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Suppression of infectious virus spread and corneal opacification by the combined use of recombinant interferon beta and interleukin-10 following corneal infection with herpes simplex virus-1 in mice

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Abstract

The effects of Interleukin-10 (IL-10) and recombinant murine interferon-β (rMuIFN-β) on experimental corneal herpes simplex virus 1 (HSV-1) inoculation in BALB/c mice were examined. The mice were inoculated with the HSV-1 strain KOS at their corneas after abrasion. IL-10 was then administered topically once a day for 10 days beginning 2 days post inoculation, while rMuIFN-β was administered once a day for 10 days beginning 1 day post inoculation. The local viral growth in the inoculated eyes and trigeminal ganglia was reduced in the rMuIFN-β-treated mice but not in the IL-10-treated mice. In the mice treated with both rMuIFN-β and IL-10, the degree of both the local viral growth and corneal opacification decreased. The establishment of HSV-1 latency in the trigeminal ganglia was partially prevented by rMuIFN-β treatment but not by IL-10 treatment. The combined use of the cytokines resulted in both the suppression of viral spread and the prevention of corneal inflammation induced by HSV-1 infection.

Keywords: Herpes simplex virus; Herpetic stromal keratitis; Immunopathology; Interferon; Interleukin-10

1. Introduction

Herpetic stromal keratitis (HSK) is caused by ocular infection with herpes simplex virus (HSV) and is also a leading cause of vision impairment

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(Whitley, 1996). The pathogenesis of HSK is not fully understood but is believed to represent an immunopathologic response of CD4+ T cells to HSV and/or self antigen(s) following tissue destruction caused by HSV propagation (Avery et al., 1995; Doymaz and Rouse, 1992). As a result, the effects on the HSV-infected cornea of cytokines and anti-inflammatory drugs as well as antiviral drugs, either alone or in combination, have been studied (Balish et al., 1992; Mestan et al., 1988; Naito et al., 1991; Tang and Hendricks, 1996). Interferon (IFN)-α (Hendricks et al., 1991; Minkovits and Pepose, 1995) and an anti-inflammatory inhibitory cytokine interleukin-10 (IL-10) (Tumpey et al., 1994) have been independently reported to be effective in the prevention and treatment of HSK in both experimental animals and humans. In this study, we showed that the combined use of IL-10 and recombinant murine IFN-β in the corneas of BALB/c mice inoculated with HSV-1 not only significantly reduced the degree of corneal opacification but also suppressed the infectious virus spread to the contralateral trigeminal ganglia.

2. Materials and methods

2.1. Cytokines

Murine recombinant IL-10 was purchased from Pharmingen (San Diego, CA). Recombinant murine IFN-β (rMuIFN-β) was obtained from Toray (Tokyo, Japan). For the in vivo topical administration, 0.025 ml (2 U for IL-10; 2.5×10^5 U for IFN-β) per eye was given every 24 h a total of 10 times beginning 1 day (IFN-β) or 2 days (IL-10) pi unless specified otherwise. A daily dose of 2 U for IL-10 was considered sufficient since the treated mice were partially protected from stromal keratitis without increasing mortality. In addition, two out of five mice given 20 U of IL-10 daily, died of encephalitis within 10 days in the preliminary experiments (data not shown). The control mice received the same volume of Eagle's minimum essential medium (MEM).

2.2. Virus and cell culture

The HSV-1 strain KOS was used as the inoculum throughout the experiments. The infectivity of each virus stock was assayed on Vero cells and expressed as plaque-forming units (PFU) per ml. Vero cells were used for virus propagation and titration, while L-929 cells were used in the IFN-β/IL-10 sensitivity assays. Both cells were grown in Eagle's MEM supplemented with 5% calf serum.

2.3. Mice and corneal inoculation of HSV-1

Five-week old BALB/c mice were obtained from Seac Yoshitomi (Yoshitomi, Japan) and inoculated at 6–8 weeks of age. For the corneal inoculation, the mice were inoculated under anesthesia with 5×10^5 PFU of HSV-1 on their right corneas after an abrasion with a 27-gauge needle had been made. Both IL-10 and IFN-β were topically administered once a day for 10 days. The control mice received MEM. The severity of HSK was determined by observations under a binocular microscope and scored as described previously (Sakai et al., 1994). After 28 days pi, the mice were killed and the trigeminal ganglia of both sides were examined for HSV-1 latency by cocultivation with Vero cells. Student's t-test and Fisher's exact test were used for the statistical analyses.

