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Enhanced Expression of Histocompatibility Antigens on Interferon-Treated Mouse Embryonic Fibroblasts[1] (40076)

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Interferon induces pronounced modifications of the surface of cells both *in vitro* (1–8) and *in vivo* (9, 10) and these changes may well be important in understanding the varied biologic effects of interferon (11). In investigating the effect of interferon on the cell surface, we showed previously that incubation of murine lymphoma and normal lymphoid cells with interferon resulted in a marked enhancement in the expression of surface histocompatibility (H-2) antigens (2, 3). Likewise inoculation of mice with interferon or interferon inducers resulted in an increase in the expression of H-2 antigens on the surface of thymocytes and splenic lymphocytes (9, 10). H-2 antigens on viral infected target cells appear to be an integral part of the recognition and destruction of these cells by cytotoxic lymphocytes (12–18). Since we have shown that interferon increases the expression of H-2 antigens on lymphoid cells and enhances the cytotoxicity of sensitized lymphocytes (19), it seemed important to determine whether interferon, a product of viral infected cells, could also modify the expression of H-2 antigens on nonlymphoid cells, or whether this effect was restricted to lymphoid cells. The results presented herein show that mouse interferon does in fact markedly enhance the expression of H-2 antigens on mouse embryonic fibroblasts.

Materials and methods. Mice. Pathogen free DBA/2, C3H and C57Bl/6 mice were obtained from the breeding colonies of the Institut du Cancer. Breeding couples of B10 and B10 D2 mice were obtained from Jackson Laboratories Bar Harbor, Maine and a colony was maintained at the Institut du Cancer.

Interferon and control preparations. Mouse interferon was prepared from C-243 cells induced with Newcastle Disease virus. The techniques of preparation, concentration and assay have been previously described (20). The control preparation consisted of medium harvested from uninduced C-243 cell cultures. The cell free supernatant was processed and concentrated in a manner identical to that used in the preparation of interferon (20). In one experiment brain interferon was prepared from mice inoculated intracerebrally with West Nile virus (21). One of our units, as expressed in the text is the equivalent of four reference units. The specific activities of the C-243-cell and brain interferon were approximately 10^6 units and 10^4 units/mg protein respectively. Human leucocyte interferon was kindly provided by Dr. K. Cantell (22) and rabbit interferon was prepared from RK_{13} cells inoculated with Newcastle Disease virus.

Sera: The origin of the sera used is summarized in Table III.

Assays of cytotoxicity. The method used for determining the specific complement dependant cytotoxicity of alloantisera on radioactive labelled chromium (^{51}Cr) target L1210 ($H\text{-}2^d$) cells have been previously described (1) as has the technique of ^{51}Cr labelling of mesenteric lymph node cells (23). The microdroplet lymphocytotoxicity tests were carried out in Terasaki plates as previously described (24).

Experimental plan. Secondary fibroblast cultures from 18-day old embryos were seeded at a concentration of 5×10^5 or 10^6 cells/ml in 3 cm diameter Falcon Petri dishes (2 ml/dish) in 5% CO_2–air at 37°. After 18–24 h in culture, the nonadherent cells were removed, the monolayer culture was washed and nutrient MEM medium with 5% calf serum was replaced containing approximately 10,000 units of mouse interferon or suitable control preparations. After 18 hr of incubation with interferon, the cell monolayer was washed 3× and incubated with twofold dilutions of 0.2 ml alloantiserum.

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After incubation of cells with antiserum for 1 hr, the residual serum was removed and assayed as described above for complement dependent cytotoxicity. Unless otherwise indicated, determinations were always carried out in duplicate.

Results. Enhanced expression of H-2 antigens on interferon treated DBA/2 embryonic fibroblasts. Secondary cultures of DBA/2 mouse embryonic fibroblasts were treated for 18 hr with 10^4 units of mouse C-243 cell interferon and then incubated with varying dilutions of a C57Bl/6 (H-2^b)-anti L1210 (H-2^d) cell serum. As illustrated in Fig. 1, interferon treated cultures absorbed a greater amount of alloantiserum (see antiserum dilution 1:32) than cultures treated with a control preparation or left untreated. Another experiment using five cultures per serum dilution showed the reproducibility of the phenomenon (experiment 1 Table I).

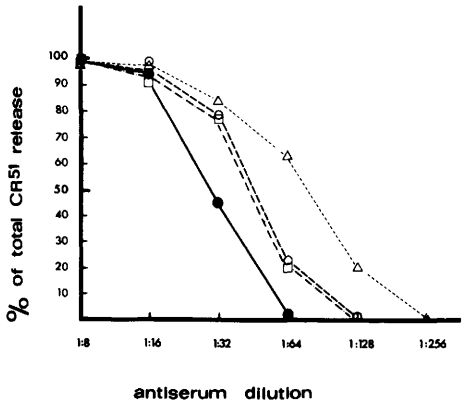

FIG. 1. Assay of residual C57Bl/6 anti-L1210 cell serum on ^{51}Cr labelled L1210 cells after absorption on untreated secondary cultures of DBA/2 embryonic fibroblasts (O-----O); or cultures treated with a control preparation (□-----□); or with cultures treated with 10^4 units of C-243 cell interferon (●——●). The activity of the antiserum prior to absorption is shown by (△——△).

