Interferon treatment of mice: Enhanced expression of histocompatibility antigens on lymphoid cells

(thymocyte/splenic lymphocyte/lymphocyte maturation/cell surface antigen/cortisone treatment)

PERNILLA LINDAHL*, ION GRESSER, PATRICIA LEARY, AND MICHAEL TOVEY

Institut de Recherches Scientifiques sur le Cancer, 94300 Villejuif, France

ABSTRACT   Treatment of young and mature mice with potent mouse interferon preparations results in a marked enhancement of the expression of histocompatibility antigens on the surface of thymocytes and splenic lymphocytes as measured by an enhanced absorption of alloantiserum. We postulate that such modifications of the cell surface may reflect an effect of interferon on lymphocyte maturation and may be relevant to the effect of interferon on lymphocyte function.

Since interferon can affect both the division and function of lymphocytes (1–17) it seems likely that it plays a role in the regulation of the humoral (6–11) and cell-mediated immune response (1, 2, 12–17). We have recently shown that incubation of mouse lymphoid cells with interferon was accompanied by a marked enhancement of the expression of surface histocompatibility antigens (18). The availability of potent semi-purified mouse interferon preparations (19) enabled us to determine whether treatment of mice with interferon would also result in modifications in the expression of histocompatibility antigens on thymocytes and splenic lymphocytes. The results of these experiments are presented herein.

MATERIALS AND METHODS

Mice. DBA/2 mice were obtained from the breeding colony of the Institut du Cancer.

Interferon and Control Preparations. Mouse interferon was prepared from mouse C-243 cells (20) cultivated in suspension culture and infected with Newcastle disease virus (NDV) (19). After concentration and partial purification by selective precipitation with ammonium sulfate (19) the preparation was assayed on mouse L cells. One unit of interferon as expressed in the *text* is approximately the equivalent of four mouse interferon reference units. The specific activity of the interferon preparations was 1×10^6 to 6×10^6 units/mg of protein. Control preparations consisted of the concentrated supernatants from uninoculated suspension cultures.

Alloantisera, Labeling of Target Cells, Cytotoxicity Assay. Alloimmune sera were prepared in C57Bl/6 mice (H-2^b) by six intraperitoneal (i.p.) injections of 5×10^7 L1210 cells (H-2^d) or in C57Bl/6 mice by six i.p. injections of 10^8 Balb/c (H-2^d) splenic lymphocytes. These alloimmune sera should also contain antibodies against non-H-2 alloantigens. However, as in the experiments to be described the sera were utilized at a limiting dilution, we assume that they should primarily detect H-2 antigens.

The techniques for culture and labeling a suspension of

Abbreviations: i.p. intraperitoneal; NDV, Newcastle disease virus.
* Present address: Department of Medical Microbiology, University of Lund, Sweden.

target L1210 cells with radioactive chromium (^{51}Cr) and the techniques used in the cytotoxicity assay have been previously described (14, 18, 21). The formula for calculating the percentage of specific lysis was:

$$100 \times \frac{\frac{^{51}\text{Cr release from target cells incubated with residual antiserum (after absorption on lymphocytes) and 1:10 dilution of rabbit serum}}{^{51}\text{Cr release from target cells incubated with 1 cytotoxic unit of antiserum and 1:10 dilution of rabbit serum}} - \frac{^{51}\text{Cr release from target cells incubated with 1:10 dilution of rabbit serum}}{^{51}\text{Cr release from target cells incubated with 1:10 dilution of rabbit serum}}}$$

Preparation of Lymphocyte Suspensions and Quantitative Determination of the Expression of Histocompatibility Antigens on Lymphocytes. The techniques have been previously described in detail (18) but may be summarized here as follows: spleen or thymus was homogenized gently in a Ten-Broek grinder with Eagle's minimal essential medium, and after sedimentation of large clumps of tissue the cell-rich supernatant was centrifuged. The cell sediment was washed once. Cell concentrations from control or interferon-treated mice were adjusted so that the initial tube contained 20×10^6 splenic lymphocytes per ml or 40×10^6 thymocytes per ml. (Cell viability was determined by the trypan blue dye exclusion method. Cell mortality was always below 10%.) Tubes containing 2-fold dilutions of cells (in 1 ml) were centrifuged for 5 min at $800 \times g$ in a refrigerated International Centrifuge. The supernatant was drained off and the cell sediment was incubated for 1 hr with an appropriate antiserum dilution corresponding to one cytotoxic unit, which is defined as ½ the dilution of antiserum which gave a 95% lytic end point in the cytotoxicity assay. The cells were again sedimented by centrifugation and 0.05 ml of the supernatant was harvested and assayed for remaining complement-dependent cytotoxicity for ^{51}Cr-labeled L1210 target cells.

