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Synergistic anti-herpes effect of TNF-α and IFN-γ in human corneal epithelial cells compared with that in corneal fibroblasts

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Abstract

In this study we compared how effectively the proinflammatory cytokines TNF-α and IFN-γ could inhibit HSV-1 replication in human corneal tissue fragments and monolayers of epithelial cells and fibroblasts derived from intact corneas, and investigated the mechanism responsible for the inhibition. Pretreatment of corneal tissue or cells derived therefrom with TNF-α (50 U/ml) and IFN-γ (5 IU/ml) consistently induced a synergistic antiviral effect. Inhibition of HSV-1 growth was most evident in fibroblasts ($>$ 1000-fold reduction) and less apparent (7–25-fold reduction) when epithelial cells were the target. Virus suppression was correlated with the induction of IFN-β because antibody to this cytokine but not IFN-α abrogated synergism. The more modest synergistic effect in epithelial cells was associated with a $\geq$ 4-fold reduction in the synthesis of IFN-β protein and mRNA, and decreased responsiveness of these cells to the antiviral effect of IFN-β. We conclude that the combination of TNF-α and IFN-γ induces a synergistic antiviral effect in corneal cells. The degree of synergism observed varies with the corneal cell type, and is correlated with the amount of IFN-β induced and the target cell responsiveness to the antiviral action of this cytokine.

Keywords: HSV-1; IFN-β; TNF-α; Human corneal epithelial cell ; IFN-γ; Human corneal fibroblast

1. Introduction

Viruses such as herpes simplex virus type 1 (HSV-1) can induce an inflammatory response at the site of infection. The induction and severity of inflammation is controlled

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in part by various cytokines operating in a cascade fashion (Arai et al., 1990). It has been postulated that cytokines released by resident tissue cells and infiltrating inflammatory cells may facilitate clearance of virus from the infected site (Ramsay et al., 1993). However, precisely which cytokines are the critical players and what mechanisms are involved remains unknown.

We have been investigating the effects of selected proinflammatory cytokines on the replication of HSV-1 in human corneal cells. Prior studies have shown that pretreatment of cultured human corneal fibroblasts with low dose tumor necrosis factor α (TNF-α) or interferon γ (IFN-γ) resulted in only modest suppression of virus growth (Chen et al., 1993). However, when the two cytokines were combined, virus growth was suppressed by some three orders of magnitude. This striking synergistic antiviral effect was demonstrable in corneal fibroblasts prepared from different donors, and occurred in cells infected with HSV type 2 as well as HSV-1.

It has recently proven possible to grow human corneal epithelial cells in tissue culture (Cubitt et al., 1993), and we have shown that HSV-1 can replicate to high titer in these cells just as it does in corneal fibroblasts (Oakes et al., 1993). It was therefore of interest to test whether the antiviral synergistic effect of TNF-α and IFN-γ was also operative in corneal epithelial cells. In this paper, we report that cytokine synergism was observed not only in epithelial cells but also in excised corneal tissue. However, the magnitude of the antiviral effect was less potent when compared with that seen in fibroblasts. An investigation into the reasons for the differences in cytokine synergistic potency between the two corneal cell types was carried out.

2. Materials and methods

2.1. Viruses

HSV-1 strain 17 was grown in Vero cells after being plaque purified. Infectious titers of virus preparations were determined by standard plaque assay on Vero cells. Vesicular stomatitis virus was grown and titered on L-929 fibroblasts.

2.2. Cells

L-929 cells (a gift from Dr. Jenifer Turco, University of South Alabama), Vero cells (Flow Laboratories, McLean, VA), and A549 cells (American Type Culture Collection, Rockville, MD) were cultured in DMEM supplemented with 10% calf serum, 0.15% sodium bicarbonate, 10 mM Hepes buffer solution, and antibiotics.

2.3. Reagents

Recombinant human TNF-α (2×10^7 units/mg; 2×10^5 units/ml) was purchased from Genzyme (Cambridge, MA). Human IFN-β was obtained from the NIH and recombinant human IFN-γ was purchased from Alpha Therapeutic Corporation (Los Angeles, CA). Interferon activity was calibrated using the World Health Organization

international reference preparations of human interferons provided by the NIH. Anti-human TNF-α monoclonal antibody was purchased from Endogen (Boston, MA). Sheep antiserum to human IFN-α, and IFN-β, and rabbit antiserum to IFN-γ were obtained from the NIH. Anti-human IFN-β monoclonal antibody was purchased from Boehringer-Mannheim (Indianapolis, IN).

