BIKEN JOURNAL Vol. 27 189-193, 1984

SHORT COMMUNICATION

EFFECT OF INTERFERONS ON DENGUE VIRUS MULTIPLICATION IN CULTURED MONOCYTES/MACROPHAGES

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(Received September 13, 1984)

The effects of interferons on dengue virus multiplication in cultured human and mouse monocytes/macrophages were studied. Interferon treatment before, but not after virus inoculation suppressed virus multiplication dose-dependently. Recombinant human leukocyte A interferon was as effective as ordinary human fibroblast interferon in suppressing dengue virus multiplication in cultured human monocytes. Human monocytes, a population of non-proliferating cell lineage, maintained their interferon-mediated antiviral state for a few days after removal of the interferons.

Dengue fever is a common disease in tropical areas. Some patients with dengue fever develop severe symptoms, including hemorrhagic manifestations and circulatory disturbances, namely dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) (Schlesinger, 1977; Halstead, 1981). No vaccines for prevention of dengue infection have yet been established because of the possible danger of antibody-mediated enhancement of infection (Halstead, 1981).

Interferons (IFN) have been used for not only therapeutic but also prophylactic purposes in various virus infections (Dunnick and Galasso, 1979; 1980; Stewart, 1981). There are only a few data available on the effect of IFN on dengue virus infection (Liu, 1978), however, and these are concerned with infection of nerve cells of mice, not of cells of monocyte/macrophage lineage, which are the most important target cells in human dengue (Halstead and O'Rourke, 1977; Halstead et al., 1977; Brandt et al., 1979; Scott et al., 1980; Daughaday et al., 1981; Halstead, 1981). In the present study, we investigated the effects of IFN on multiplication of dengue virus in cultured monocytes/macrophages from both humans and mice to obtain fundamental data for the possible clinical application of IFN.

The following three IFN preparations were kindly provided by Dr. M. Kohase, National Institute of Health, Tokyo: (i) Recombinant human leukocyte A IFN (IFN-α; Hoffman-

La Roche Inc., Nutley, NJ., U.S.A.) produced by *Escherichia coli* by means of recombinant genetic technology, (ii) human IFN-β (BM 532; Toray Co., Ltd., Tokyo), which was induced by poly I: poly C in human foreskin fibroblast cultures, and (iii) murine IFN (types α–β mixture) produced in Newcastle disease virus-treated L929 cell cultures.

The following three macrophage systems were used to examine the effects of IFN on dengue virus infection: (i) Cultured human peripheral blood monocytes obtained from healthy donors as described previously (Böyum, 1968; Hotta et al., 1984b), and cultivated in complete RPMI 1640 medium containing 20% heat-inactivated fetal calf serum. (ii) Cultured mouse peritoneal macrophages obtained from C57 BL/6N mice, and cultivated as described previously (Hotta and Hotta, 1982; Hotta et al., 1983). These macrophages were treated with phytohemagglutinin-P (PHA, 10 μg/ml; Difco Lab., Mich., U.S.A.) for 3 days before virus inoculation, as PHA treatment increases the susceptibility of cultures to dengue virus infection (Hotta and Hotta, 1982). (iii) A mouse macrophage cell line, Mm1 (Yodoi et al., 1978; Kyoizumi et al., 1982; Hotta et al., 1984a), kindly donated by Dr. T. Masuda, Institute for Immunology, Faculty of Medicine, Kyoto University, Kyoto, and maintained in Eagle's minimum essential medium supplemented with 5% heat-inactivated fetal calf serum.

Untreated cultured cells, or cells treated with IFN were inoculated with dengue type 2 virus (D2V; Trinidad 1751 strain) at input multiplicities of 2 to 5 plaque-forming units (PFU) per cell. Since anti-dengue antibodies are reported to enhance infection of monocytes/macrophages (Halstead and O'Rourke, 1977; Halstead et al., 1977; Brandt et al., 1979; Daughaday et al., 1981; Halstead, 1981; Hotta et al., 1984a; Hotta et al., 1984b), in the present study, cells were infected with D2V both in the presence and absence of the infection-enhancing antibody (Hotta et al., 1984a; Hotta et al., 1984b). After virus adsorption for 90 min, the cells were washed with Hanks' balanced salt solution and refed with the complete medium. Portions of the culture fluids were removed every day thereafter, and stored at −80 C for titration of viral infectivity.

Antibody-mediated and unmediated D2V multiplication in cultured human monocytes was effectively suppressed by treatment of the cells with either human IFN-α or human IFN-β for 22 h before D2V inoculation

TABLE 1. *Effect of human IFN on D2V multiplication in cultured human peripheral blood monocytes*[a]

IFN	Concn. (IU/ml)	Virus yield after inoculation with:	
		D2V alone	D2V+antibody
None	—	1.1×10^3 (100)[b]	1.4×10^4 (100)
IFN-α	10	1.0×10^2 (9.1)	1.0×10^3 (7.1)
	100	5 (0.5)	1.7×10^2 (1.2)
	1,000	<5 (<0.5)	3.5×10^1 (0.2)
IFN-β	10	1.6×10^2 (14.5)	1.7×10^3 (11.8)
	100	1.5×10^1 (1.3)	1.9×10^2 (1.3)
	1,000	<5 (<0.5)	5.0×10^1 (0.4)

[a] Cultured human monocytes were pretreated with IFN for 22 h and infected with D2V in the presence or absence of infection-enhancing anti-dengue antibody (Hotta et al., 1984a; Hotta et al., 1984b). Maximum virus yields in their culture fluids were measured and are shown in PFU/ml.

