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Effect of interferon on herpes simplex virus replication in murine macrophage-like cell lines

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Summary

The effect of interferon on the replication of herpes simplex virus types 1 and 2 was studied in two murine macrophage-like cell lines which differ in their ability to synthesize interferon. Higher titers of herpes simplex virus type 1 occurred in PU5-1.8 cell cultures where interferon was not produced than in J774A.1 cell cultures where low amounts of interferon were produced. Herpes simplex virus type 2 replicated poorly in both types of cell cultures. Interferon synthesis was induced in J774A.1 cell cultures but not in PU5-1.8 cell cultures. Exogenously added interferon was shown to inhibit virus replication, however the restrictiveness of these cells to HSV replication was not relieved by treating cell cultures with anti-interferon serum. These results show that factors other than induced interferon regulate the replication of herpes simplex viruses in these cells and suggest that induced interferon synthesis does not affect herpes simplex virus replication in macrophages.

herpes simplex virus; interferon; macrophage; macrophage-like

Introduction

The ubiquitous nature of mononuclear phagocytes makes them possibly the first cells encountered by viruses invading a host. One of many possible outcomes of virus–mononuclear phagocyte interactions is the production of interferon (IFN) which may directly inhibit virus replication or indirectly control virus dissemination within an animal. Results of studies using IFN [2] and antiserum against IFN [4] show that IFN modulates the pathogenicity of herpes simplex virus (HSV) in mice. The im-

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portance of IFN has also been shown by using the mouse model of intraperitoneal infections with HSV. Higher titers of IFN were produced in serum and in spleen cell cultures of HSV type 1 (HSV-1) infected resistant mice than of susceptible mice suggesting a correlation of resistance with IFN production [18]. Similarly, the genetically determined resistance of mice to HSV type 2 (HSV-2) may also be mediated by IFN production [14]. The IFN-producing cells of mice resistant to HSV-1 were partially identified as B lymphocytes [3], but more detailed studies indicate that they may be cells of macrophage lineage [8]. Studies by Brucher et al. [1] support this possibility because peritoneal macrophages from resistant mice produced more IFN and less virus than those from susceptible mice, and resistant splenic macrophages could be made susceptible by treating them with anti-IFN serum. In contrast, Hirsch et al. [5] reported that mouse peritoneal macrophages treated with exogenous HSV-induced macrophage IFN did not inhibit HSV replication in macrophages. A similar finding has been reported for African swine fever virus replication in porcine macrophages [17]. These results suggest that mouse macrophages may be refractory to the IFN they produce.

To better understand the importance of IFN on HSV replication in macrophages, we examined the replication of HSV-1 and HSV-2 in murine macrophage-like cell lines. In contrast to primary cell cultures of macrophages, these cell lines are not contaminated with other cell types that may also produce IFN or regulate IFN production and their functional characteristics are not dependent upon the method of eliciting cells from the mouse peritoneal cavity. The macrophage-like cell lines differ in degree of maturity and functional properties as indicated by the presence or absence of various surface properties, lysosomal enzymes, secretory activities, immunoregulatory activities, and other characteristics [11,15]. They also differ in response to IFN inducers and in the amount of IFN produced [16]. In this study we used two macrophage-like cell lines, one of which is a good producer of IFN and the other a very poor producer of IFN to study the effect of induced IFN synthesis on the replication of HSV-1 and HSV-2.

Materials and Methods

Cells

Murine macrophage-like cell lines PU5-1.8 and J774A.1, were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown as monolayers in 16-oz prescription bottles containing Dulbecco's modified Eagle's medium (GIBCO Laboratories, Grand Island, NY) supplemented with 8% (v/v) fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml gentamicin. Cell cultures were incubated at 37°C in an atmosphere of 10% CO_2/90% air. To subculture, the cells were scraped from the surface with a rubber policeman into 10 ml of fresh growth medium and then added to culture vessels at a split ratio of 1:4 to 1:20, dependent upon needs.

