Brief Definitive Report

INTERFERON ENHANCES THE SUSCEPTIBILITY OF VIRUS-INFECTED FIBROBLASTS TO CYTOTOXIC T CELLS

By JACK F. BUKOWSKI AND RAYMOND M. WELSH

From the Departments of Pathology and of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, Massachuetts 01605

Interferon (IFN) induced during virus infection may limit disease by direct mediation of antiviral effects in cells or by modulation of the host response. IFN induces the activation and proliferation of natural killer (NK) cells (1–3), enhances macrophage-mediated phagocytosis (4) and cytotoxicity (5), and may be required for the generation of cytotoxic T cells (CTL) (6). IFN also alters the membranes of target cells, rendering them resistant to NK cell–mediated lysis (7) and inducing the expression of cell surface proteins, including major histocompatibility (MHC) antigens (8, 9). CTL recognize viral antigens in the context of syngeneic class I MHC antigens (10). It is thus possible, though not previously shown, that IFN may condition target cells for increased susceptibility to antiviral CTL by inducing MHC antigen expression. We show here that IFN greatly enhances the susceptibility of virus-infected mouse embryonic fibroblasts (MEF) to CTL-mediated lysis, and that this correlates with increased expression of mouse MHC (H-2) antigens.

Materials and Methods

Animals. C57BL/6 and BALB/c mice were purchased from The Jackson Laboratory, Bar Harbor, ME. C3H/St mice were purchased from West Seneca Laboratories, West Seneca, NY. Mice of either sex, 6–16 wk old, were used in these experiments.

Cells. Mouse embryonic fibroblasts (MEF) from BALB/c (H-2^d) or C57BL/6 (H-2^b) mice were prepared as described (16), and maintained in minimal essential medium (MEM) (Gibco Laboratories, Grand Island, NY) supplemented with antibiotics, glutamine, and 10% heat-inactivated fetal bovine serum (FBS) (M. A. Bioproducts, Walkersville, MD). The continuous cell lines L-929 (H-2^k) and MC57G (H-2^b) were also maintained on MEM. Baby hamster kidney (BHK) cells were grown in Dulbecco's MEM with 10% tryptose phosphate broth as an additive.

Viruses. Lymphocytic choriomeningitis virus (LCMV), Armstrong strain, was grown in BHK cells. Vaccinia virus (VV), strain WR, was grown in mouse L-929 cells.

Treatment of Target Cells. Target cells were dispensed in 60 mm Petri dishes and some were infected with LCMV at a multiplicity of infection (MOI) of 0.05 and incubated for 2 d. VV was added at an moi of 5.0 and incubated for 10 h. IFN-β (Lee Biomolecular, San Diego, CA) was then added to some of the virus-infected and uninfected cultures at 10,000 U/ml, and the incubation was continued for an additional 12–24 h. In some experiments, supernatant containing 64 U/ml IFN-γ generated from concanavalin A (Con A)–stimulated spleen cells was added. The cells were then used as targets in cytotoxicity assays.

Cytotoxicity Assay. Assay medium was RPMI 1640 medium supplemented with 0.1 M Hepes (Sigma Chemical Co., St. Louis, MO), 10% FBS, glutamine, and antibiotics. The assay was performed as described (1). Briefly, target cells labeled with 100 μCi

This research was supported by U. S. Public Health Research grants AI-17672 and CA 334161.

[^{57}Cr]sodium chromate (New England Nuclear, Boston, MA) for 1 h at 37°C were washed and mixed with various numbers of effector cells in round-bottomed microtiter wells at 10^4 target cells per well. For spontaneous release determination, medium was added to the wells; 1% Nonidet P-40 was added for maximum release determination. Plates were incubated for 6–8 h at 37°C in a humidified atmosphere of 5% CO_2, 95% air. At the end of the incubation, plates were centrifuged at 200 *g* for 5 min, and 0.1 ml of the supernatant was collected and counted for radioactivity in a Beckman Gamma 5500 Counter (Beckman Instruments, Palo Alto, CA). Data are expressed as percent specific release: 100 × [(cpm experimental − cpm spontaneous)/(cpm maximum − cpm spontaneous)]. Spontaneous release was 16–28%. Standard deviations of quadruplicate replica samples were <10% of the mean, and are not shown.

