 1963; Kulkarni and Srinivasan, 1987; Weinstein, 1962). Indometacin (IND) and other non-steroidal anti-inflammatory drugs are important and widely used in the treatment of the inflammatory diseases. The basic mechanism of their action is the blockade of the metabolism of arachidonic acid to prostaglandins (PGs) by the inhibition of the enzyme cyclooxygenase (Shaw et al., 1988). Thus the drugs are most effective where the inflammation is largely due to, or is amplified by, the synthesis of PGs.

The role of PGs in inflammation due to HSV is a matter of considerable concern, especially as a possible relationship between PGs and HSV reactivation was suggested by the fact that many of the stimuli that provoke a recurrence of overt HSV infection are associated with elevated levels of PGs (Baker et al., 1982; Blyth et al., 1976; Harbour et al., 1978; Hill and Blyth, 1976; Trofatter and Daniels, 1980). Such a reactivation may follow a decline of cellular immunity in response to infections, exposure to sunlight, fever, emotional stress, or immunosuppressive therapy (Duesberg, 1987; Mims and White, 1984). Many of these factors are associated with a local or systemic increase in PG levels (Hill and Blyth, 1976).

Using a human cell line, Trofatter and Daniels (1980) reported no effect of PGE_2 on HSV growth, in contrast to Harbour's and Baker's groups who demonstrated an enhancing effect of PGE_2 on HSV replication in VERO cells (Baker et al., 1982; Harbour et al., 1978). They argued that their results differ from those obtained with nonhuman cells because of the origin of the cells used, and differences in the metabolic effects PGs have depending on cell type (Pelus and Strausser, 1977). The same authors suggested that differences may also exist in the effects that PG have on HSV growth in human cells of different tissue origins (Trofatter and Daniels, 1980). Therefore, it was of interest to determine the effect of PGE_2 on HSV-1 infection of different human lymphoid cell lines, including one with an established persistent in vitro HSV-1 infection.

Conceivably, PG could indirectly enhance HSV multiplication locally by suppressing defense mechanisms such as IFN that ordinarily curtail virus replication (Trofatter and Daniels, 1980), or even by inducing suppressor T cells (Fischer et al., 1981). Hence, if PG inhibits the antiviral effect of IFN, the development of anti-PG agents with improved anti-inflammatory reaction and potential therapeutic value, without potentiating HSV growth as is the case with steroids (Kulkarni and Srinivasan, 1987), would be of great interest. This approach involves studies of lymphocytes, lymphokines such as IFN, PGs and PG inhibitors. We decided to investigate the role of PGE_2, IFN and IND, a potent anti-PG drug, separately or in combination, on HSV-1 replication in human lymphoid cell lines.

Our data indicate that both IND and IFN inhibit, and that PGE_2 increases, HSV-1 replication. The inhibitory effect of IND was not reversed by the addition of exogenous PGE_2. Furthermore, the data also suggest that the combination of IFN and IND results in an additional inhibitory effect on HSV-1 replication.

Materials and Methods

Cell cultures

Raji cell line is a Burkitt lymphoma-derived lymphoid cell line (Pulvertaft, 1965). The chronically HSV-infected Raji cell line, referred to hereafter as Raji-HSV, was derived from an in vitro infection of Raji cells with HSV-1 DNA (Seigneurin et al., 1976). This cell line has the property of continuously producing HSV-1 particles (Bourkas and Menezes, 1979). Both cell lines were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Flow Laboratories), penicillin (100 μg/ml), gentamicin (10 μg/ml) and amphotericin-B (0.25 μg/ml). This culture medium referred to hereafter as RPMI-10 was changed twice weekly.

The VERO cells (African green monkey kidney cell line) used for HSV-1 production and titration, were propagated in Eagle's minimum essential medium (MEM) supplemented with 10% FBS and antibiotics as described above. HSV-1 infected cells were maintained in the same medium supplemented with only 2% of FBS.

All cultures were incubated at 37°C in 95% air and 5% CO_2.

HSV-1 infection of Raji cells

HSV-1 stock (McIntyre strain, Bourkas and Menezes, 1979) was prepared by rapidly freezing and thawing HSV-infected VERO cells, and titered in VERO cell monolayers by plaque assay (Menezes and Bourkas, 1980).