Mouse L929 cells and Vero monkey kidney cells were purchased from Flow Laboratories, Inc. (McLean, VA). The growth medium consisted of Eagle's minimum essen-

tial medium with Hank's salts (GIBCO Laboratories), 10% (v/v) calf serum, and antibiotics as listed above. To subculture, cells were washed with PBS (0.01 M phosphate, 0.137 M NaCl, 0.003 M KCl, 0.001 M $MgCl_2$, pH 7.2), treated with a trypsin-EDTA solution, and then resuspended in growth medium to seed other culture vessels.

Viruses

Herpes simplex virus type 1, strain KOS, was provided by Robert G. Hughes, Roswell Park Memorial Institute, Buffalo, NY. Herpes simplex virus type 2, strain 196, was provided by Edward J. Young, Baylor College of Medicine, Houston, TX. Stock suspensions were prepared by infecting Vero cells at a multiplicity of 0.01 plaque-forming units (PFU)/cell. After advanced cytopathic effects, culture fluids were harvested and centrifuged at a low speed to remove cellular debris. The supernatant was filtered through a 0.2 μM Metricel membrane (Gelman Instrument Co., Ann Arbor, MI) and the filtrate stored in aliquots at -70°C. Thawed samples contained $1\text{-}2 \times 10^7$ PFU/ml.

Stocks of vesicular stomatitis virus (VSV), strain Indiana, were prepared in L929 cell cultures and stocks of Newcastle disease virus (NDV), strain California 11914, were prepared in embryonated chicken eggs as described previously [16].

Interferons and antiserum

A laboratory standard of IFN consisted of culture fluid of L929 cell cultures infected with NDV as described previously [16]. A mouse reference IFN (Reagent No. G-002-904-511) was provided by the Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD. In our laboratory the titer of this reference IFN was 1.5 times greater than the expected titer. Virus induced J774A.1 cell IFNs were prepared by inoculating monolayers of J774A.1 cells in 60-mm dishes and 1-dram vials with NDV and HSV-1, respectively. After 24 h of incubation at 37°C the culture fluids were harvested and centrifuged. The supernatants were then dialyzed for 48 h at 4°C against Sorensen's glycine I buffer (pH 2.0) to inactivate viruses. Following additional dialysis against Hank's balanced salt solution at 4°C for 24 h, these IFN preparations were stored at -70°C until needed.

Sheep antiserum to mouse L cell IFN (Reagent No. G-024-501-568) was provided by the Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health. The neutralization titer, reciprocal of the dilution of antiserum that reduces the IFN titer by a factor of ten as suggested by Kawade [6], was 94 000 against NDV-induced L929 cell IFN and 55 000 against NDV-induced J774A.1 cell IFN [16]. A 1 : 1000 dilution of the anti-IFN serum in maintenance medium was used in experiments.

Plaque assay

Serial ten-fold dilutions of HSV-1 or HSV-2 suspensions were made in maintenance medium and assayed in 24-well plates (Falcon 3047, Becton Dickinson and Co., Oxnard, CA) containing monolayers of Vero cells. Each dilution was tested in quadruplicate by inoculating each of four wells with 0.1 ml of the dilution. After an adsorption

period of 1.5 h at 37°C, 0.8 ml of maintenance medium containing 0.5% agarose type I (Sigma Chemical Co., St. Louis, MO) was added to each well. All cultures were then incubated at 37°C. After 3 days of incubation, 0.1 ml of 0.25% neutral red in PBS was added to each well and then plaques were counted the next day.

Interferon assay

Serial two-fold dilutions of sample culture fluids were assayed in L929 cell cultures to determine the dilution necessary to inhibit 50% of the PFU of a VSV suspension as described previously [16]. A laboratory standard of IFN that has been compared to the mouse reference IFN was included in each assay to express the IFN titer as international units (IU)/ml.