Quantitation of Cell Surface Antigens. For analysis of LCMV surface antigens, 5×10^5 target cells were treated with mouse anti-LCMV antiserum at a final dilution of 1:50 in a volume of 150 μl, and incubated 45 min at 4°C. The cells were washed and then treated with 100 μl fluorescein isothiocyanate–conjugated goat anti–mouse Ig (Cappell Laboratories, Cochranville, PA) at 1:40 for 45 min at 4°C. The cells were washed and subjected to analysis by flow cytometry. Surface H-2 antigens were quantitated by the same method, using congenic anti-H-2^b (B10.A anti-B10) or anti-H-2^d (B10 anti-B10.D2) antisera obtained from Dr. Rolf Kiessling, Karolinska Institute, Stockholm, Sweden, and used at a final dilution of 1:60.

Results and Discussion

We inoculated C57BL/6 (H-2^b), C3H/St (H-2^k), or BALB/c (H-2^d) mice intraperitoneally with 10^7 plaque-forming units (PFU) VV strain WR or 8×10^4 PFU LCMV, strain Armstrong. 7 d later, the mice were killed and their spleen leukocytes were used as CTL effectors in cytotoxicity assays. The target cells were low-passage C57BL/6 or BALB/c MEF, or the continuous cell lines, L-929 (H-2^k) and MC57G (H-2^b). Cells were either untreated, treated with IFN, infected with VV or LCMV, or infected and later treated with IFN, as described in Materials and Methods. After radiolabeling with chromate, we used them as targets for CTL. The results in Fig. 1, *A–C* indicate that pretreatment of VV- or LCMV-infected MEF with 10,000 U/ml IFN-β resulted in a substantial increase in the sensitivity of these targets to lysis by virus-specific CTL. IFN-β did not enhance the low levels of lysis observed with uninfected cells. Pretreatment with supernatants containing 64 U/ml IFN-γ generated from Con A–stimulated spleen cells yielded virtually identical results (data not shown). Lysis of the virus-infected IFN-β-treated targets was mediated by CTL, as it was H-2 restricted, and eliminated by pretreating the effectors with monoclonal anti-Thy-1.2 antibody and complement (C) (data not shown). In contrast to the results with T cell killing, IFN-β induced protection of both uninfected and LCMV-infected target cells against lysis by activated NK cells (Fig. 1, *D* and *H*). This demonstration of IFN-mediated protection against NK cells indicates that IFN did not increase target cell sensitivity to lysis in general. Further, IFN-treated cells did not exhibit greater spontaneous release of label, and they were equally resistant to anti-LCMV antibody plus C–mediated lysis as compared with controls (data not shown).

We observed IFN-induced enhancement of sensitivity to CTL lysis with both C57BL/6 and BALB/c MEF, which suggests that this phenomenon may be a general property of low-passage MEF (Fig. 1, *A–C*). However, IFN-β pretreatment of virus-infected continuous cell lines, i.e., LCMV-infected L-929 (Fig. 1*E*), VV-infected L-929 (data not shown), LCMV-infected MC57G (Fig. 1*F*),