Unless otherwise specified, all the following experiments were carried out using a multiplicity of infection (m.o.i.) of 10 plaque forming units per cell (pfu/cell). HSV-1 was adsorbed for a period of 1 h, the cells were then washed twice and resuspended in RPMI-10. HSV-infected and control cultures were harvested at 48 h and 72 h post-infection, frozen and thawed 3 times, centrifuged for 20 min at 2000 RPM, and the supernatant was stored at −70°C until titered in triplicate on VERO cell monolayers cultivated in Petri dishes; titers were expressed as pfu/ml.

Reagents

A stock solution of IND was prepared by dissolving IND powder (Indocid, Sigma Chemical Co., lot no. 75F-0557) in ethanol (10 mg/ml), and filtered through a 0.22 μm membrane filter (Millipore Corp., Bedford, MA, U.S.A.). Further dilutions were made in culture medium to get final concentrations of 2 to 20 μg/ml.

Freeze-dried PGE_2 (Sigma Chemical Co., St Louis, U.S.A.) was dissolved in 100 μl of 95% ethanol. Subsequent dilutions were made in culture medium so as to avoid cell damage due to the ethanol solution. The final concentrations of PGE_2 used in cell cultures were 10^{-5}, 10^{-6} and 10^{-7} M, which correspond to physiologic doses of PGE_2 (Chouaib and Fradelizi, 1982).

Partially purified human leukocyte IFN containing 2.5×10^6 international units (IU) per ml (10^5 IU/mg protein) produced in Sendai virus-treated leukocytes was kindly supplied by Dr K. Cantell (Central Public Health Laboratory, Helsinki, Finland). Since the minimal inhibitory concentration of IFN against HSV in various in vitro systems is known to vary between 91 to 120 IU/ml, concentrations of 50 to 500 IU of IFN/ml of culture medium were used (Bourkas and Menezes, 1979; Lerner and Bailey, 1974; Moran et al., 1985).

Sheep antibodies to human leukocyte interferon (Natl. Inst. Allergy & Infectious Diseases, Bethesda, MD, catalog no. G-026-502-568) was reconstitued with physiological saline and used at 100 IU/ml of culture medium.

Effect of IND, PGE_2 and IFN on HSV-1 replication in Raji and Raji-HSV

Pre-treatment: Raji cells were treated for 24 h before HSV-1 infection with IND (0 to 20 μg/ml) or PGE_2 (10^{-5} to 10^{-7} M) or for 72 h before HSV-1 infection with IFN (50 to 500 IU/ml) (Bourkas and Menezes, 1979). IND, PGE_2, and IFN at the same concentrations, were also added to the culture medium after adsorption of virus. At these concentrations, neither IND, PGE_2 nor IFN had any detectable effect on cell viability, as determined using trypan blue dye exclusion test.

Treatment: IND, PGE_2 or IFN were added to the medium only after the virus infection procedure. IFN preparation incubated with anti-IFN antibodies was also included as control.

To study the effect of IND, PGE_2 and IFN separately on Raji-HSV, the reagent was kept in the culture medium throughout the entire experiment.

The effects of the following combinations were also tested on HSV-1 replication in Raji and Raji-HSV: IND (20 μg/ml) with PGE_2 (10^{-5} to 10^{-7} M); IFN (100 IU/ml) with IND and IFN with PGE_2. Different combinations of IND and PGE_2 were used. In one experiment, the cells were treated with both agents 24 h prior to virus infection. In another experiment, Raji cells were treated with PGE_2 24 h before infection and IND treatment. Raji cells treated for 24 h before infection with IND or PGE_2 or untreated Raji, served as controls in both experiments. To test the effect of IND (20 μg/ml) and PGE_2 (10^{-6} M) on the IFN-induced antiviral state in both Raji and Raji-HSV cells, these reagents were added simultaneously with the IFN (100 IU/ml), or 72 h after the treatment of cells with IFN.

Number of tests and statistical analysis

To establish the reproducibility of our results, each experiment was performed at least 3 times. Thus, the figures presented in the results represent the means of three or more separate experiments carried out each time in triplicate. Standard

deviations were always within 5% of the mean.

Our results were evaluated by means of ANOVA test (F test), which consists of variance analyses to compare mean values. Comparisons have been made according to Tukey intervals, and the difference was considered statistically significant when $P < 0.05$.