2.4. Assay for the effect of cytokine pretreatment on HSV-1 replication in human corneas

Human corneas, obtained from the National Disease Research Interchange (Philadelphia, PA), were placed in RPMI-1640 medium (Irving Scientific, Santa Ana, CA) with 1% Fung-Bact Solution. After trimming the cornealscleral rim and peeling off the endothelial cell layer, the corneas were cut into four equal pieces. Two pieces of corneal tissue from different eyes of one donor were pooled in 1 ml of DMEM medium supplemented with 2% fetal bovine serum and exposed to cytokines, or left untreated. Twenty-four hours later, the medium was removed and then the corneal tissue pieces were infected with 2×10^4 PFU of HSV-1 strain 17 in 1 ml of medium. After absorption for 1 h at room temperature, the infected corneal tissue pieces were washed 3 times with medium, incubated in fresh 1 ml medium at 37°C in 5% CO_2 for 24 h without cytokines, and then the samples were collected and frozen at -70°C. The samples were prepared and assayed for virus content on Vero cell monolayers as previously described (Chen et al., 1993). The fold reduction of virus growth after cytokine treatment was calculated by using the formula: fold reduction = (virus titer of untreated tissue/virus titer of cytokine-treated tissue). To evaluate the combined effect of two cytokines, the criteria for synergism described previously were used (Chen et al., 1993).

2.5. Assay for the effect of cytokine pretreatment on HSV-1 replication in human corneal epithelial cells and fibroblasts

Confluent monolayers of human corneal epithelial cells and fibroblasts prepared as described previously (Cubitt et al., 1993) were incubated in DMEM medium containing 10% fetal bovine serum for 2 days. Then the human corneal cells were exposed to 1 ml of fresh medium containing the desired concentration of cytokines, or left untreated. Twenty-four hours later, the cells were infected with HSV-1 at a multiplicity of infection of 0.01. After virus adsorption, the cells were washed and incubated in 1 ml of medium without cytokine(s) for 24 h. The cell preparations were collected by scraping with a rubber policeman and frozen until assayed for infectious virus content.

2.6. IFN assay

The IFN activity in cell lysates was measured using a bioassay as described previously (Chen et al., 1993). The assay involved protection of A549 cells against infection with vesicular stomatitis virus as assessed by inhibition of the cytopathic effect. An internal human IFN-β standard from the NIH was included with each titration

and served as the reference for calculating IFN-β units in each test sample. IFN-β activity was confirmed by neutralization with a monoclonal antibody to human IFN-β.

2.7. Construction of competitive IFN-β control RNA for competitive reverse transcription-polymerase chain reaction (RT-PCR)

The cDNA which encodes human IFN-β was obtained from ATCC and subcloned into pBlueprint phagemid SKII + (Stratagene, La Jolla, CA) which has a T3 RNA polymerase promoter site. The restriction endonuclease *Bst*N1 was used to remove 164-bp of internal sequence from the IFN-β cDNA and then cohesive ends of the cleavage product were joined using T4 DNA ligase (BRL, Gaithersburg, MD). The plasmid containing the shortened IFN-β cDNA was used to transform XL-1 Blue *E. coli* strain (Stratagene, La Jolla, CA) in order to prepare plasmid stock. The plasmid was then linearized with a restriction endonuclease *Hin*cII and in vitro RNA transcripts were prepared with commercially available kits using T3 RNA polymerase. The competitive IFN-β RNA transcripts were then treated with DNase to remove template DNA, purified by the acid guanidinium isothiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987), and determined to be essentially free of degradation products by Northern blot analysis. Final preparation of competitive IFN-β control RNA in water was measured by 260 nm spectrophotometry and the number of RNA molecules was calculated. The competitive IFN-β control RNA added to each reaction was RT-PCR amplified with the same primers used to amplify the naturally producing IFN-β mRNA found in the total cellular RNA. The RT-PCR product of the competitive IFN-β control RNA was 164 bp shorter than IFN-β mRNA and was sufficiently lower in molecular weight to allow the derived PCR products to be readily resolved by electrophoresis.