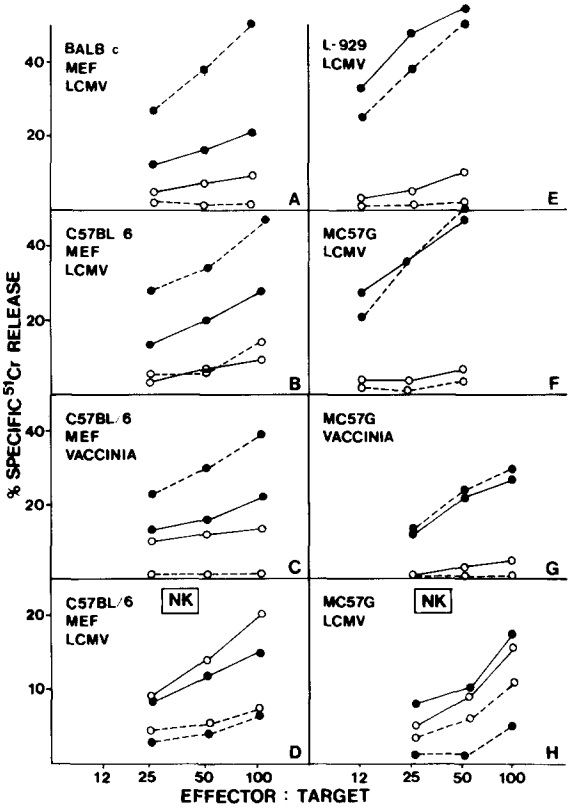

FIGURE 1. Enhancement of LCMV- and VV-specific T cell–mediated lysis by pretreatment of MEF with IFN. (●—●) Virus-infected targets. (●—●) Virus-infected targets pretreated with IFN-β. (○—○) Uninfected targets. (○—○) Uninfected targets pretreated with IFN-β. (*A*) BALB/c day-7 LCMV-immune spleen cells were used as CTL effectors against LCMV-infected or uninfected BALB/c MEF. (*B*) Same as *A*, except CTL were C57BL/6 and targets were C57BL/6 MEF. (*C*) C57BL/6 day-7 VV-immune spleen cells were used as effectors against VV-infected or uninfected C57BL/6 MEF. (*D*) C57BL/6 spleen cells 3 d after LCMV infection were used as a source of NK cell effectors (1) against LCMV-infected or uninfected C57BL/6 MEF. (*E*) C3H/St day-7 LCMV-immune spleen cells were used as CTL effectors against LCMV-infected or uninfected L-929 cells. (*F*) C57BL/6 day-7 LCMV-immune spleen cells were used as CTL effectors against LCMV-infected or uninfected MC57G target cells. (*G*) Same as *C*, except targets were MC57G. (*H*) Same as *D*, except targets were MC57G.

VV-infected MC57G (Fig. 1 *G*), did not enhance their sensitivity to lysis by CTL. This result was anticipated, as these targets are already highly sensitive to lysis by CTL.

The structures recognized by virus-specific CTL are virus-induced surface proteins in association with class I MHC antigens (10). IFN is known to enhance the cell surface expression of both MHC (8, 9) and certain viral antigens (11) in some systems. To investigate the possibility that IFN was altering surface antigen expression on MEF, we quantitated surface H-2 and LCMV antigens by treating MEF with fluorescein-labeled antibodies and analyzing the cells by flow cytometry. IFN-β pretreatment of MEF had no significant effect on the expression of surface LCMV antigens (data not shown), but both uninfected and LCMV-infected MEF had substantial increases in surface H-2 expression (Fig. 2, *A*, *C*, and *F*). Analysis of the continuous cell line MC57G also showed that surface

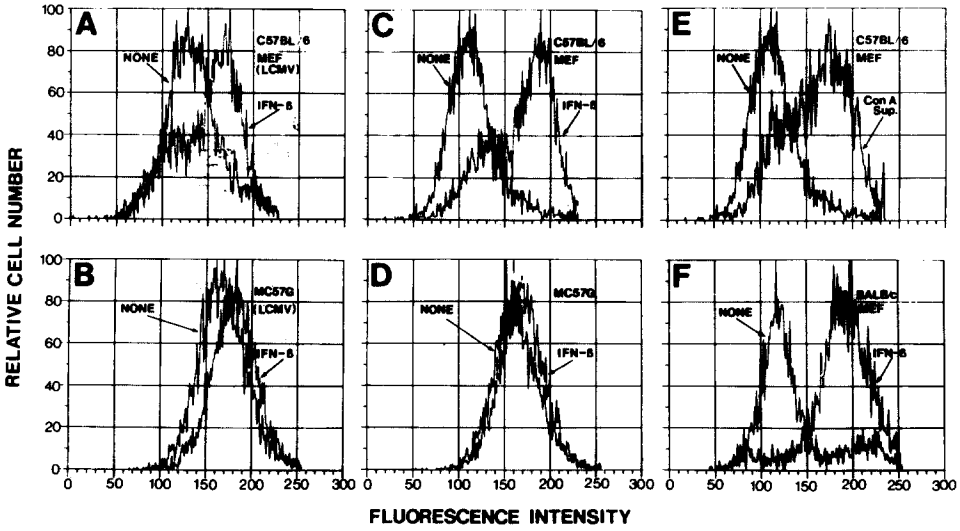

FIGURE 2. Surface expression of H-2 antigens. (*A*) LCMV-infected C57BL/6 MEF. (*B*) LCMV-infected MC57G. (*C*) Uninfected C57BL/6 MEF. (*D*) Uninfected MC57G. (*E*) Uninfected C57BL/6 MEF. (*F*) Uninfected BALB/c MEF.