Results

Effect of IND on HSV-1 multiplication in Raji and Raji-HSV

Our initial experiment involved the study of the effect of different concentrations of IND on HSV-1 growth in Raji and Raji-HSV. The results given in Figs. 1 and 2 show that culture of both cell lines in medium containing IND resulted in a dose-dependent inhibition of HSV-1 replication. The same effect of IND was observed with Raji cells that were treated immediately after virus infection. We also observed a similar reduction in yield of HSV-1 using VERO cells (data not shown). Ethanol

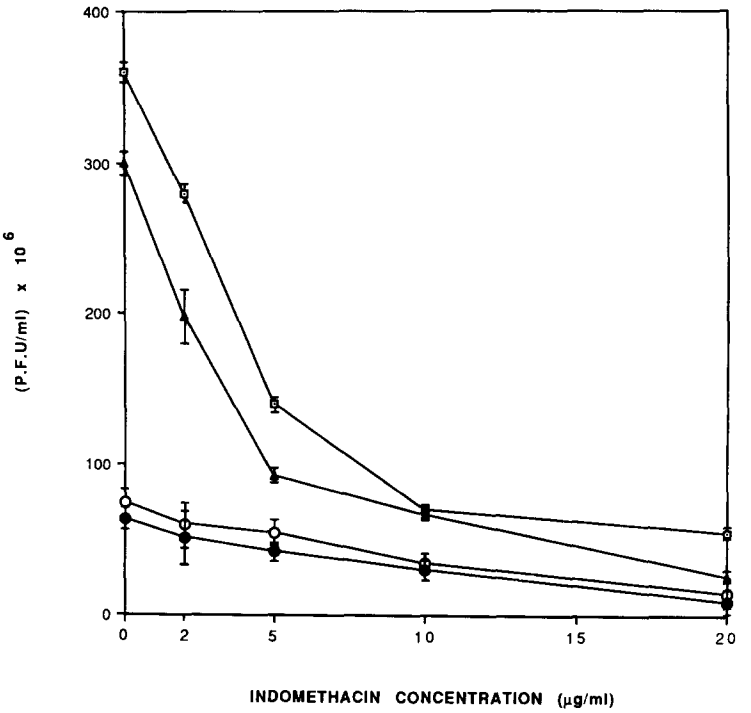

Fig. 1. Effect of IND on growth of HSV-1 in Raji cells. Cells were treated with different concentrations of IND for 24 h pre-infection, and growth of HSV-1 was measured at 48 h (○), and 72 h (□). Raji cells were infected with HSV-1 and treated simultaneously with the same concentrations of IND, then HSV-1 growth was examined at 48 h (●) and 72 h (▲) post-infection. An moi of 100 pfu/cell was used in these experiments.