2.8. Analysis of IFN-β mRNA induction by competitive RT-PCR after dual cytokine treatment

For extraction of total cellular RNA, the human corneal cells were pretreated with the desired cytokines and then the total cellular RNA was isolated from the cell cultures by the acid guanidinium isothiocyanate phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). The concentration of RNA in the preparations was determined, and then the RNA was fractionated on a formaldehyde agarose gel and stained with ethidium bromide to confirm the integrity of the RNA. For cDNA synthesis, 10^6 copies of competitive IFN-β control RNA and 0.5 μg of total cellular RNA were mixed with random hexamer primers and M-MLV reverse transcriptase from a GeneAmp RNA PCR kit (Perkins Elmer, Norwalk, CT) according to the manufacturer's instructions. PCR reactions were performed with AmpliTaq DNA polymerase and excess amounts of complementary primers specific for IFN-β mRNA sequences. Three sets of reactions were thermocycled for three different numbers of cycles to insure that the amounts of product were within the linear range of the assay. A 10 μl volume of each PCR reaction mixture (40 cycles) was separated by electrophoresis on a 1.5% agarose gel. Gels were stained with ethidium bromide for visualization under ultraviolet illumination and then photographed using Polaroid 667 instant film. Negatives were used to quantify band

intensities of full length and competitive control PCR product using a Zeineh laser densitometer (Biomed Instruments, Fullerton, CA). In these experiments, it would be labor-intensive to perform a competitor titration for each individual sample, so instead a constant amount of the competitive IFN-β RNA was used to correct for amplification efficiency in each tube as described by Siebert and Larrick (1992). Therefore, the ratio of the fluorescent intensity of the IFN-β product and the competitive product was equal to the relative fold induction of IFN-β mRNA in each sample. To ensure that equal amounts of total cellular RNA were added to each sample, the mRNA of the house keeping gene-human glyceraldehyde 3-phosphate dehydrogenase (GAPD) was used as the internal reference for RT-PCR amplification (25 cycles).

The following primers were used:

(i) Human IFN-β primers (563 bp product): Sense 5′-ACATGACCAACAAGTG-TCTCC-3′ (base −2 to 19). Anti-sense 5′-GTTTCGGAGGTAACCTGTAAG-3′ (base 441 to 561, Ohno and Taniguchi, 1981).

(ii) Human GAPD primers (878 bp product): Sense 5′-GTAAAGTGGATATTGT-TGCCATCA-3′. Anti-sense 5′-AAATTCGTTGTCATACCAGGAAAT-3′ (Cubitt et al, 1994).

3. Results

3.1. Synergistic antiviral effect of TNF-α and IFN-γ on HSV-1 replication in human corneal tissue

The concentrations of IFN-γ and TNF-α used in the present experiments were those previously demonstrated to produce a strong synergistic antiviral effect in corneal fibroblasts (Chen et al., 1993). We first investigated whether intact corneal tissue pretreated with the indicated cytokine(s) would become less susceptible to HSV-1 replication. Fig. 1 shows the results of individual experiments using tissue from four different donors. It can be seen that IFN-γ at 5 IU/ml inhibited virus growth by 7–15-fold, while 50 U/ml TNF-α produced a quite modest antiviral effect ($<$ 5-fold reduction in titer) in all but one donor. In contrast, dual cytokine pretreatment resulted in 140–1433-fold reduction in virus titer. Thus, combined cytokine treatment proved to be some 10–30 times more inhibitory than single cytokine treatment in 4 of 4 trials.