Only treatment of cells with partially purified mouse C-243 cell interferon or crude mouse brain interferon resulted in an increased absorption of alloantiserum. Human and rabbit interferon preparations were ineffective (experiment 2, Table I) as were mouse control preparations (experiment 1, Table I).

In the above experiments sera were absorbed with 10^6 cells treated with 10^4 units of interferon but the enhancing effect of interferon on the expression of H-2 antigens was also observed when fewer cells (2.5×10^5), a smaller amount of interferon (10^3 units of interferon) were used. (Minimal dose of interferon appeared to be approximately 10^2–10^3 units).

Determination of the number of viable and dead cells in the fibroblast cultures of the different groups showed that interferon had not affected cell multiplication in the first 24 hr. This finding was in accord with previous work of Lindahl-Magnusson and coworkers who found that the inhibitory effect of interferon on the multiplication of mouse embryonic fibroblasts was not observed until after several days of culture (25).

Evidence that the effect of interferon is on the expression of H-2 antigens. To define the

TABLE I. ENHANCED EXPRESSION OF H-2 ANTIGENS ON MOUSE INTERFERON TREATED DBA/2 EMBRYONIC FIBROBLASTS.

Exp.	Antiserum[a] absorbed on 10^6 DBA/2 embryonic fibroblasts	Treatment of fibroblasts[b]	% Specific ^{51}Cr release from L1210 target cells
1	No	—	86% ± 11
	Yes	None	78% ± 7
	Yes	Control preparation	66% ± 6
	Yes	Mouse C-243 cell interferon (10^4 units)	46% ± 8
2	No	—	100%
	Yes	None	100%
	Yes	Human leucocyte interferon (10^4 units)	93%
	Yes	Rabbit RK13 cell interferon (10^2 units)	97%
	Yes	Mouse C-243 cell interferon (10^4 units)	46%
	Yes	Mouse brain interferon (10^3 units)	56%

[a] C57Bl/6 anti-L1210 cell serum diluted 1:20 and 1:32 in experiments 1 and 2 respectively. Specific ^{51}Cr release determined for residual antiserum after absorption on DBA/2 fibroblasts.

[b] There were five cell cultures per serum dilution in experiment 1 and duplicate cultures in experiment 2.

optimal experimental conditions for demonstrating enhancement by interferon of the expression of H-2 antigens on embryonic fibroblasts and to show that interferon itself was responsible we have used in the preceding experiments a crude C57Bl/6 anti-L1210 tumor cell serum absorbed on DBA/2 fibroblasts and assayed on ^{51}Cr labelled L1210 tumor cells. In the following series of experiments we have shown by the use of different sera, different embryonic fibroblasts and different target cells in the assays that interferon did enhance the expression of antigens coded by the H-2 complex. Firstly, the results of the experiment summarized in Panel A, Table II showed that enhancement in the expression of H-2 antigens occurs in an experimental system in which neither tumor antigens nor tumor cells were present, ie., an antiserum directed against C57Bl/6 lymphocytes. Secondly, in the experiment illustrated in Panel B (Table II) we used a commercially available monospecific anti-H-2^d serum, absorbed on DBA/2 fibroblasts and assayed on ^{51}Cr labelled mesenteric B10 D2 lymph node cells. In a second experiment (Panel C Table II) we used a crude C57Bl/6 (H-2^b) anti-DBA/2 (H-2^d) sera absorbed on B10 D2 fibroblasts and assayed on B10 D2 lymphocytes. B10 D2 mice (H-2^d) are congenic with B10 mice (H-2^b) differing from these only at the H-2 locus. Thus by testing the C57Bl/6 sera on B10 D2 fibroblasts we determined specifically expression of H-2 antigens. (Preliminary tests showed that this serum did not exhibit any toxicity for B10 lymphocytes).

Effect of interferon on the expression of H-2K, H-2D, and Ia antigens on C3H embryonic fibroblasts. We have previously observed that interferon exerted a differential effect on the expression of H-2K, H-2D and Ia antigens on splenic lymphocytes (10). Likewise as can be seen in Fig. 2, incubation of 10^6 C3H embryonic fibroblasts with 5×10^3 units of C-243 cell interferon resulted in a clear cut increase in the expression of H-2^k (Panel A), H-2D^k (Panel C), H-2K^k (Panel D) but exerted no effect on the expression of antigens detected by an antiserum directed against I^{k}G^{k}S^{k}TL (Panel B).