2.4. Determination of the virus titers in various types of tissues

The tissue virus titers were determined as described previously (Li et al., 1995). The mice were killed at various times after virus inoculation and tissue samples including the eyes, trigeminal ganglia and brain were collected and stored at -70°C until assayed. Three mice per point were examined. The tissue specimens to be titered were thawed and homogenized in phosphate-buffered saline (PBS) with either a Teflon homogenizer or a mortar and pestle with quartz sand. The homogenized tissue specimens were centrifuged at $800 \times g$ for 10 min and a plaque assay of the supernatants was performed on Vero cell mono-

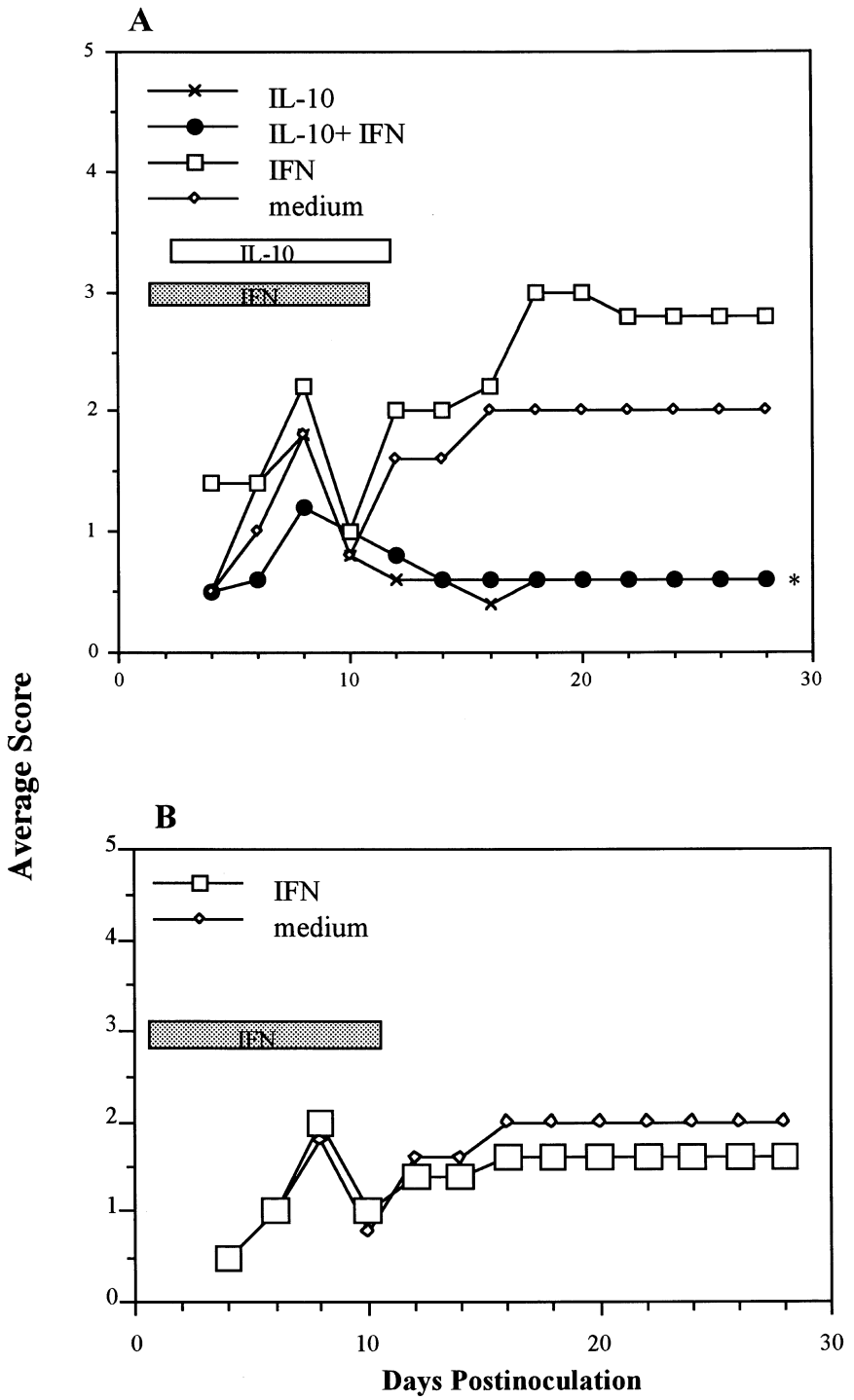

Fig. 1. The effects of IL-10, IFN-β, IL-10 + IFN-β, and medium on the HSK lesion score of BALB/c mice inoculated with HSV-1 (KOS). The average scores of five mice per group from two