To test the reliability (i.e., reproducibility) of this technique, a cell suspension was prepared from a pool of four spleens and several series of 2-fold dilutions were prepared from the same original cell suspension. Two such experiments comprised six series of 2-fold cell dilutions from a given cell suspension and a third experiment comprised eight series of 2-fold cell dilutions. It was found that the absorption curves (% specific ^{51}Cr release from target cells after incubation with residual antiserum) were virtually superposable and the percent standard error at the 50% reduction point was 8%, 11%, and 15%, respectively.
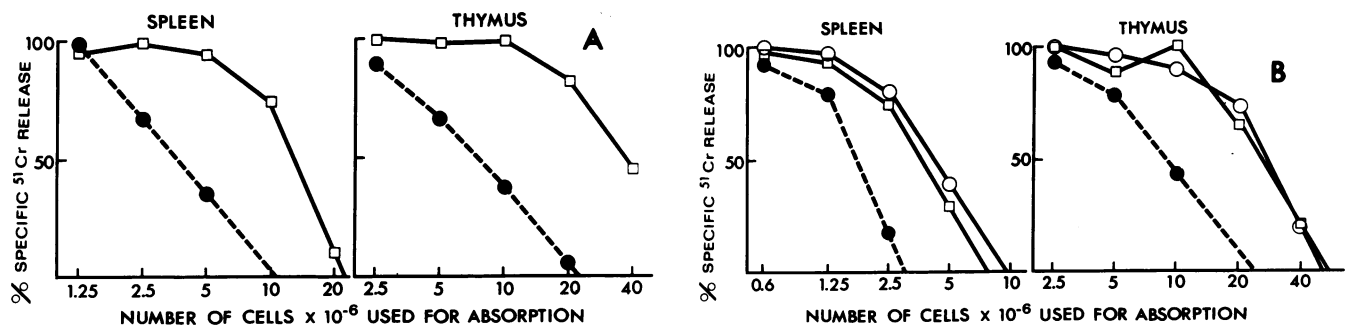
FIG. 1. Quantitative absorption of a C57Bl/6 mouse anti-L1210 cell serum by various numbers of splenic and thymic cells from 18-day-old DBA/2 mice treated with interferon (● - - - ●) or left untreated (□———□) (Panel A); or from 6-week-old DBA/2 mice treated with interferon (● - - - ●) or control preparation (O———O), or left untreated (□———□) (Panel B).

RESULTS

Interferon treatment of young and mature DBA/2 mice: Effect on the expression of histocompatibility antigens of thymocytes and splenic lymphocytes

Four 18-day-old DBA/2 mice were injected four times at approximately 12 hr intervals intraperitoneally (i.p.) with 0.25 ml of an interferon preparation titering 1.6×10^{-6} (that is, the preparation diluted by this factor gave one unit of activity) or left untreated. In another experiment, four 6-week-old male DBA/2 mice were injected four times i.p. with 0.25 ml of an interferon preparation titering 8×10^{-5}, or four times i.p. with 0.25 ml of a control mock interferon preparation, or left untreated. Forty-eight hours after the first injection the mice in each experiment were sacrificed, the thymuses and spleens from mice in each group were pooled, and various numbers of cells were incubated with alloantiserum diluted to contain one cytotoxic unit (see *Materials and Methods*).

As can be seen in Fig. 1A and B, thymocytes and splenic lymphocytes from interferon-treated mice absorbed more antibody than the same number of cells from control mice. Interferon treatment of the animal did not affect cell viability of suspensions as judged by the trypan blue dye exclusion method.