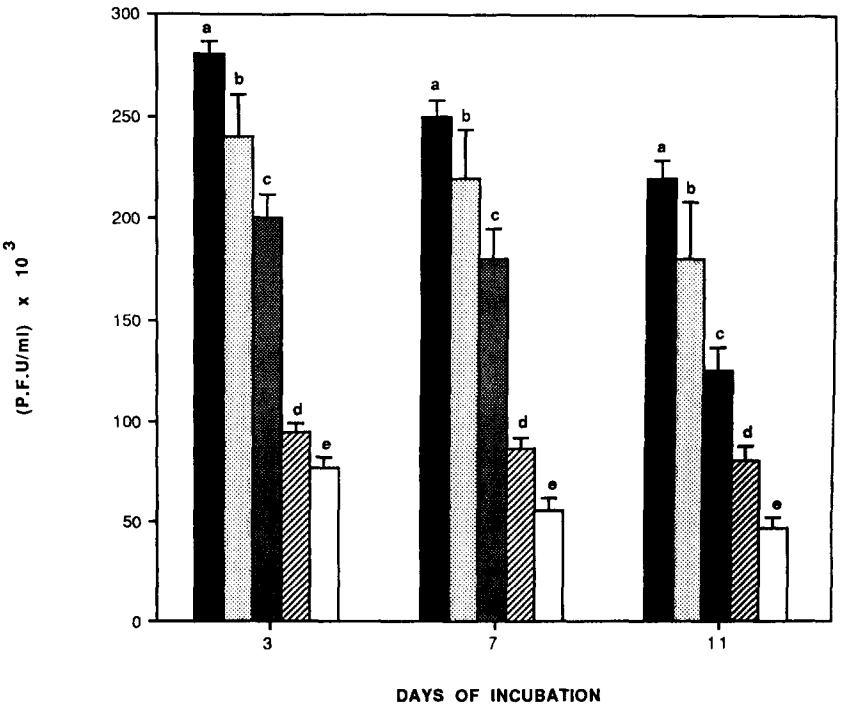

Fig. 2. Effect of IND on growth of HSV-1 in Raji-HSV. a: Cells + virus (no IND); b: cells + virus + IND (2 μg/ml); c: cells + virus + IND (5 μg/ml); d: cells + virus + IND (10 μg/ml); e: cells + virus + IND (20 μg/ml).

alone (at the same concentration as used to dissolve IND) was used as control and had no effect on HSV-1 replication.

Effect of PGE_2 on HSV-1 growth in Raji and Raji-HSV

The effect of PGE_2 on HSV-1 multiplication in Raji and Raji-HSV was then investigated. As shown in Fig. 3, PGE_2 activated HSV-1 replication in a statistically significant manner ($P = 0.01$), both in Raji treated with PGE_2 24 h before or simultaneously to virus infection as well as in Raji-HSV cells. PGE_2 effect on HSV-1 replication in Raji cells was dose-dependent. This effect was more pronounced with an moi of 10 pfu/cell than with 100 pfu/cell (data not shown).

Combined effect of IND and PGE_2 on HSV-1 growth in Raji and Raji-HSV

As shown in Fig. 4, PGE_2 was incapable of overcoming the inhibitory effect of IND. Cells treated simultaneously with IND and PGE_2 showed the same inhibitory effect as with the IND alone, whereas PGE_2 alone increased virus yield. The same effects were observed with Raji cells where the PGE_2 and the IND were

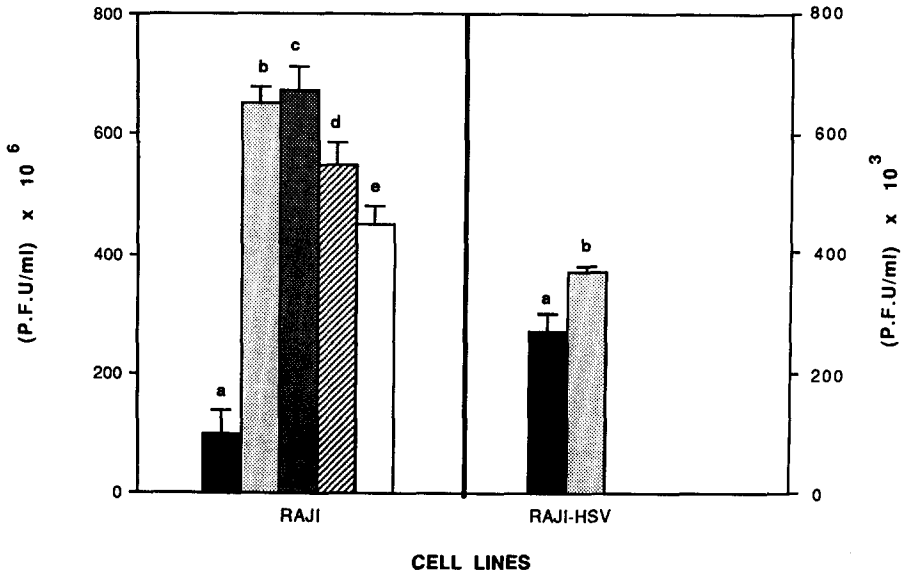

Fig. 3. Effect of PGE_2 on growth of HSV-1 in Raji cells infected with an moi of 10 pfu/cell, and in Raji-HSV cell line at 48 h and 72 h, respectively. a: Untreated cells; b: cells pre-treated with 10^{-6} M PGE_2 for 24 h; c: (10^{-5} M); d: (10^{-6} M) and e: (10^{-7} M) represent combinations in which cells were treated (PGE_2) and infected (HSV-1) simultaneously.

incorporated in the culture medium, respectively before and after the infection, and in Raji-HSV cells treated simultaneously with PGE_2 and IND (data not shown).