representative separate experiments are shown. The effects of IL-10, IFN-β, IL-10 + IFN-β, medium (panel A) and the effects of IFN-β and medium (panel B) on the HSK lesion score of BALB/c mice inoculated with HSV-1 (KOS). Significant differences were observed in the scores 28 days post infection between the medium control group and the IL-10 group ($P < 0.01$), and the control group and the IL-10 + IFN group ($P < 0.01$), the control group and the IFN group in panel A ($P = 0.02$) but not between the control group and the IFN group in panel B ($P = 0.34$), respectively.

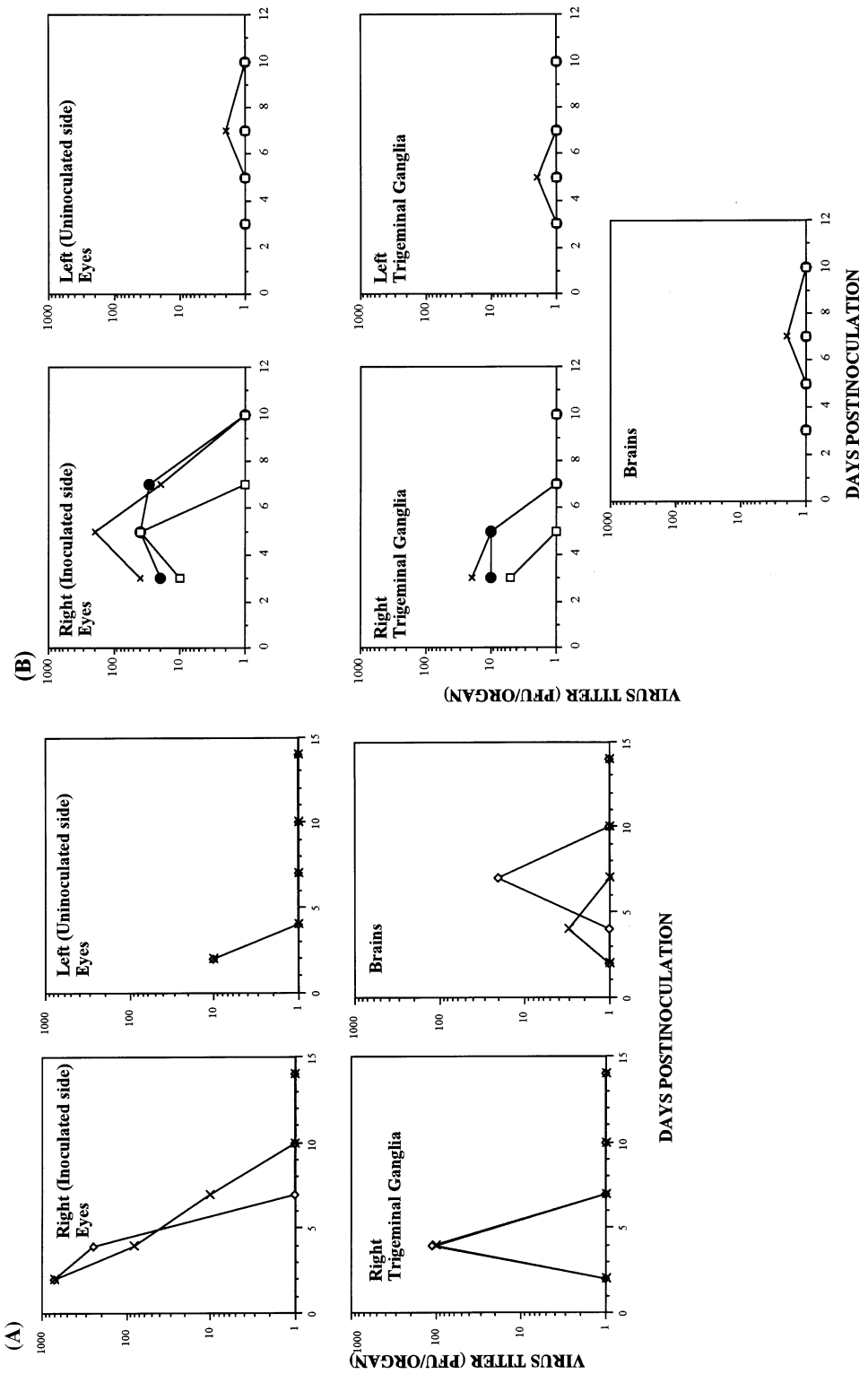
(A)
Right (Inoculated side) Eyes
Left (Uninoculated side) Eyes
Right Trigeminal Ganglia
Brains
VIRUS TITER (PFU/ORGAN)
DAYS POSTINOCULATION
(B)
Right (Inoculated side) Eyes
Left (Uninoculated side) Eyes
Right Trigeminal Ganglia
Left Trigeminal Ganglia
Brains
VIRUS TITER (PFU/ORGAN)
DAYS POSTINOCULATION

