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Neutralization of Interferon Activity in Homologous and Heterologous Cells with Homologous and Heterologous Antibody (40953)

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Abstract. Human fibroblast interferon (HFIF) and human leukocyte interferon (HLIF) can protect mouse L-929 cells against the cytopathic effects of vesicular stomatitis virus (VSV), although HLIF is about 60 times and HFIF about 1200 times less active than mouse interferon (MIF). This relationship suggests that, with repsect to the mouse L-929 receptor, there exists a greater similarity between the active sites of MIF and HLIF than between MIF and HFIF. Employing antibodies directed against MIF, HFIF, and HLIF as a probe, we found interferon–cell interactions to be stronger for homologous than for heterologous combinations. A variable-fit model is proposed to explain the differences that interferons display in cross-species protection. Thus, the closer the fit between interferon and receptor, the greater the antiviral response.

Interferons of one species have been shown to protect cells of other species, albeit to a diminished degree as compared to homologous interferon, viz. human leukocyte interferon (HLIF)[3] on bovine, porcine, and murine cells (1); mouse interferon (MIF) on human cells (2, 3); avian interferons on other avian cells (4); porcine interferon on bovine and human cells (5); and bovine interferon on porcine (6), monkey, and human cells (7). Moreover, some interferons of heterologous origin have been reported to exhibit greater activity than homologous interferons. Examples of this are human fibroblast interferon (HFIF) on rabbit cells (8), human leukocyte interferon on bovine cells (9), bovine interferon on lamb cells (10), and the low molecular weight component of human leukocyte interferon on feline cells (11).

We now present definitive evidence that HFIF is capable of protecting mouse cells against viral infection. When compared to HLIF and MIF, it is about 20 and 1200 times less active, respectively.

In light of our own findings that human interferon can protect mouse cells, and of the previous knowledge that mouse interferon can protect human cells (2), we have also examined the effects of neutralization of human and mouse interferons by both homologous and heterologous antisera in both homologous and heterologous cells. The interactions between interferon, antibody and cell receptor are discussed, and two additional models regarding the mechanisms of cross-species specificity of interferons are proposed.

Materials and methods. Cells and viruses. L-929 cells and human diploid skin fibroblasts (VGS strain) were grown in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal calf serum (FCS). The Kumarov strain of Newcastle disease virus (NDV) was used for interferon induction. The stock virus was pre-

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[3] Abbreviations used: anti-HFIF, antibodies directed against human fibroblast interferon; anti-HLIF, antibodies directed against human leukocyte interferon; anti-MIF, antibodies directed against mouse interferon; EMEM, Eagle's minimum essential medium; FCS, fetal calf serum; HFIF, human fibroblast interferon; HLIF, human leukocyte interferon; L-929, transformed mouse cell line derived from embryo fibroblasts; MIF, mouse interferon; NDV, Newcastle disease virus; VGS, human diploid fibroblast cell line derived from a human skin biopsy; VSV, vesicular stomatitis virus.

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pared in the allantoic fluid of 10-day-old chick embryos. The challenge virus used for all assays was the Indiana strain of vesicular stomatitis virus (VSV). The stock virus was propagated in BSC-1 cells.

Interferons. Crude MIF was produced by a modification of the procedure described by Knight (12). L-929 cells, grown to near confluency in 0.5-gal roller bottles, were primed for 2 hr at 37° with 100 units/ml of MIF in EMEM containing 10% FCS. The primer was decanted and replaced with 1.4 $\times 10^8$ PFU/ml of NDV in EMEM. After incubation for 1 hr at 37°, the NDV was discarded, and the cells were washed in phosphate-buffered saline (PBS) containing magnesium and calcium. The bottles were then replenished with EMEM and incubated at 37° for 18 hr.

HFIF and HLIF were the generous gifts of Dr. A. Billiau, Leuven, Belgium, and Dr. K. Cantell, Helsinki, Finland, respectively.