HSV infection of macrophages

Macrophage-like cells were grown in 1 ml of growth medium in 1-dram shell vials fitted with cork stoppers. When complete monolayers formed, cell cultures were washed twice with PBS and then inoculated with 0.1 ml of either HSV-1 or HSV-2 at a specific multiplicity of infection (MOI). Cell cultures contained $2\text{–}3 \times 10^5$ cells/vial. After 1.5 h at 37°C for virus adsorption, the inoculum was aspirated and each culture was washed two times with PBS before adding 1 ml of maintenance medium for further incubation at 37°C. At various times post infection (PI), cultures were examined for cytopathic effects (CPE) and then frozen at −70°C. Thawed samples were sonicated and centrifuged. The supernatants were either diluted to determine the number of PFU/ml or processed to determine the amount of IFN. Before assaying for IFN the supernatants were dialyzed against Sorensen's glycine I buffer (pH 2.0) at 4°C for 24 h and then dialyzed against Hanks' balanced salt solution at 4°C for 24 h.

Results

Replication of HSV in low and high IFN-producing macrophage cell lines

When inoculated with NDV, PU5-1.8 cells produce approximately 20 IU/10^6 cells of IFN and J774A.1 cells produce approximately 10 000 IU/10^6 cells within 24 h [16]. These low and high IFN-producing cell lines were inoculated with HSV-1 or HSV-2 at a low and a high MOI to determine if HSV replication in macrophages is affected by IFN induced during HSV replication. The yields of HSV-1 were greater by 1–2 $\log_{10}$ PFU/ml in PU5-1.8 cell cultures than in J774A.1 cell cultures (Table 1). Interferon was not detectable in PU5-1.8 cell cultures; however, IFN was detectable in J774A.1 cell cultures at 48 h inoculated at a low MOI and at 4, 24, and 48 h inoculated at a high MOI.

The yield of HSV-2 in PU5-1.8 and J774A.1 cell cultures was considerably lower than that of HSV-1, in spite of the similarity of CPE (Table 1). Unlike HSV-1 infected cultures, the yield of HSV-2 in both types of cell cultures was the same. Interferon was produced only in J774A.1 cell cultures inoculated at a high MOI.

A high MOI of either virus induced IFN in J774A.1 cell cultures as early as 4 h PI. When considering the maximum amount of IFN produced, this is a relatively early

TABLE 1

Replication of HSV and the amount of IFN produced (IU/ml) in PU5-1.8 and J774A.1 cells inoculated at a low and a high MOI[a]

Virus	Hours PI	low MOI						high MOI					
		PU5-1.8			J774A.1			PU5-1.8			J774A.1		
		CPE[b]	PFU/ml[c]	IU/ml[c]	CPE	PFU/ml	IU/ml	CPE	PFU/ml	IU/ml	CPE	PFU/ml	IU/ml
HSV-1	4	-	1.1×10^2	<8	-	$<2.5 \times 10^1$	<8	-	1.5×10^3	<8	-	1.3×10^3	20
	24	-	2.3×10^5	<8	-	2.1×10^4	<8	2+	2.0×10^7	<8	1+	1.1×10^6	70
	48	2+	2.9×10^7	<8	2+	3.2×10^5	120	4+	2.8×10^6	<8	3+	4.4×10^4	110
HSV-2	4	-	$<2.5 \times 10^1$	<10	-	$<2.5 \times 10^1$	<10	-	1.4×10^2	<10	-	1.4×10^2	15
	24	1+	4.2×10^3	<10	-	4.0×10^3	<10	2+	7.7×10^3	<10	2+	1.4×10^3	20
	48	2+	5.6×10^3	<10	2+	2.9×10^3	<10	3+	$<2.5 \times 10^1$	<10	4+	$<2.5 \times 10^1$	20

[a] MOI: HSV-1, low = 0.03, high = 3.0; HSV-2, low = 0.1, high = 10.

[b] CPE: - = 0%, 1+ = <25%, 2+ = 25–50%, 3+ = 50–75%, 4+ = >75%.

[c] Average titer of two experiments each assayed two times.

time period to detect virus-induced IFN. The possibility of the presence of a non-viral inducer such as bacterial endotoxin was investigated since maximum amounts of IFN are produced within 4 h after treating J774A.1 cells with lipopolysaccharide from *Escherichia coli* [16]. When the virus inoculum, HSV-1 or HSV-2, was heated at 60°C for 30 min prior to inoculating cells, IFN was not detectable at 4 h or at 24 h PI (data not shown). When 50 μg/ml of polymyxin B sulfate which binds lipids and lipopolysaccharides [13] was added to the virus inoculum, IFN was detected sometimes at 4 h and consistently at 24 h PI. Although the detection of IFN at 4 h with or without polymyxin B sulfate treatment of the virus inoculum was variable, these results suggest that when IFN is produced at 4 h PI it is probably due to HSV infection rather than possible bacterial endotoxins.