Fig. 2.

layers in 24-well plates (Becton-Dickinson Labware, Lincoln Park, NJ). The infectious virus titer of each tissue specimen was expressed as plaque-forming units (PFU) per tissue (Fig. 2).

2.5. In vitro virus inhibition assay

The in vitro sensitivity of HSV to cytokines was assessed by a plaque-reduction assay using L-929 cells essentially as described previously (Minagawa et al., 1987). Monolayer cultures of L-929 cells were incubated for 18 h with medium containing various amounts of IFN-β and/or IL-10, washed, and then inoculated with HSV-1. After adsorption for 1 h, the cells were overlaid with medium containing 1% methylcellulose. The cells were then further incubated for 3 days before fixation with 10% formalin, staining with crystal violet and determining the plaque count.

3. Results

3.1. In vivo effects of IFN-β and IL-10

The infected control mice with the MEM treatment beginning 1 or 2 days pi developed HSK comparable to that seen in the untreated infected mice. The effects of IFN-β treatment from 1 day pi and of IL-10 treatment from 2 days pi are demonstrated in Fig. 1. and Fig. 2. As shown in Fig. 1, the IL-10 treatment reduced the severity of HSK (Fig. 1A). However, the viral growth in the inoculated tissue specimens and the viral spread to distant organs were neither reduced nor exacerbated by the IL-10 treatment (Fig. 2A). In some mice treated with IL-10 the infectious virus was detected from the brain (Fig. 2).

In contrast, IFN-β treatment without IL-10 suppressed the virus growth and the virus spread (Fig. 2B) without a significant reduction in the degree of corneal opacification (Fig. 1). Another experiment similar to the one shown in Fig. 1B using five mice per group gave results similar to Fig. 1B, i.e. IFN-β treatment did not exacerbate HSK compared to the medium control (data not shown). The combined use of IL-10 and IFN-β reduced both the degree of corneal opacification (Fig. 1) and the virus spread (Fig. 2B). The establishment of HSV-1 latency in the trigeminal ganglia of the left (uninoculated) side was prevented in the IFN-β-treated groups (Table 1). The difference between the IFN-β-treated groups (both IFN-β alone and IL-10 + IFN-β) and the IL-10 treated group was significant ($P = 0.0476$).

3.2. In vitro sensitivity of KOS to IFN-β and IL-10

Plaque reduction was observed only in L-929 cells treated with IFN-β (Fig. 3). The 50% effective dose for plaque reduction of the IFN-β on L-929 cells with HSV-1 (KOS) infection was 2.0 U/ml with or without IL-10 (25 U/ml). The addition of IL-10 to IFN-β treatment did not affect

Table 1
The results of cocultivation with Vero cells of trigeminal ganglia (TG) excised from HSV-1-infected mice

Treatment	Right TG (HSV+/Total)	Left TG (HSV+/Total)
Medium	5/5	3/5
IL-10	5/5	4/5
IFN-β	3/5	0/5*
IL-10 + IFN-β	3/5	0/5*

*Significant difference ($P = 0.0476$) compared to IL-10-treated mice and $P = 0.1667$ compared to medium-treated mice.

Fig. 2. The effects of (A) IL-10 (X) and control medium (◇), and (B) IL-10 (X), IFN-β (□), IL-10 + IFN-β (●) on infectious virus spread following inoculation on the right cornea with HSV-1 (KOS). The geometric mean virus titers of three mice per point are shown. Only each one of the three IL-10 treated mice examined was HSV-1-positive for the brain (4 days post infection in panel A and 5 days post infection in panel B, respectively). In the experiment shown (panel A), one IL-10-treated mouse and one control mouse were HSV-1-positive for the left eye. No mice examined in the experiment (panel A) were HSV-1-positive in their left (uninoculated side) ganglia. In the experiment shown in panel B, only one IL-10-treated mouse was HSV-1-positive for both the left trigeminal ganglion and the left eye (7 days post infection). All the other groups were either all positive or all negative for HSV-1.