Anti-interferons. Antibodies directed against MIF (anti-MIF) and HFIF (anti-HFIF) were produced in goats (I. A. Braude, Ph.D. thesis, K. U. Leuven, 1978). Anti-HLIF was the generous gift of Dr. K. Cantell, Helsinki, Finland.

Interferon assays. MIF was assayed by the inhibition of VSV-induced cytopathogenicity (CPE) in L-929 cells. All assays included an internal standard calibrated against the National Institute of Health mouse reference standard G 002-904-511.

Both HLIF and HFIF were titrated in a similar VSV CPE inhibition assay on VGS cells. All assays included an internal standard calibrated against the British Research Standard for interferon, MRC B69/19.

For all assays, except those involving VGS cells treated with MIF, heterologous interferon activity was detected by VSV CPE inhibition. However, this CPE inhibition assay was not sensitive enough to detect MIF activity on VGS cells. Therefore, a virus yield reduction assay was employed to titrate MIF on VGS cells. Human diploid fibroblasts, grown to confluency in 60 $\times$ 15-mm tissue culture dishes (Falcon), were incubated with MIF for 18 hr at 37°. Cells were then washed three times with EMEM and challenged with 1.2 $\times 10^4$ PFU/ml VSV. After 1 hr at 37°, the cells were washed once in EMEM and incubated for a further 18 hr at 37°. Cells were then frozen at −70°, thawed, and scraped, and progeny were titrated in a plaque assay using L-929 cells. Only cell-associated VSV was measured in these assays, as it was considered to be a more reliable parameter of virus replication than the virus released from the cells in the supernatant fluid.

Anti-interferon neutralization assays. The neutralization end points of antibodies directed against interferons were determined by a modification of the microtiter technique described by Havell *et al.* (13). Prior to incubation with cells, antibody–antigen mixtures were incubated for 4 hr at 37°. The titers were expressed as the highest twofold dilution that restored partial antiviral activity (i.e., first indication of protection) multiplied by the final concentration of interferon used.

Results. Activities of mouse interferon (MIF), human fibroblast interferon (HFIF), and human leukocyte interferon (HLIF) on homologous and heterologous cells. Table I presents a comparison of the antiviral activities of mouse and human interferon on homologous and heterologous cells. Although HFIF is active on L-929 cells, it is approximately 20 times less so than HLIF. Thus, with respect to the mouse cell, MIF provides greater protection than HLIF, which in turn is more active than HFIF.

Neutralization of the antiviral activities of MIF, HFIF, and HLIF on homologous and heterologous cells by homologous and heterologous antibody. Titers representing the neutralization of the antiviral activities of MIF, HFIF, and HLIF by anti-MIF, anti-HFIF, and anti-HLIF, as assayed on mouse L-929 and human VGS cells, are shown in Table II.

In order to equate titers expressed on L-929 and VGS cells, a ratio, based on the results presented in Table I, has been established. One unit per milliliter of MIF activity on L-929 cells is approximately equivalent to $10^{3.1}$ units/ml of HFIF and $10^{1.8}$ units/ml of HLIF. Also, $10^{4.0}$ units/ml of MIF are equivalent to 1 unit/ml of activity on VGS cells. Thus, the neutralization titer is expressed as the product of the highest dilution of antibody restoring partial

TABLE I. TITERS OF INTERFERON WHEN ASSAYED ON EITHER HOMOLOGOUS OR HETEROLOGOUS CELL CULTURES

Interferon	Titer ($\log_{10}$ units/ml)		
	Mouse L-929 cells	Human VGS cells	Difference
MIF	6.5	2.5	4.0
HFIF	2.9	6.0	3.1
HLIF	5.2	7.0	1.8

protection times the number of units per milliliter of interferon used, as expressed on the cells tested.

The expected neutralization titer is what the end point would supposedly have been if tested in the other cell system. Therefore, for example, based on the ratio of activities for MIF assayed on both L-929 and VGS cells, a titer of $10^{5.6}$ neutralizing units/ml for anti-MIF tested toward MIF on L-929 cells is expected to have a titer of $10^{1.6}$ neutralizing units/ml if assayed on VGS cells; and a titer of $10^{3.0}$ neutralizing units/ml for anti-MIF tested toward MIF on VGS cells is expected to have a titer of $10^{7.0}$ neutralizing units/ml if assayed on L-929 cells (Table II).