3.2. Comparison of dual cytokine treatment on HSV-1 replication in human corneal epithelial cells and fibroblasts

The foregoing results indicated that a synergistic anti-HSV-1 effect could be demonstrated in human corneal tissue pretreated with TNF-α and IFN-γ. However, although the superiority of dual versus single cytokine treatment was clearly evident, it was not as pronounced as that observed in our earlier studies in which the two cytokines inhibited HSV-1 replication by $>$ 1000-fold when human corneal fibroblasts were the target cells (Chen et al., 1993). The major cell type in the cornea, besides the fibroblast, is the epithelial cell. It is known from one step growth curve experiments that HSV-1 grows to

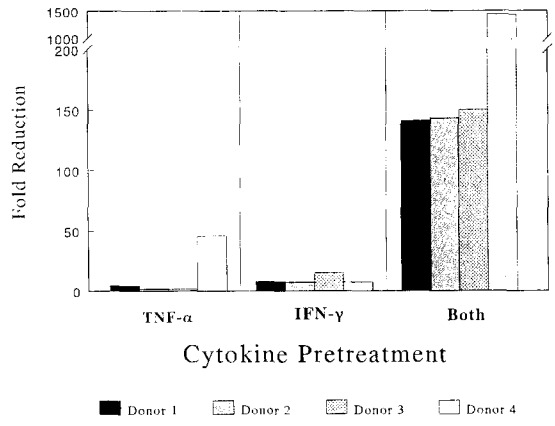

Fig. 1. Synergistic antiviral effect of TNF-α and IFN-γ on HSV-1 replication in human corneal tissue. Dissected quadrants of human corneas in 1 ml medium were exposed to the indicated individual or combined cytokines for 24 h before being infected with HSV-1. Twenty-four hours post-infection, the samples were harvested and virus titers were determined. * The fold reduction of the combined treatment of TNF-α and IFN-γ was significantly greater than that of the individual cytokine ($P < 0.04$) for all four donors.

a similar titer in the two cell types (Oakes et al., 1993). Thus, our results could be accounted for if epithelial cells were less able to resist HSV-1 growth following dual cytokine treatment. To test this hypothesis, experiments were performed using pure cultures of corneal epithelial cells and fibroblasts prepared as described previously (Cubitt et al., 1993). Fig. 2 summarizes the data from four independent experiments.

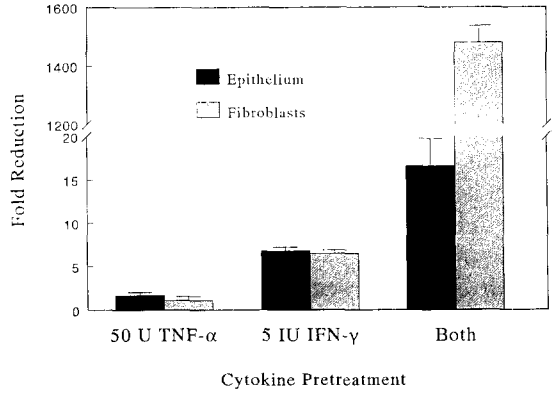

Fig. 2. Effect of TNF-α and IFN-γ pretreatment on HSV-1 replication in human corneal epithelial cells and fibroblasts. Confluent monolayers of human corneal epithelial cells and fibroblasts derived from the same donor cornea were exposed to the indicated cytokines for 24 h or left untreated. The cells were then infected with HSV-1 and 24 h later the samples were harvested and virus titers were determined. The bars represent the mean fold reductions $\pm$ S.E. of the means from four independent experiments using human corneal cell cultures established from different donors. The fold reduction of combined treatment of TNF-α and IFN-γ in fibroblasts was significantly higher than that in epithelial cells ($P < 0.02$).

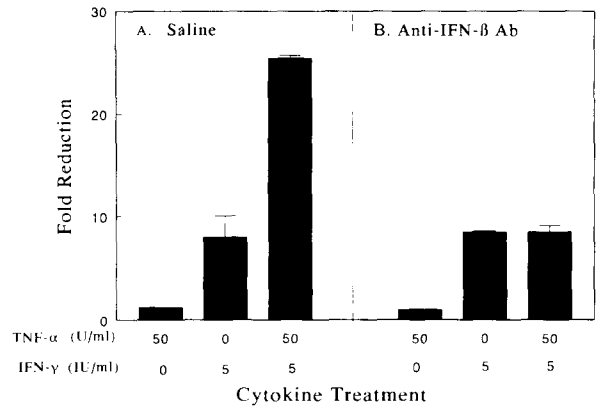

Fig. 3. Effect of anti-IFN-β antibody treatment on the synergistic antiviral activity of TNF-α and IFN-γ in human corneal epithelial cells. Human corneal epithelial cells were exposed to (A) saline or (B) 250 neutralizing units (NU)/ml of sheep antiserum to human IFN-β 30 min prior to cytokine treatment. Twenty-four hours after antibody and cytokine treatment, the cells were infected with HSV-1. Following virus infection, the cells were washed and incubated in fresh medium supplemented with 250 NU/ml of desired antibody or saline. After 24 h of incubation, the samples were harvested and virus titers were determined. The bars represent the mean fold reductions of samples titered in triplicate. Error bars indicate standard errors of the mean.