expression of LCMV antigens remained unchanged after IFN-β pretreatment (data not shown), but, in contrast to the MEF, MC57G cells had similar levels of surface H-2 antigens whether or not they were pretreated with IFN-β (Fig. 2, *B* and *D*). Infection with LCMV had only a minor effect on this observation. MC57G cells were sensitive to other IFN-mediated effects, as IFN protected these cells from NK cell–mediated lysis (Fig. 1*H*). The level of surface H-2 on the untreated MC57G cells was similar to that on IFN-treated MEF, suggesting that MC57G cells may already express a level of surface H-2 antigens high enough for efficient association with viral antigens to be good targets for CTL. Treatment of MEF (Fig. 2*E*) or MC57G cells (data not shown) with supernatants containing 64 U/ml IFN-γ generated from Con A–stimulated spleen cells yielded nearly identical results.

IFN-induced enhancement of surface H-2 expression was dose dependent, and supernatants containing IFN-γ were more potent than IFN-β as inducers of H-2 antigens (Table I), in agreement with previously published observations (12). Similarly, fewer units of IFN-γ were needed to render targets more susceptible to lysis by CTL (Table I).

Since MHC class I–restricted, virus-specific T cells are known to eliminate virus in vivo (13), it is possible that IFN enhances this process by increasing the sensitivity of virus-infected cells to lysis by CTL. The source of the IFN could be either virus-induced IFN-α or IFN-β, or IFN-γ produced by T cells upon recognition of a target (14). T cell–produced IFN-γ may locally enhance MHC expression in focal areas of infection, thereby increasing the sensitivity of T cell recognition of virus-infected tissue. Recent findings (15) show that IFN treatment of mice infected intracranially with LCMV leads to increased mortality. Since death in this system has been shown to be caused by virus-specific T cell–dependent destruction of brain tissue (16), it is possible that IFN may be enhancing H-2 antigen expression on the surface of brain tissue (12), leading to

TABLE I

Enhancement of H-2 Expression and of Sensitivity to CTL-mediated Lysis by Various Concentrations of IFN-β and IFN-γ

IFN		Mean fluorescence intensity	Percent cells fluorescing above channel 175	Percent specific ^{51}Cr release
	U/ml			
IFN-β	10,000	1,038 (426)	78 (45)	41 (31)
	3,333	1,120 (508)	77 (44)	39 (29)
	1,111	1,120 (508)	80 (47)	35 (25)
	370	1,078 (466)	80 (47)	36 (26)
	123	893 (281)	69 (36)	29 (19)
	41	860 (248)	61 (28)	29 (19)
	14	739 (127)	49 (16)	27 (17)
	4.6	712 (100)	46 (13)	27 (17)
	1.5	660 (48)	40 (7)	15 (5)
	0.0	612 (0)	33 (0)	9.9 (0)
IFN-γ	64	1,302 (690)	85 (52)	35 (25)
	21	963 (351)	68 (35)	34 (24)
	7.1	1,000 (388)	70 (37)	33 (23)
	2.4	963 (351)	70 (37)	29 (19)
	0.8	ND*	ND	25 (15)
	0.3	797 (185)	54 (21)	29 (19)
	0.09	686 (74)	44 (11)	17 (7)
	0.00	612 (0)	33 (0)	9.9 (0)

Various concentrations of IFN-β and Con A supernatant containing IFN-γ activity were added to LCMV-infected MEF. 24 h later, the MEF were used as targets in a CTL assay and also quantitated for surface H-2 expression by flow cytometry as described in Materials and Methods. Fluorescence is expressed as the percentage of cells fluorescing above channel 175, or as the mean fluorescence intensity of all the cells in the sample. 61 channels are one order of magnitude of fluorescence intensity; the gain was set at 1.7. The numbers in parentheses represent the differences between the IFN-treated cells and the untreated control cells.

* ND, not done.

more destruction of LCMV-infected cells by virus-specific T cells. This IFN-induced enhancement of MHC antigens on virus-infected cells may thus augment T cell–dependent immunopathology as well as T cell–dependent clearance of virus.