The deviation of the actual from the expected results was used as a tool to evaluate the interaction between anti-interferon and interferon on both homologous and heterologous cells. Interferon/anti-interferon mixtures tested on heterologous (with respect to the interferon) cells gave a neutralization titer that was lower than that found on homologous (with respect to the interferon) cells: compare, for example, the neutralization titers observed for the combination HLIF/anti-HLIF on VGS and L-929 cells (Table II). When interferon–antibody mixtures were assayed on heterologous cells (with respect to the interferon), the observed neutralization titer was always greater than the expected one, whereas the converse was true when the assays were run on homologous cells: compare, for example, the neutralization titers observed and expected for the combination HFIF/anti-HFIF on L-929 and VGS cells (Table II).

In support of some of our findings, Gresser *et al.* (14) reported that anti-HLIF, having a titer of $10^{5.3}$ neutralizing units/ml against HLIF on human cells, had a titer of

TABLE II. NEUTRALIZATION TITERS OF ANTI-INTERFERON SERA WHEN ASSAYED ON EITHER HOMOLOGOUS OR HETEROLOGOUS CELL CULTURES

Anti-interferon	Interferon	Neutralization titer ($\log_{10}$ units/ml)[a]			
		Mouse L-929 cells		Human VGS cells	
		Observed	Expected	Observed	Expected
Anti-MIF	MIF	5.6	7.0	3.0	1.6
	HFIF	3.5	−2.1	1.0	6.6
	HLIF	3.1	0.4	2.2	4.9
Anti-HFIF	MIF	<1.0	4.3	0.3	<−3.0
	HFIF	4.7	1.9	5.0	7.8
	HLIF	<1.2	<−2.8	<1.0	<3.0
Anti-HLIF	MIF	<2.1	6.7	2.7	<−1.9
	HFIF	3.6	2.5	5.6	6.7
	HLIF	4.8	4.2	6.0	6.6

[a] As determined by multiplying the highest dilution of antibody restoring partial interferon activity with the number of interferon units used. If this number of interferon units was derived from the same cells as those employed for the neutralization assay, the neutralization titer is listed under "Observed." If the number of interferon units was derived from the other cells, taking into account the differences in interferon titer between the homologous and heterologous cell systems (see Table I), the neutralization titer is listed under "Expected."

$10^{1.0}$ neutralizing units/ml when assayed with MIF on mouse cells.

It is also noteworthy that, as previously reported (15, 16), anti-HLIF neutralized HFIF, while the converse was not observed (Table II). In fact, anti-HLIF was found to neutralize HFIF to a far greater extent than previously described (15, 16). A high neutralization end point was also observed on heterologous cells. It is possible that the anti-HLIF batch used in our assays contained some anti-HFIF, since the buffy coats that served as the source of HLIF may have yielded a small proportion of HFIF, which could have acted as a contaminant immunogen. (HFIF, produced from $(I)_n \cdot (C)_n$-induced cell cultures, would contain no HLIF.)

Antisera adsorbed on cells had essentially the same titer as the nonadsorbed antisera (Table II). This eliminates the possibility that the results obtained could be attributed to antibodies directed against antigenic determinants of cellular origin.

Discussion. In line with prior reports (17, 18) alluding to some (e.g., priming) activity of human interferon on mouse L-929 cells, we have now demonstrated that human interferon can indeed protect mouse cells against viral infection. The fact that HLIF is more active than HFIF on L-929 cells may reflect a greater structural similarity between the active sites of MIF and HLIF than between MIF and HFIF. Although L-929 cells distinguish between HLIF and HFIF, anti-MIF does not. This implies that the active sites may not be very antigenic with respect to anti-MIF, which is possible if they were located within pockets of the molecule and thus not accessible to the antibody.