When TNF-α and IFN-γ were tested individually, HSV-1 replication was only modestly inhibited (< 7-fold) in both cell types. Dual cytokine treatment of epithelial cells led to a 17-fold reduction in virus yield, a modest synergistic effect. However, in fibroblasts treatment with both cytokines inhibited HSV-1 growth by more than 1400-fold. Thus, combined cytokine treatment was some 82-fold more inhibitory for HSV-1 replication in fibroblasts than in epithelial cells. These results suggest that the human corneal fibroblast is the cell type primarily responsible for the synergism observed in the human corneal tissue fragments.

3.3. Neutralizing antibodies to IFN-β abrogated TNF-α and IFN-γ synergism observed in epithelial cells

Our previous studies had established that the synergistic anti-HSV-1 effect of TNF-α and IFN-γ in human corneal fibroblasts was associated with IFN-β synthesis (Chen et al., 1993). We therefore investigated whether induction of this cytokine might also contribute to the modest synergism observed in epithelial cells. Human corneal epithelial cells were exposed to dual cytokine treatment in the presence of antibody to human IFN-β or saline. Fig. 3 shows that the synergistic antiviral activity of TNF-α and IFN-γ was completely abrogated by anti-IFN-β antibody ($P < 0.03$). An antiserum to human IFN-α had no effect (data not shown). These results, which were reproduced in a second independent experiment, indicated that the IFN-β induced after dual cytokine treatment plays an important role in the mechanism of synergism observed in epithelial cells as well as in fibroblasts.

3.4. Analysis of IFN-β activity and mRNA production in human corneal epithelial cells and fibroblasts after dual cytokine treatment

The foregoing results indicated that treatment with TNF-α and IFN-γ could induce IFN-β synthesis in both corneal epithelial cells and fibroblasts. We hypothesized that IFN-β induction in fibroblasts was substantially higher than in epithelial cells, and this is one reason why the synergism was more pronounced in the former. To investigate this hypothesis, we compared IFN-β activity levels in both cell types. Fig. 4 shows that 20 $IU/10^5$ cells of IFN-β was detected in fibroblast cell lysates 24 h after dual cytokine treatment. Only 5 $IU/10^5$ cells of IFN-β was detected in an equivalent number of epithelial cells. Less than < 2.5 $IU/10^5$ cells was detected in control and single cytokine-treated samples. This experiment was repeated using corneal cells derived from another donor. Again 20 $IU/10^5$ cells of IFN-β was detected in fibroblasts, while < 2.5 $IU/10^5$ cells was found in epithelial cells. The IFN activity was specifically neutralized by monoclonal antibody to human IFN-β but not by antibodies to human IFN-α, IFN-γ, or TNF-α (data not shown). These results indicated that fibroblasts lysates had $\geq$ 4-fold more IFN-β activity than did epithelial cell lysates after dual cytokine treatment.

To further analyze these results total cellular RNA was extracted at 3, 6, 12, and 18 h after dual cytokine treatment, and the kinetics of IFN-β mRNA synthesis were monitored by competitive RT-PCR amplification. Constant amounts of competitive IFN-β control RNA, which differs from the target sequence of interest by virtue of an introduced internal deletion, were co-amplified with same amount of total cellular RNA from each sample using the same primers. The competitive control RNA PCR product was used to correct for amplification efficiency as described in Section 2.