Discussion. The results of previous experiments showed that interferon treatment of

TABLE II

	PANEL		
	A	B	C
Antiserum prepared:	*DBA/2 anti-C57Bl/6*	*B10Br anti-B10 D2 (Searle)*	*C57Bl/6 anti-DBA/2*
Dilution	1:258	1:10	1:15
Absorbed on embryonic fibroblasts from:	C57Bl/6	DBA/2	B10 D2
Antiserum assayed on target cell:	C57Bl/6 splenic lymphocytes	B10 D2 mesenteric lymph node cells (labeled with ^{51}Cr)	B10 D2 mesenteric lymph node cells (labelled with ^{51}Cr)
Results expressed as:	% of lysed cells (trypan blue)	% specific ^{51}Cr release	% specific ^{51}Cr release
Antiserum not absorbed	81% ± 12	96%	84% ± 13
Antiserum absorbed on fibroblasts —Not treated	71% ± 11	36.3% ± 5	62% ± 3
Antiserum absorbed on fibroblasts —Treated with a control preparation	68% ± 9	–	74% ± 8.5
Antiserum absorbed on fibroblasts —Treated with mouse C-243 cell interferon (10^4 u)	39% ± 5	23.3% ± 2.5	27% ± 6

TABLE III.

Laboratory of origin	Mouse strain	Directed against
Author	C57Bl/6 anti-DBA/2*	H-2^d
Author	DBA/2 anti-C57Bl/6	H-2^b
Searle Diagnostic	B10 Br anti-B10 D2	H-2^d
Dr. H. O. McDevitt	C_3H.SW anti-C3H-DiSn	H-2^k
Dr. H. O. McDevitt	(A.TL × C3H.OL) F1 anti-C3H	H-$2K^k$
Dr. H. O. McDevitt	(A.TL × B10.A) F1 anti-B10.Br	H-$2D^k$
Dr. H. O. McDevitt	A.TH anti-A.TL	$I^kG^kS^k$TL

* One antiserum was made by injecting L1210 (H-2^d) tumor cells and another by injecting normal DBA/2 splenic lymphocytes. All other sera were produced by injection of normal lymphoid cells.

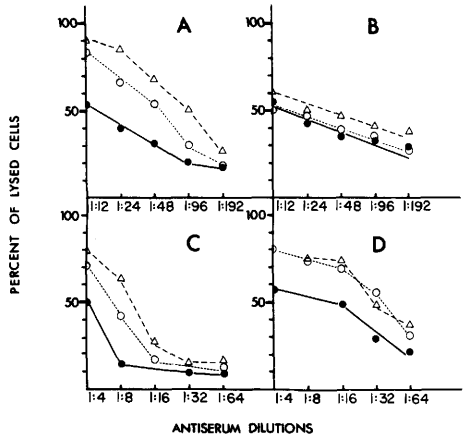

FIG. 2. Assay of residual sera on C3H splenic lymphocytes (expressed as % of trypan blue positive cells) after incubation with untreated (○——○) or interferon treated (5 × 10^3 units of C-243 cell interferon (●——●). The activity of the antiserum prior to absorption is illustrated by (△——△). Panel A, anti-H-2^k. Panel B, anti-$I^kG^kS^k$TL. Panel C, anti-H-$2D^k$. Panel D, anti-H-$2K^k$

malignant or normal lymphoid cells resulted in an increase in the expression of H-2 antigens (2, 3, 9, 10) It seemed important to us to determine whether this effect was restricted to lymphoid cells or was of more general relevance and could be observed on normal nonlymphoid cells. The results presented herein clearly show that interferon enhanced the expression of H-2 antigens on embryonic fibroblasts prepared from different strains of mice. In previous work alloantisera were often assayed on chromium labelled tumor cells and probably contained antibody to antigens other than H-2 antigens (2, 3). In this study we showed that interferon treatment was accompanied by an enhancement in the expression of H-2 antigens in experimental systems where tumor antigens were not involved either in the preparation of sera or in the assay. The use of congenic mice differing only at the H-2 locus, and monospecific and restricted sera against determinants of the H-2 complex permit us to affirm, with the confidence that these reagents and techniques allow, that we are measuring the expression of H-2 antigens, and furthermore that the effect of interferon appears to be specific for antigens coded for by the K and D region of the H-2 complex. No effect was observed on the expression of Ia antigens.

These experiments and those previously reported raise a number of questions. Does the enhanced expression of H-2 antigens on interferon treated lymphoid or non lymphoid cells reflect an increase in the amount of H-2 antigens due either to an increase in synthesis or a decrease in shedding, or a difference in the spatial presentation of H-2 antigens on the cell surface? To date we have observed an increase in the expression *only* of H-2 antigens on interferon treated cells. Interferon treatment has not resulted in any modification in the expression of Thy-1 (3), Ia (10) or TL (Lindahl, P., unpublished observations) antigens. What is the explanation for the seeming selectivity of this effect? Lastly what is the relationship between the effect of interferon on the expression of H-2 surface antigens and other effects of interferon on cells ie. antiviral action, inhibition of cell division and enhancement of cell function.

Summary. Treatment of mouse embryonic fibroblasts with mouse interferon enhances the expression of surface histocompatibility antigens as demonstrated by quantitative absorption of anti-H2 sera. Interferon exerts a differential effect on the expression of H-2D, H-2K and Ia antigens.

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