Effect of IFN on HSV-1 growth in Raji and Raji-HSV

Figure 5 shows the results of experiments comparing the response of HSV-1 outgrowth to IFN in Raji cells. All concentrations of IFN tested inhibited virus replication. Surprisingly, the inhibition of HSV-1 multiplication was seen only with a low moi of 10 pfu/cell ($P = 0.01$). Based on these results, a concentration of 100 IU/ml of IFN and an moi of 10 pfu/cell were used in subsequent experiments. Anti-IFN antibodies inhibited IFN effect on HSV growth indicating that the antiviral effect observed was indeed due to IFN.

IFN was added to Raji-HSV at a concentration of 100 IU/ml, and about three-fold decrease of virus yield was observed (Fig. 6c).

Combined effect of IFN with either IND or PGE_2 on HSV-1 growth in Raji and Raji-HSV

The inhibition of HSV-1 replication was as marked in cultures of Raji cells treated with IFN in the presence of IND (20 μg/ml) as in cultures treated with IFN or IND alone; interestingly, the addition of PGE_2 to cells treated with IFN did not

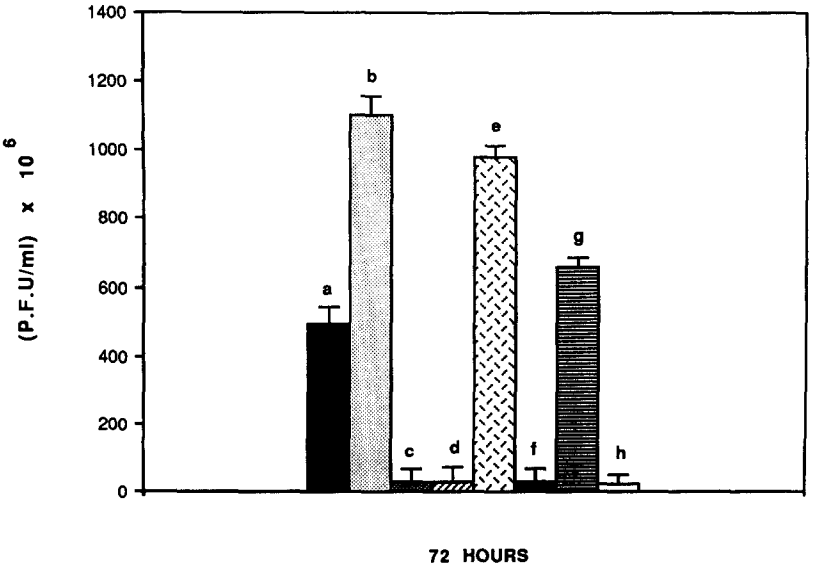

Fig. 4. Combined effect of PGE_2 and IND on growth of HSV-1 in Raji cells, 72 h post-infection. a: Untreated cells; b: cells + PGE_2 (10^{-6} M); c: cells + IND (20 μg/ml); d: cells + IND (20 μg/ml) + PGE_2 (10^{-6} M); e: cells + PGE_2 (10^{-5} M); f: cells + IND (20 μg/ml) + PGE_2 (10^{-5} M); g: cells + PGE_2 (10^{-7}M); h: cells + IND (20 μg/ml) + PGE_2 (10^{-7} M). An moi of 10 pfu/cell was used in these experiments.

reverse the inhibitory effect of IFN, whereas in the absence of IFN the addition of PGE_2 resulted in an increase in virus yield (data not shown). With Raji-HSV, combining effects of IFN (100 IU/ml) with IND (20 μg/ml) or with PGE_2 (10^{-6} M) gave the same results as with HSV primary infection of Raji cells (Fig. 6).

Discussion

To our knowledge, this is the first report showing that IND inhibits multiplication of HSV-1 in human lymphoid cells. Our findings also agree with those of Newton (1979) who used mouse L cells. Baker et al. (1982), and Harbour et al. (1978) have also obtained evidence of a decrease in plaque size and yield of HSV-1 or HSV-2 in VERO cells treated with IND.