Fig. 5A shows that IFN-β mRNA was not detectable in untreated epithelial cells and only seen at 6 h in cytokine treated cultures. In treated fibroblasts IFN-β mRNA was evident at all time points. At 12 h the level of IFN-β mRNA in fibroblasts was 6-fold

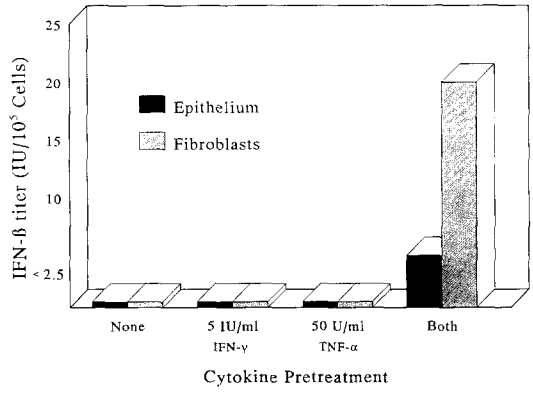

Fig. 4. Comparison of IFN-β activity in human corneal epithelial cells and fibroblasts. Cultures were exposed to the indicated cytokines or left untreated. The samples were harvested and assayed for antiviral activity 24 h later. The IFN-β activity was calibrated using a World Health Organization international reference preparation of human IFN-β from the NIH and confirmed by neutralization with a monoclonal antibody to human IFN-β.

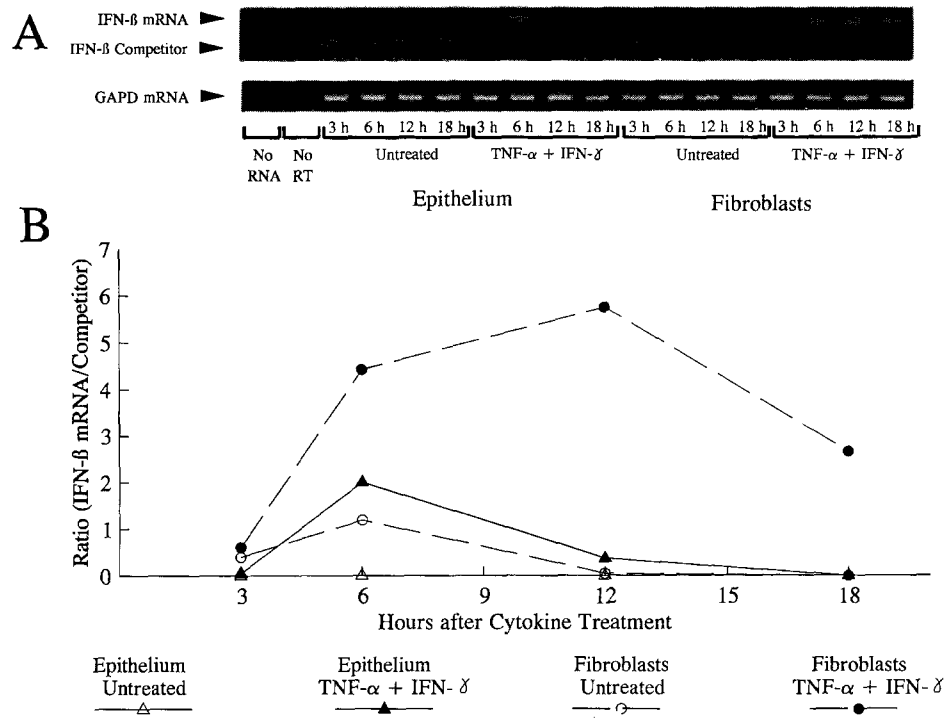

Fig. 5. Comparison of IFN-β mRNA levels in human corneal epithelial cells and fibroblasts after dual cytokine treatment. The two types of human corneal cells were exposed to the combination of TNF-α and IFN-γ, or left untreated, and total cellular RNA was isolated from samples at the indicated times. The presence of IFN-β mRNA was determined by competitive RT-PCR. (A) Constant amounts of competitive IFN-β control RNA transcribed from cDNA which has an internal 164 base pair deletion were co-amplified with 0.5 μg of total cellular RNA using the same primers. The PCR product was electrophoretically separated, and then the agarose gel was stained with ethidium bromide. The mRNAs encoding the house keeping gene GAPD were comparable in all the reactions thus ensuring that equal amounts of total cellular RNA were used in the different reactions. (B) Plot of the optical density ratio of IFN-β mRNA to competitor obtained by computer-based laser image analysis of the gel shown in panel A.