Inhibition of HSV-1 replication by IFN

To determine if the replication of HSV-1 in macrophages is sensitive to IFN, J774A.1 cell cultures as well as L929 cell cultures to serve as a non-macrophage cell control were pretreated with different IFN preparations and then inoculated with HSV-1. The yield of HSV-1 after 24 h of incubation was greatly reduced with IFNs from either NDV-induced L929 cells, NDV-induced J774A.1 cells or HSV-1 induced J774A.1 cells (Table 2). The replication of HSV-1 was equally sensitive to IFN in both J774A.1 cells and L929 cells.

Replication of HSV in the presence of anti-IFN serum

To determine if IFN produced during HSV replication affects the yield of HSV, J774A.1 cell cultures were treated during virus replication with anti-IFN serum. There was a slight increase in yield of HSV-1 and HSV-2 at 48 h but not at 24 h (Table 3). Cell

TABLE 2

Effect of IFN pretreatment of J774A.1 and mouse L929 cells on HSV-1 replication[a]

IFN source	Concentration (IU/ml)	Cell type	% Inhibition
NDV-L929	50 ± 10	J774A.1	99 ± 0
		L929	95 ± 4
	120 ± 20	J774A.1	>99
		L929	99 ± 0
NDV-J774A.1	40 ± 10	J774A.1	98 ± 4
		L929	99 ± 2
	140 ± 40	J774A.1	>99
		L929	>99
HSV-1 J774A.1	20 ± 5	J774A.1	95 ± 5
	30 ± 10		99 ± 1

[a] Cultures of either J774A.1 or L929 cells were treated with or without IFN for 18–20 h at 37°C, inoculated with HSV-1 at an MOI of 0.1, and then incubated for 24 h at 37°C. Results are expressed as the average ± S.D. of 2–3 assays.

TABLE 3

Effect of anti-IFN serum on the replication of HSV-1 and HSV-2 in J774A.1 cells inoculated at an MOI of 0.1

Virus	Antiserum treatment	PFU/ml[a] at		
		4 h	24 h	48 h
HSV-1	None	8.0×10^1	8.0×10^3	1.1×10^5
	Pretreatment	NT[b]	7.2×10^3	9.8×10^4
	During	NT	2.4×10^3	6.2×10^5
	Pretreatment and during	NT	6.5×10^3	5.0×10^5
HSV-2	None	2.6×10^1	1.3×10^2	8.8×10^1
	Pretreatment	NT	8.0×10^2	9.5×10^1
	During	NT	1.2×10^2	5.5×10^2
	Pretreatment and during	NT	1.4×10^3	2.5×10^2

[a] Average titer of two experiments. S.D. ranged from 4% to 27% of the average titers.
[b] Not tested.

cultures were also pretreated with anti-IFN serum for 18–20 h to ascertain if non-detectable amounts of possible endogenous IFN regulated the permissiveness of these cells to HSV replication. The yield of HSV-1 was not affected but there was a slightly increased yield of HSV-2 at 24 h by those cultures pretreated with anti-IFN serum. At 48 h the yields of HSV-2 were essentially identical whether the cultures were pretreated with antiserum, treated during virus replication or subjected to treatment both before and during replication.

Discussion

In this study two macrophage-like cell lines were used, one a poor producer of IFN and the other a good producer of IFN, to determine the effect of IFN on the replication of HSV-1 and HSV-2 in macrophages. We have shown that the yield of HSV-1 is greater by 1–2 $\log_{10}$ PFU/ml in PU5-1.8 cells than in J774A.1 cells. Since IFN was not detectable at 48 h in PU5-1.8 cell cultures inoculated with a low MOI but was detected in J774A.1 cell cultures under the same conditions, the decreased yield of virus in J774A.1 cell cultures may be a result of this induced IFN. In support of this are the results showing that the replication of HSV-1 is inhibited by IFN (Table 2). This is the first report showing that exogenously added IFN, including HSV-1 induced IFN, inhibits the replication of HSV-1 in macrophages. Hirsch et al. [5] reported previously that HSV-induced mouse peritoneal macrophage IFN protected mouse embryo fibroblasts against virus infection but did not protect macrophages against HSV. Furthermore, we found that the sensitivity of HSV replication in J774A.1 cells to IFN was almost equal to that of VSV, a virus considered very sensitive to the antiviral effects of IFN (data not shown).