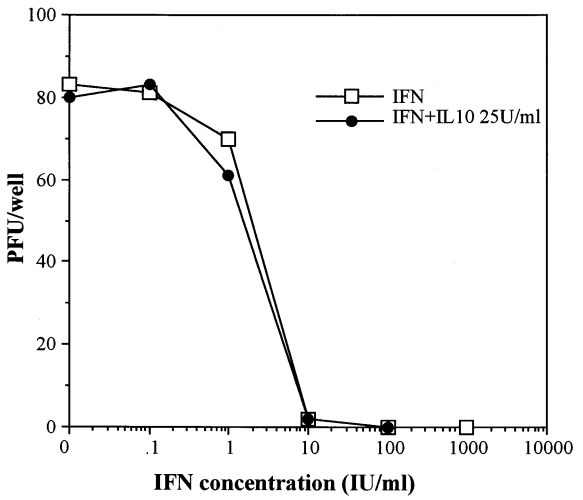

Fig. 3. The in vitro effects of IL-10 and/or IFN-β on HSV-1 (KOS) plaque formation on L-929 cells. Average counts of three wells are shown. The standard deviations were all less than 0.6 plaque counts per well.

the plaque count. The plaque count on the cells treated with 25 U/ml of IL-10 and without IFN-β is shown in Fig. 3. In those wells treated with IL-10 alone with 250 U/ml, the average plaque count per well was 83.7.

4. Discussion

Immunopathology, which possibly includes some type of autoimmunity (Avery et al., 1995) induced by viral replication (Babu et al., 1996), has been thought to play an important part in the pathogenesis of HSK (Doymaz and Rouse, 1992). We therefore attempted to selectively suppress the CD4+ Th1 immune response in infected corneal tissue by the topical application of IL-10. As reported earlier by Tumpey et al. (1994), IL-10 treatment suppressed the development of HSK without any significant alteration of the HSV-1 clearance rate compared with the infected controls without IL-10 treatment (Fig. 2A). Nevertheless, the viral replication and spread to distant neural tissues should both be inhibited as far as possible in order to prevent herpes simplex encephalitis (Ikemoto et al., 1995) and the establishment of HSV-1 latency. We therefore attempted to combine use of IFN-β with IL-10 in order to lessen the infectious virus spread (Kumano et al., 1987) with less immunopathological consequences reported compared to the application of IFN-γ instead of IFN-β. The in vitro plaque assays shown in Fig. 3 revealed that IL-10 did not either increase or decrease the HSV-1 plaque counts on L-929 cells. There was neither any apparent synergism nor antagonism in the plaque assay between the recombinant murine IL-10 and rMuIFN-β (Fig. 3).

It has been well established that T cell immunity is essential for both the clearance of replicating HSV and the subsequent recovery from infection (Doymaz and Rouse, 1992). Among other molecules produced by T cells, IFN-γ has been reported to be critically important (Smith et al., 1994). On the one hand, IFN-γ has been reported to be pathologic in the corneas of HSV-1-infected mice (Hendricks et al., 1992; Tang and Hendricks, 1996). On the other hand, IFN-β, which is available for both humans and mice, has been reported to be effective against HSV infection, when administered alone (Ophir et al., 1995), or in combination with antiviral nucleosides (Fraser-Smith et al., 1984), with antibodies (Kumano et al., 1987) or with tumor necrosis factor-α (Schmitt et al., 1992). Recently, Chen et al. (1994) reported that the synergistic anti-herpes effect of TNF-α and IFN-γ in the human cornea correlated with the induction of IFN-β. In this study, topical IFN-β treatment suppressed both local viral growth and viral spread to the brain (Fig. 2) but did not suppress corneal opacification (Fig. 1). The combined use of IL-10 and IFN-β reduced the degree of corneal opacification without exacerbating the viral spread. In addition, the establishment of an HSV-1 latent infection was also apparently prevented in the trigeminal ganglia of the left (uninoculated) side of the IFN-β-treated mice (Table 1).

Based on these findings, the combined use of IL-10 and IFN-β seemed to be more beneficial than the therapy using either of the cytokines alone. Further studies are required in the combined use of anti-inflammatory cytokines and antiviral agents including IFNs for the prevention and treatment of HSK.

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