higher than in epithelial cells as assessed by laser densitometer analysis (Fig. 5B). Interestingly, low levels of IFN-β mRNA were also detected in untreated fibroblasts (Fig. 5A). Message was repeatedly observed in independent experiments but only at early time points (3 or 6 h), and no protein product was detected (Fig. 4). Apparently, the simple act of adding fresh medium with 10% fetal calf serum to corneal fibroblast cultures can trigger transient IFN-β mRNA synthesis. Collectively, these results indicated that the steady-state level of IFN-β mRNA was greater and persisted longer in cytokine-treated fibroblasts than in epithelial cells.

3.5. Anti-HSV effect of IFN-β in human corneal epithelial cells and fibroblasts

It was possible that the reduced synergistic effect observed in epithelial cells might also reflect decreased responsiveness of these cells to the antiviral action of IFN-β. To

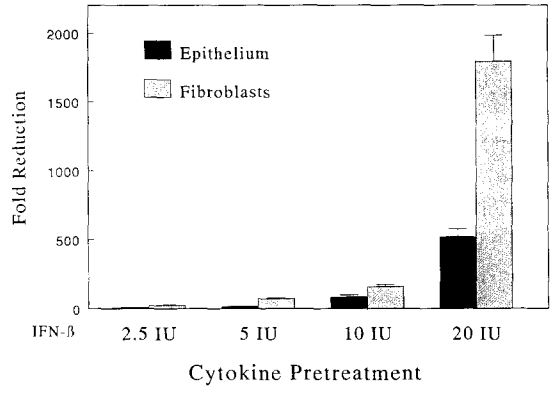

Fig. 6. Inhibitory effect of IFN-β pretreatment on the growth of HSV-1 in human corneal epithelial cells and fibroblasts. Human corneal cell cultures were exposed to the indicated units of IFN-β or left untreated. Twenty-four hours later, the cells were infected with HSV-1. Following an additional 24 h incubation in fresh medium, the samples were harvested and virus titers were determined. The bars represent the mean fold reduction $\pm$ S.E. of the means from three independent experiments.

test this hypothesis we compared how effectively IFN-β pretreatment could suppress HSV-1 growth in the two cell types. The results of three independent dose-response experiments using cell cultures from 3 different donors are summarized in Fig. 6. It was found that at the different concentrations tested IFN-β was 2 to 4 times more effective at suppressing HSV-1 growth in fibroblasts than in epithelial cells.

4. Discussion

In this study, we have shown that a mixture of TNF-α and IFN-γ could induce an elevated antiviral state in human corneal epithelial cells, and in corneal tissue recently removed from the donor. We have also confirmed our previous observation that TNF-α and IFN-γ acted in a synergistic manner to inhibit HSV-1 replication in human corneal fibroblasts (Chen et al., 1993). The degree of synergism obtained in the two corneal cell types was very different. In fibroblasts virus growth was usually inhibited $>$ 1000-fold, whereas in epithelial cells the reduction was much more modest, varying from 7–25-fold. The magnitude of the inhibitory effect in corneal tissue was higher than in epithelial cells presumedly reflecting the superior synergism seen in corneal fibroblasts.

In our earlier study it was concluded that the antiviral synergism observed after dual cytokine treatment of fibroblasts was largely, and probably entirely, due to induction of IFN-β synthesis (Chen et al., 1993). The present work confirmed that exposure of these cells to TNF-α and IFN-γ resulted in the induction of IFN-β mRNA and protein synthesis. Treatment of epithelial cells with these two cytokines also induced both IFN-β mRNA and protein. This finding indicated that cytokine treatment was not toxic, and suggested that the antiviral activity of IFN-β also accounted for the synergism observed in epithelial cells. This hypothesis is strongly supported by the demonstration

that antibody to IFN-β but not IFN-α could completely abrogate the antiviral synergistic effect. The association of IFN-β induction with the synergistic effect is further strengthened by the finding that the cell type (fibroblasts) producing the most IFN-β was the most effective at suppressing virus growth.