When interferon–antibody mixtures were assayed on homologous cells (with respect to the interferon), the actual neutralization titer was always less than the expected one, whereas the converse was true when the assays were run on heterologous cells. This might be anticipated if the interactions between interferon and cells were strong for the homologous combination, but weak for the heterologous. If the interferon–cell associations were weak, the antibody could more effectively neutralize the interaction. When tested on homologous cells, the actual titer obtained was lower than predicted from the heterologous cell results, because the interferon–cell interactions were stronger and therefore less neutralization occurred. But, when tested on heterologous cells, the actual titer obtained was higher than predicted from the homologous cell results, because the interferon–cell interactions were weaker and therefore more neutralization occurred.

The neutralization of interferon by its antibody may take place by two mechanisms. Antibody, once having interacted with the antigen, could either sterically hinder the antigen's ability to associate with a cell receptor, or shift its tertiary structure so that it is no longer recognized by the cell.

Paucker and his colleagues (18) have proposed an attractive model to explain the cross-species activities of interferon. They contend that interferons, or at least HFIF and HLIF, possess multiple active sites, and thus each interferon molecule is capable of interacting with cells of another species. The number and distribution of these sites would vary considerably according to the source of interferon, and this variation would account for the differences in activity observed (18).

There are, however, two other possible mechanisms by which cross-species activities could occur. The first considers interferons of a given species to be comprised of subpopulations which, although physicochemically similar, contain slight variations within specific regions. This would be analogous to the situation of antibodies. One, or many, of these subpopulations may be able to interact fully with cells of certain other species, but, as they comprise only a minor fraction of the whole interferon population, the overall heterologous activity of the interferon preparation may be relatively low. For mouse interferon a subspecies has been isolated which is antigenically related to HLIF and which might be responsible for part, if not all, of the heterologous activity of MIF on human cells (3, 19).

The second model considers only one interferon within a given species. The ac-

tive sites between species would be similar and the degree of cell protection conveyed would be dependent upon the physicochemical fit between interferon and cell receptor. By this arrangement the receptor, in concert with some amplification (20) and/or activator (21) system, would modulate the cells' response to interferon. The closer the fit, the greater the signal conveyed, and the more protection conferred. In addition, this model would also apply for cell hybrids which contain the receptor of one species but the antiviral machinery of another (22).

The evidence presented in this report supports the last model. As one would anticipate when interferon is added to heterologous cells, a weak fit would create both greater neutralization by antibody and a diminished antiviral signal. Conversely, as seen with interferon applied onto homologous cells, less neutralization and a greater antiviral signal would suggest a close fit between interferon and its receptor. Both the "multiple active site" model and the "subpopulation" model (see above) require interaction between interferon and the cell to be uniform, since differences in heterologous activities would be accounted for by relevant molecular sites or populations, rather than interferon–receptor affinities.

A logical sequel to the studies reported here would be to examine the neutralization effect of anti-interferon sera on those interferons that are more active on heterologous cells than on homologous cells (8–11), particularly with antisera derived from the same animal as the heterologous cell. It would also be interesting to test the ability of anti-interferon antibody to neutralize the other acknowledged biological properties of interferon, such as cell growth inhibition (23–27), enhancement of cytotoxicity to double-stranded RNA (28, 29), and particularly, the modulating (suppressive and enhancing) effects of interferon on the immune response (30–33). Perhaps, with this approach a distinction could be discerned between the multiple functions of interferon.