In contrast to HSV-1 replication, the yields of HSV-2 in PU5-1.8 and J774A.1 cell

cultures inoculated at a low MOI were lower by 2–4 $\log_{10}$ PFU/ml, IFN was not detectable, and there was no difference in the amount of virus produced between the two cell types. In addition the yield of HSV-2 at 48 h was not greater than that at 24 h even though CPE increased with time of incubation. It is possible low levels of IFN that could not be detected prevented further replication of HSV-2, however we feel that this is rather unlikely for reasons discussed below.

Macrophage-like cell cultures were also inoculated at a high MOI to characterize the single-cycle replication of HSV-1 and HSV-2 in macrophages. Higher titers were obtained at 24 h than at 48 h. Some of the decrease was probably due to thermal inactivation because we have found an inactivation rate of about 1 $\log_{10}$ PFU/ml per 24 h for both viruses in cell-free maintenance media at 37°C (data not shown). The difference in titers of HSV-1 in PU5-1.8 and J774A.1 cell cultures was the same as that in cultures inoculated at a low MOI. For HSV-2 the titer was five times greater in PU5-1.8 than in J774A.1 cell cultures. Both viruses induced IFN in J774A.1 cells as early as 4 h PI, but not in PU5-1.8 cells. Interferon was also detected early in HSV-infected mouse peritoneal macrophages [1] and human monocytes [10]. Although HSV titers were lower in J774A.1 than in PU5-1.8 cell cultures, this reduction is probably not due to the IFN produced because cell cultures were infected with an MOI permitting only one cycle of replication. Since these virus titers were similar to those of cell cultures infected with a virus dose 100 times lower, requiring several cycles of virus replication, IFN does not appear to influence the replication of HSV in PU5-1.8 and J774A.1 cells.

Results with anti-IFN serum support our conclusion that HSV-induced IFN does not affect the replication of either HSV-1 or HSV-2 in macrophage-like cells since treatments with anti-IFN serum did not significantly increase virus yields. The antiserum used in our studies effectively neutralizes acid stable NDV-induced IFN in PU5-1.8 and J774A.1 cells [16] and contains antiglobulins to both alpha and beta IFN [7]. Cultures of J774A.1 cells were treated with antiserum before virus infection, during replication, and in combination. At 48 h PI there was a slightly higher titer of HSV-1 infected cultures treated with antiserum during virus replication, however the titer was considerably lower than that in PU5-1.8 cell cultures. At 24 h PI there were also slightly higher titers of HSV-2 infected cultures pretreated with antiserum than in non-treated cultures. This difference was less at 48 h PI. These differences are much lower than those reported by Brucher et al. [1] who showed that the titer of HSV-1 increased from 10^3 to 10^6 PFU/culture when splenic macrophages from resistant DBA/2 mice were treated with anti-IFN serum during HSV-1 infection. In contrast to this and similar to our results, the yield of HSV did not increase when human WISH cells or Chang human conjunctival cells were treated with anti-IFN serum [9].

Factors other than IFN appear to cause restrictiveness to HSV-1 replication in J774A.1 cells in comparison with PU5-1.8 cells. The genetic origin of these cells is not a factor because both cell types were derived from BALB/c mice. The restrictiveness of both cell types to HSV-2 replication in comparison to HSV-1 replication is also not caused by IFN. Further investigations as to the cause of the restrictiveness of these cell lines are warranted to better understand the relationship of virulence with the ability of some strains of HSV to replicate in macrophages as reported by others (reviewed in Ref. 12).