[b] Numbers in parentheses indicate percentages of virus yield in the untreated control.

TABLE 2. *Effect of the time of addition of IFN and the length of IFN treatment on D2V multiplication in cultured human peripheral blood monocytes*[a]

Expt. no.	Time of IFN treatment	Suppression ratio[b]	
		IFN-α	IFN-β
1	−72 h to −48 h	5.1	6.9
	−48 h to −24 h	4.5	4.5
	−24 h to 0 h	1.3	1.3
	−6 h to 0 h	3.8	8.4
2	−24 h to 0 h	2.2	NT[c]
	+2 h to +28 h	26.0	NT
	+8 h to +28 h	28.0	NT

[a] Cultured human monocytes were treated with human IFN (100 IU/ml) at various times of infection for various periods, and were inoculated with D2V in the presence of infection-enhancing anti-dengue antibody (Hotta et al., 1984a; Hotta et al., 1984b).

[b] Suppression ratio = (Virus yield in IFN-treated cultures / Virus yield in untreated control cultures) × 100.
Virus yields in the untreated control cultures were 5.6×10^4 PFU/ml and 1.4×10^4 PFU/ml in Expt. 1 and Expt. 2, respectively.

[c] Not tested.

(Table 1). The suppressive effects of the IFNs were dose-dependent, and doses of 100 IU/ml reduced virus production to about 1% of that in untreated controls. Recombinant IFN-α was as effective as ordinary IFN-β in suppressing D2V multiplication in the monocytes.

The antiviral state of IFN-treated human monocytes was rather stable, decreasing slowly in 48 h after removal of IFN (Table 2). This stability might be related to the fact that human blood monocytes are not proliferating cells (van Furth et al., 1980). This stability is consistent with the finding that the IFN-mediated antiviral state could be maintained for several days in non-growing chick cells after removal of IFN (Isaacs and Westwood, 1959; Stewart, 1981). On the other hand, the antiviral state was incomplete when IFN was added after D2V inoculation (Table 2).

TABLE 3. *Effect of mouse IFN on D2V multiplication in cultured mouse macrophages*

IFN[a] (IU/ml)	PFU/ml[b]	
	Peritoneal macrophages[c]	Mml
None	1.1×10^8 (100)[d]	1.4×10^5 (100)
1	6.4×10^2 (58.2)	NT[e]
10	2.4×10^2 (21.8)	1.7×10^4 (11.8)
100	4.0×10^1 (3.6)	1.8×10^3 (1.3)
1,000	<10 (<1.0)	3.6×10^2 (0.2)

[a] Cultured mouse macrophages were treated with mouse IFN for 22 h before D2V inoculation.

[b] Virus yields in culture fluids 2 days after infection.

[c] Mouse peritoneal macrophages were treated with PHA (10 μg/ml) for 3 days before D2V inoculation.

[d] Numbers in parentheses indicate percentages of the virus yield in the untreated control.

[e] Not tested.

TABLE 4. *Comparison of suppressive effects of human IFN and mouse IFN on D2V multiplication*

IFN preparation[a]	Suppression ratio[b]	
	Human monocytes	Mml (mouse)
Human IFN	1.3	47.3
Mouse IFN	94.6	1.3

[a] Cultured cells (human monocytes or Mml) were treated with human IFN-α (100 IU/ml) or mouse IFN (100 IU/ml) for 22 h before D2V inoculation.

[b] Suppression ratio = (Virus yield in IFN-treated cultures / Virus yield in untreated control cultures) × 100.
Virus yields in the untreated control cultures of human monocytes and Mml cells 2 days after infection were 5.6×10^4 PFU/ml and 1.4×10^5 PFU/ml, respectively.

Suppressive effects of IFN on D2V multiplication were also observed in murine sys-

tems; mouse IFN suppressed virus production in cultures of both mouse peritoneal macrophages and Mm1 (Table 3), and the dose-dependent suppressions were comparable with those observed in the human system.
Mouse IFN was not effective in the human system and *vice versa* (Table 4).

The present results showed that IFN induced suppression of D2V multiplication in cultured monocytes/macrophages. For clinical use in prevention of aggravation of human dengue large amounts of IFN are needed and genetically recombinant IFNs should be useful for this purpose, if they have sufficient antiviral effects. We found that recombinant human leukocyte A IFN exerted the same extent of suppression as ordinary human fibroblast IFN on D2V multiplication in human monocytes.

IFN production in cultured green monkey kidney cells by dengue virus itself was reported (Russell et al., 1966). If endogenous IFN can be induced by the virus in patients with dengue, the time and dosage of exogenous IFN administered in vivo trials should be carefully determined. These factors are now under investigation.

ACKNOWLEDGMENTS

The authors are grateful to Dr. M. Kohase, National Institute of Health, Tokyo, for providing the IFN preparations used in this study. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (no. 57770344).

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