Summary

Interferon (IFN) pretreatment of low-passage mouse embryonic fibroblasts (MEF) infected with lymphocytic choriomeningitis virus or vaccinia virus rendered these cells two to three times more susceptible to lysis by H-2 restricted, virus-specific cytotoxic T lymphocytes (CTL) than control, virus-infected MEF. The increased sensitivity to lysis correlated with increased expression of surface H-2 antigens, but not viral antigens. Continuous cell lines already highly sensitive to CTL-mediated lysis and already expressing high levels of surface H-2 antigens were unaffected by IFN pretreatment. These results suggest that IFN treatment, by increasing surface H-2 levels, may result in increased association of surface H-2 and virus antigens, leading to enhanced recognition and lysis by virus-

specific CTL.

We thank Ms. Marcia McFadden for performing the flow cytometry, and Ms. Dottie Walsh for typing the manuscript.

Received for publication 5 July 1984 and in revised form 10 October 1984.

References

1. Welsh, R. M. 1984. Interferon and natural killer cells. *CRC Crit. Rev. Immunol.* 5:55.
2. Biron, C. A., L. A. Turgiss, and R. M. Welsh. 1983. Increase in NK cell number and turnover rate during acute viral infection. *J. Immunol.* 131:1539.
3. Biron, C. A., G. Sonnenfeld, and R. M. Welsh. 1984. Interferon induces natural killer cell blastogenesis in vivo. *J. Leuk. Biol.* 35:31.
4. Huang, K. Y., R. M. Donahoe, F. B. Gordon, and H. R. Dressler. 1971. Enhancement of phagocytosis by interferon-containing preparation. *Infect. Immun.* 4:581.
5. Schultz, R. M., and M. A. Chirigos. 1979. Selective neutralization by anti-interferon of macrophage activation by L-cell interferon, Brucella abortus ether extract, Salmonella typhimurium lipopolysaccharide, and polyanions. *Cell. Immunol.* 48:52.
6. Farrar, W. L., H. M. Johnson, and J. L. Farrar. 1981. Regulation of the production of immune interferon and cytotoxic T lymphocytes by interleukin-2. *J. Immunol.* 126:1120.
7. Trinchieri, G., and D. Santoli. 1978. Anti-viral activity induced by cultivating lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferons and antagonistic inhibition of susceptibility of target cells to lysis. *J. Exp. Med.* 147:1314.
8. Lindahl, P., I. Gresser, P. Leary, and M. Tovey. 1976. Interferon treatment of mice. Enhanced expression of histocompatability antigens on lymphoid cells. *Proc. Natl. Sci. USA.* 73:1284.
9. Wong, G. H. W., I. Clark-Lewis, J. L. McKimm-Breschkin, A. W. Harris, and J. W. Schrader. 1983. Interferon γ induces enhanced expression of Ia and H-2 antigens on B lymphoid, macrophage, and myeloid cell lines. *J. Immunol.* 131:788.
10. Zinkernagel, R. M., and P. C. Doherty. 1975. H-2 compatibility requirement for T cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. Different cytotoxic T-cell specificities are associated with structures coded for in H-2^k or H-2^d. *J. Exp. Med.* 141:1427.
11. Chang, E. H., S. J. Mims, T. J. Triche, and R. M. Friedman. 1977. Interferon inhibits mouse leukaemia virus release: an electron microscope study. *J. Gen. Virol.* 34:363.
12. Wong, G. H. W., P. F. Bartlett, I. Clark-Lewis, F. Battye, and J. W. Schrader. 1984. Inducible H-2 and Ia antigens on brain cells. *Nature (Lond.).* 310:688.
13. Zinkernagel, R. M., and R. M. Welsh. 1976. H-2 compatibility requirement for virus-specific T cell-mediated effector functions in vivo. *J. Immunol.* 117:1495.
14. Morris, A. G., Y. L. Yin, and B. A. Askonas. 1982. Immune interferon release when a cloned cytotoxic T cell line meets its correct influenza-infected target cell. *Nature (Lond.).* 295:150.
15. Jacobson, S., R. M. Friedman, and C. J. Pfau. 1981. Interferon induction by lymphocytic choriomeningitis viruses correlates with maximum virulence. *J. Gen. Virol.* 57:275.
16. Cole, G. A., N. Nathanson, and R. A. Prendergast. 1972. Requirement for θ-bearing cells in lymphocytic choriomeningitis virus-induced central nervous system disease. *Nature (Lond.).* 238:335.
17. Selgrade, M. J. K., and J. E. Osborn. 1973. Divergence of mouse brain interferon response following virulent or avirulent Newcastle disease virus inoculation. *Proc. Soc. Exp. Biol. Med.* 143:12.