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1. Paucker, K., Dalton, B. J., Törmä, E. T., and Ogburn, C. A., J. Gen. Virol. 35, 341 (1977).
2. Bodo, G., Palese, P., and Lindner, J., Proc. Soc. Exp. Biol. Med. 137, 1392 (1971).
3. Havell, E. A., Virology 92, 324 (1979).
4. Moehring, J. M., and Stinebring, W. R., Nature (London) 226, 360 (1970).
5. Carter, W. A., Davis, L. R., Jr., Chadha, K. C., and Johnson, F. H., Jr., Mol. Pharmacol. 15, 685 (1979).
6. Ahl, R., and Rump, A., Infect. Immunity 14, 603 (1976).
7. Tovey, M. G., Bandy, M.-T., Begon-Lours, J., Brouty-Boyé, D., and Gresser, I., J. Gen. Virol. 36, 341 (1977).
8. Desmyter, J., Rawls, W. E., and Melnick, J. L., Proc. Nat. Acad. Sci. USA 59, 69 (1968).
9. Gresser, I., Bandu, M. T., Brouty-Boyé, D., and Tovey, M., Nature (London) 251, 543 (1974).
10. Rinaldo, C. R., Jr., Isackson, D. W., Overall, J. C., Jr., Glasgow, L. A., Brown, T. T., Bistner, S. I., Gillespie, J. H., and Scott, F. W., Infect. Immunity 14, 660 (1976).
11. Desmyter, J., and Stewart W. E., II, Virology 70, 451 (1976).
12. Knight, E., Jr., J. Biol. Chem. 250, 4139 (1975).
13. Havell, E. A., Vilcek, J., Falcoff, E., and Berman, B., Virology 63, 475 (1975).
14. Gresser, I., Tovey, M. G., Bandu, M. T., Maury, C., and Brouty-Boyé, D., J. Exp. Med. 144, 1305 (1976).
15. Havell, E. A., Berman, B., Ogburn, C. A., Berg, K., Paucker, K., and Vilcek, J., Proc. Nat. Acad. Sci. USA 72, 2185 (1975).
16. Berg, K., Ogburn, C. A., Paucker, K., Mogensen, K. E., and Cantell, K., J. Immunol. 114, 640 (1975).
17. Levy-Koenig, R. E., Golgher, R. R., and Paucker, K., J. Immunol. 104, 791 (1970).
18. Paucker, K., Dalton, B. J., Ogburn, C. A., and Törmä, E., Proc. Nat. Acad. Sci. USA 72, 4587 (1975).
19. Stewart, W. E., II, and Havell, E. A., Virology 101, 315 (1980).
20. Chany, C., Biomedicine 24, 148 (1976).
21. Chany, C., Grégoire, A., Vignal, M., Lemaitre-Moncuit, J., Brown, P., Besançon, F., Suarez, H., and Cassingena, R., Proc. Nat. Acad. Sci. USA 70, 557 (1973).

22. Slate, D. L., Shulman, L., Lawrence, J. B., Revel, M., and Ruddle, F. H., J. Virol. **25**, 319 (1978).
23. Buffett, R. F., Ito, M., Cairo, A. M., and Carter, W. A., J. Nat. Cancer Inst. **60**, 243 (1978).
24. De Maeyer-Guignard, J., Tovey, M. G., Gresser, I., and De Maeyer, E., Nature (London) **271**, 622 (1978).
25. Knight, E., Jr., Nature (London) **262**, 302 (1976).
26. Stewart, W. E., II, Gresser, I., Tovey, M. G., Bandu, M.-T., and Le Goff, S., Nature (London) **262**, 300 (1976).
27. Tovey, M., Brouty-Boyé, D., and Gresser, I., Proc. Nat. Acad. Sci. USA **72**, 2265 (1975).
28. De Clercq, E., and De Somer, P., J. Gen. Virol. **27**, 35 (1975).
29. Stewart, W. E., II, De Clercq, E., and De Somer, P., J. Gen. Virol. **18**, 237 (1973).
30. Gisler, R. H., Lindahl, P., and Gresser, I., J. Immunol. **113**, 438 (1974).
31. Huang, K. Y., Donahoe, R. M., Gordon, F. B., and Dressler, H. R., Infect. Immunity **4**, 581 (1971).
32. Lindahl, P., Leary, P., and Gresser, I., Proc. Nat. Acad. Sci. USA **69**, 721 (1972).
33. Sonnenfeld, G., Mandel, A. D., and Merigan, T. C., Cell. Immunol. **34**, 193 (1977).

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