We were also able to show that the addition of exogenous PGE_2 (10^{-5} to 10^{-7} M) 24 h pre-infection or immediately after HSV-1 infection of Raji, results in a dose-dependent increase of virus yield; PGE_2 also increases HSV-1 replication in Raji-HSV. Similarly, Harbour et al. (1978) reported increased yield of HSV-1 at a low moi (0.01 pfu/cell) but not at a higher moi (10 pfu/cell) in VERO cells pre-treated with PGE_2. These investigators attributed the increased yield of HSV to enhanced cell-to-cell spread of the virus. Baker et al. (1982) also reported an enhancement of cell-to-cell spread of HSV-2 at an moi of 0.01 pfu/cell in VERO

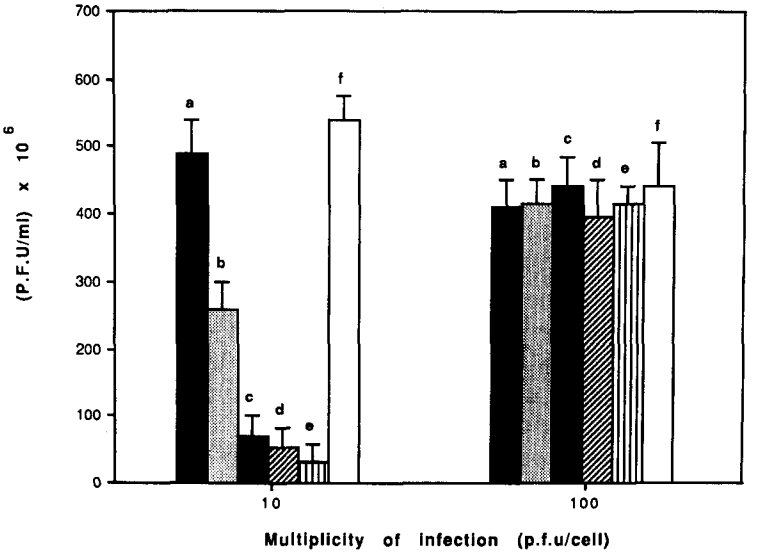

Fig. 5. Effect of IFN on growth of HSV-1 in Raji cells 72 h post-infection. a: Untreated cells; b-e: cells pre-treated with IFN (b: 50, c: 100, d: 200, e: 500 IU/ml); f: cells treated with IFN (100 IU/ml) pre-incubated with anti-IFN antibodies.

cells. On the other hand, using a human skin fibroblast cell line and an moi of 10 pfu/cell, Trofatter and Daniels (1980) did not find HSV enhancement with

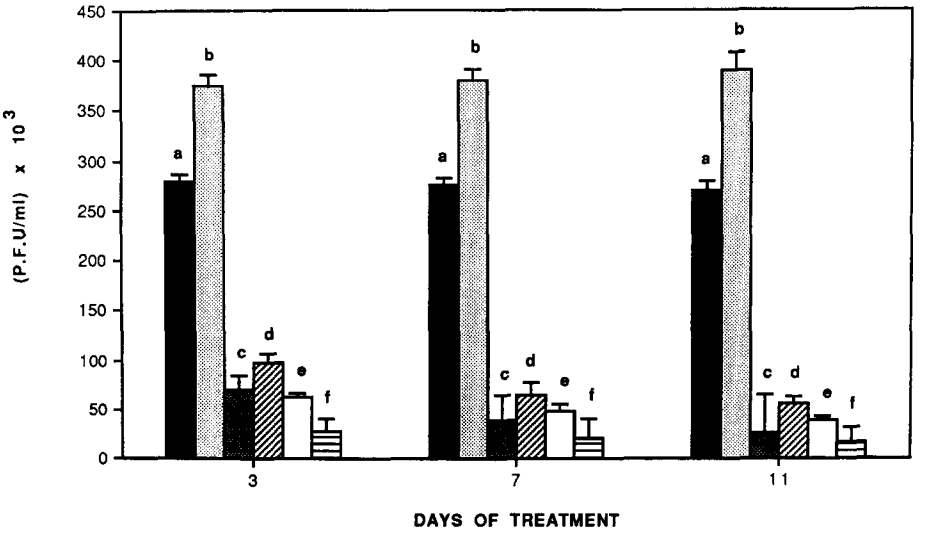

Fig. 6. Combined effect of IFN (100 IU/ml) with either IND (20 μg/ml) or PGE_2 (10^{-6} M) on HSV-1 multiplication in Raji-HSV cell line. a: Untreated cells; b: cells + PGE_2, c: cells + IFN; d: cells + IFN + PGE_2, e: cells + IND; f: cells + IFN + IND.