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References

1 Brucher, J., Domke, I., Schroder, H. and Kirchner, H. (1984) Experimental infection of inbred mice with herpes simplex virus. IV. Effect of interferon on in vitro virus replication in macrophages. Arch. Virol. 82, 83–93.

2 De Clercq, E. and Luczak, M. (1976) Intranasal challenge of mice with herpes simplex virus: an experimental model for evaluation of the efficacy of antiviral drugs. J. Infectious Dis. 133 (Suppl.), A226–A235.

3 Engler, H., Zawatzky, R., Goldbach, A., Schroder, C.H., Weyand, C., Hammerling, G.J. and Kirchner, H. (1981) Experimental infection of inbred mice with herpes simplex virus. II. Interferon production and activation of natural killer cells in the peritoneal exudate. J. Gen. Virol. 55, 25–30.

4 Gresser, I., Tovey, M.G., Maury, C. and Bandu, M.-T. (1976) Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, Maloney sarcoma, vesicular stomatitis, Newcastle disease, and influenza viruses. J. Exp. Med. 144, 1316–1323.

5 Hirsch, M.S., Zisman, B. and Allison, A.C. (1970) Macrophages and age-dependent resistance to herpes simplex virus in mice. J. Immunol. 104, 1160–1165.

6 Kawade, Y. (1980) An analysis of neutralization reaction of interferon by antibody: a proposal on the expression of neutralization titer. J. Interferon Res. 1, 61–70.

7 Kawade, Y. (1983) Characterization of mouse interferon molecules. In: Humoral Factors in Host Defense. Eds.: Yamamura, Y., Kishimoto, T., Hayashi, H., Muramatsu, M., Honjo, T. and Osawa, T. (Academic Press, New York) pp. 175–189.

8 Kirchner, H., Engler, H., Schroder, C.H., Zawatzky, R. and Storch, E. (1983) Herpes simplex virus type 1-induced interferon production and activation of natural killer cells in mice. J. Gen. Virol. 64, 437–441.

9 Langford, M.P., Villarreal, A.L. and Stanton, G.J. (1983) Antibody and interferon act synergistically to inhibit enterovirus, adenovirus, and herpes simplex virus infection. Infect. Immun. 41, 214–218.

10 Linnavuori, K. and Hovi, T. (1983) Restricted replication of herpes simplex virus in human monocyte cultures: role of interferon. Virology 130, 1–9.

11 Morahan, P.S. (1980) Macrophage nomenclature: where are we going? J. Reticuloendothel. Soc. 27, 223–245.

12 Morahan, P.S. (1984) Interactions of herpesviruses with mononuclear phagocytes. In: Immunobiology of Herpes Simplex Virus Infection. Eds.: Rouse, B.T. and Lopez, C. (CRC Press, Inc., Boca Raton, Florida) pp. 71–89.

13 Morrison, D.C. and Jacobs, D.M. (1976) Binding of polymyxin B to the lipid A portion of bacterial lipopolysaccharides. Immunochemistry 13, 813–818.

14 Pedersen, E.B., Haahr, S. and Mogensen, S.C. (1983) X-linked resistance of mice to high doses of herpes simplex virus type 2 correlates with early interferon production. Infect. Immun. 42, 740–746.

15 Ralph, P. (1984) Diversity and regulatory events in human and murine macrophage-related cell lines. In: Mononuclear Phagocyte Biology. Ed.: Volkman, A. (Marcel Dekker, Inc., New York) pp. 333–372.

16 Salo, R.J., Bleam, D.K., Greer, V.L. and Ortega, A.P. (1985) Interferon production in murine macrophage-like cell lines. J. Leukocyte Biol. 37, 395–406.

17 Wardley, R.C., Hamilton, F. and Wilkinson, P.J. (1979) The replication of virulent and attenuated strains of African swine fever virus in porcine macrophages. Arch. Virol. 61, 217–225.

18 Zawatzky, R., Hilfenhaus, J., Marcucci, F. and Kirchner, H. (1981) Experimental infection of inbred mice and herpes simplex virus type 1. I. Investigation of humoral and cellular immunity and of interferon production. J. Gen. Virol. 53, 31–38.