Why was the antiviral synergism less robust in epithelial cells? Preliminary studies in our laboratory have established that the number and affinity of TNF-α receptors on epithelial cells and fibroblasts were comparable (Cubitt et al., 1994). In addition, we found that the antiviral effect of IFN-γ in epithelial cells did not differ significantly from that in fibroblasts. These results suggest that the reduced synergism was not due to decreased expression or lower affinity of TNF-α receptors, nor the inability of IFN-γ to activate signal transduction mechanisms. Quantitative measurements revealed that epithelial cells upon dual cytokine stimulation made at least 4-fold less IFN-β activity than did fibroblasts. Furthermore, the epithelial cells were 2–4-fold less sensitive to the anti-HSV-1 effect of IFN-β. Collectively these findings may account for the reduced antiviral synergism observed in epithelial cells.

How might TNF-α synergize with IFN-γ to induce significant IFN-β synthesis? The regulatory region for the IFN-β gene is known to be composed of both positive and negative regulatory domains (Williams, 1991). These domains interact with various proteins, some of which can be induced by TNF-α and IFN-γ (Fujita et al., 1989; Meichle et al., 1990; Osborn et al., 1989). Presumably the two cytokines act in synergy to remove repressors and induce transcription factors which promote IFN-β gene expression. The reduced ability of epithelial cells to produce IFN-β might be due to fewer or less efficient tissue specific transcription factors in this cell type.

Although epithelial cells can exert antiviral effects after exposure to IFN-β, their sensitivity to the antiviral activity of IFN-β was much reduced when compared to fibroblasts. This decreased sensitivity may be due to a lower concentration of IFN-β receptors, reduced activation of a signal pathway (John et al., 1991) or, lower production of antiviral proteins after exposure to IFN-β (Sekiya et al., 1987). The stage at which IFN-β inhibits the replication cycle of HSV-1 has been controversial. Recent evidence indicates that IFN-β suppresses HSV growth by blocking the synthesis of virus immediate early proteins (Mittnacht et al., 1988; Klotzbücher et al., 1990).

TNF-α has been reported to produce an antiviral effect by itself in selected HSV-1 infection models in vitro (Ito and O'Malley, 1987) and in vivo (Rossol-Voth et al., 1991). However, in our human corneal cell studies TNF-α pretreatment led to only minimal virus inhibition ($<$ 5-fold), except in the case of donor #1 (Fig. 1). Also, five IU IFN-γ produced only a relatively low (6–15-fold) reduction in virus replication. In contrast, IFN-β at the 5 IU dose could suppress the virus yield by some 65–121-fold, and so was clearly superior to either TNF-α or IFN-γ at inducing an antiviral effect. Thus, the strategy of two low potency antiviral cytokines inducing a high potency antiviral cytokine would clearly be beneficial to the host.

It is not possible to directly determine whether cytokine-induced antiviral synergism occurs in vivo in man. However, there is precedent that cytokines can act in a synergistic fashion to protect the experimental host against fatal HSV-1 infection (Schmitt et al., 1992). The work presented in the present paper shows that TNF-α and IFN-γ synergism can be demonstrated not just in cultured cells but also in corneal tissue

recently obtained from human donors. Only a low concentration of each cytokine was needed for induction of IFN-β. The doses used were well within the concentrations which can be expected to be found in human tissue infection sites (Hamzaoui et al., 1990; Ohga et al., 1993). Cells infiltrating the cornea during HSV-1 infection such as natural killer cells (Pepose, 1991), T-cells (Niemialtowski and Rouse, 1992a; Hendricks and Tumpey, 1990), and Langerhans cells (Asbell and Kamenar, 1987) have the capacity to synthesize and release IFN-γ and/or TNF-α (Trinchieri, 1989; Niemialtowski and Rouse, 1992b; Larrick et al., 1989). In addition, there is evidence in the HSV-murine ocular infection model that IFN-α/β can exert a potent antiviral effect (Su et al., 1990; Hendricks et al., 1991). Finally, it has been reported that IFN-γ and TNF-α acting together can induce transcription of human genes coding for factors important in promoting inflammation (Barker et al., 1990). Thus, cytokine synergism may orchestrate defense mechanisms designed to terminate HSV-1 replication in ocular tissue and other sites of virus infection.

Acknowledgments

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