PGE_2. These conflicting results may relate to variation in experimental protocols or to the varied effects of PGs on cells of different species as suggested by Pelus and Strausser (1977). Our results however contradict this hypothesis and support Trofatter and Daniels's suggestion that the effects of PG may differ with tissue origin.

Exogenous PGE_2 even at high concentrations (i.e. up to 10^{-5} M) was unable to counteract the inhibitory effect of IND on HSV-1 multiplication, in both Raji and Raji-HSV cells. These results agree with those of Newton in mouse L cells. Harbour et al. (1978), on the other hand, could partly reverse the inhibitory effect of IND on VERO cells by the addition of exogenous PGE_2. It is known that the IND has no blocking action on preformed PGE_2 (Belfort et al., 1976), thus no effect on exogenous PGE_2 should be expected (Irving, 1974). We can conclude from our results that IND may inhibit HSV replication by non-PG related mechanisms, possibly by its effect on coupling mechanisms, or other effects that would be independent of its inhibitory action on cyclooxygenase.

Our studies with IFN have confirmed its inhibitory effect on HSV-1 multiplication in both primary and persistent infection in Raji cells. IFN has been long recognized as a defense mechanism that can limit HSV infections both in vitro (Lodmell and Notkins, 1974) and in vivo (Gresser et al., 1976; Guillon and Gresser, 1978). It was demonstrated in a previous report from our laboratory that IFN inhibits HSV-1 multiplication both in primary and chronic HSV-infected Raji cells (Bourkas and Menezes, 1979). Our present data show that IFN at 100 IU/ml significantly decreases the virus yield, and the maximum rate of inhibition is obtained with a low moi (i.e. 10 pfu/cell, Fig. 5); this suggests that the antiviral state may be overcome by a high enough inoculum of infectious virus. The same phenomenon has been observed with IND. In the case of PGE_2, its activation effect on HSV-1 multiplication is likewise more pronounced with an moi of 10 pfu/cell. The fact that anti-IFN serum reversed the inhibitory effect of the IFN on HSV replication confirmed that the inhibitory effect was indeed IFN specific.

We attempted to study the combined effect of IFN with IND or IFN with PGE_2 on the induction of a resistant state to HSV-1 multiplication by the IFN. Trofatter and Daniels (1980) found that although it had no direct stimulatory effect on HSV replication, PG could indirectly enhance virus growth by inhibiting both the action and the production of IFN in human fibroblast cells, via the induction of cyclic AMP. Our studies revealed that IND or PGE_2 added either simultaneously with IFN or three days after the induction of the antiviral state by the IFN, did not show an inhibition of the antiviral action of IFN. In contrast, cells treated with both IND and IFN showed an increased inhibitory effect on HSV-1 multiplication.

As such, the results we have obtained using human lymphoid cell lines agree with those of Tovey et al. (1981; 1982) and contradict the observations of Pottathil et al. (1980) concerning the effect of IND-IFN association on HSV replication. Our results contradict Trofatter and Daniels's observations (1980) concerning IFN and PGE_2 interaction. Since we did not use the same cells and, in some cases, the same virus, and bearing in mind that the amount of resistance developed by IFN varies greatly, both with the type of cell and the virus used (Levy et al., 1969), a direct

comparison is probably not proper. Chandrabose et al. (1981) reported that a cell line selected for resistance to the antiviral effect of IFN is virtually devoid of fatty acid cyclooxygenase activity, thus explaining the variability of sensitivity between different cells. Pottathil et al. (1980) also suggested that fatty acid cyclooxygenase is an essential component in the optimal induction of the IFN antiviral state.

Based on our results and the literature reports, we can conclude that IND has an inhibitory effect on HSV-1 replication independently of cell type or origin, while PGE_2's effect depends on these factors. Moreover, the effects of PGE_2, IND and IFN seem to be dependent on the moi. We have also shown that PGE_2 even at relatively high concentrations can not counteract the inhibitory effects of both IFN and IND on the lymphoid cells we studied. However, when IND is combined with IFN, there was a higher inhibitory effect which lets us believe in the beneficial effect of combined therapy. However, further work to clarify the way IND decreases, and PGE_2 increases virus yield is required and may contribute to the understanding of the mechanism of viral reactivation.

Acknowledgements

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