Since in these experiments cell suspensions were prepared from a pool of organs from four mice in the different groups, it was of interest to repeat the experiment testing cells from individual interferon-treated or control mice. Accordingly, in one experiment eight 15-day-old DBA/2 mice (from two litters) received four injections i.p. of 0.25 ml of an interferon preparation titering 1.6×10^{-6} and eight siblings (from the same two litters) received four injections i.p. of 0.25 ml of a control preparation. A similar experiment was undertaken using six 6-week-old male DBA/2 mice in each group and injecting 0.25 ml of an interferon prepara-

tion titering 1.3×10^{-6} of a control preparation. Forty-eight hours after the first injections the mice were sacrificed and spleen cell suspensions were prepared from each mouse.

As can be seen from Fig. 2A and B there was little variation between the curves of alloantibody absorption for splenic lymphocytes from individual mice within each group. A significant difference in the alloantibody absorption was observed, however, between mice treated with interferon and those treated with a control preparation.

Relation between amount of interferon injected and enhancement of expression of histocompatibility antigens on splenic lymphocytes

The results of some experiments indicated that a single injection of an interferon preparation titering 10^{-6} into young mice (24 or 48 hr before sacrifice) would result in some increase in the antibody-absorbing capacity of thymocytes and splenic lymphocytes. A consistent and marked enhancement was, however, always observed when mice were injected four times during a 48 hr period. Accordingly, in the following experiment 17-day-old mice were injected four times i.p. with 0.25 ml of 10-fold dilutions of an interferon preparation and sacrificed 48 hr after the beginning of the injections. As can be seen in Fig. 3 the splenic lymphocytes of mice receiving injections of an interferon preparation titering 10^{-6} or 10^{-5} showed an enhanced absorption of alloantiserum, whereas no effect was observed in mice injected with the more dilute interferon preparations.

Evidence that interferon is responsible for enhanced absorption of alloantibody

Use of Heterologous and Inactivated Homologous Interferon Preparations. Two-week-old DBA/2 mice from three litters were distributed into six groups and treated (0.25 ml injected i.p. four times at 12 hr intervals) as follows
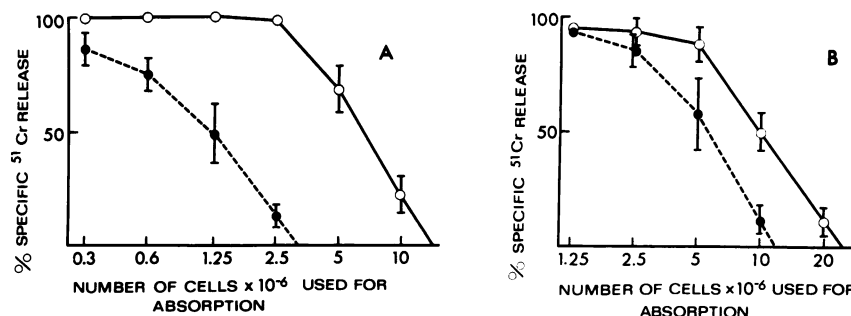

FIG. 2. Quantitative absorption of a C57Bl/6 mouse anti-L1210 cell serum by various numbers of splenic cells prepared from each of eight 15-day-old DBA/2 mice treated with interferon (● - - - ●), or from each of eight siblings treated with control preparation (O———O) (Panel A); or from each of six 8-week-old DBA/2 mice treated with interferon or control preparation (Panel B). The bars indicate SEM.
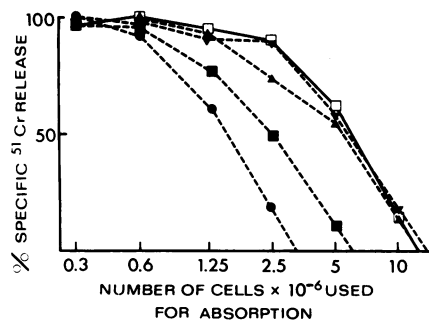
FIG. 3. Quantitative absorption of a C57Bl/6 mouse anti-L1210 cell serum by various numbers of splenic cells from 17-day-old DBA/2 mice injected with interferon preparations titering 10^{-6} (● - - - ●); 10^{-5} (■ - - - ■); 10^{-4} (▲ - - - ▲); 10^{-3} (▼ - - - ▼); or left untreated (□——□).

(three mice in each group): (*1*) a mouse interferon preparation titering 1.6×10^{-6}; (*2*) the same preparation inactivated by incubation with crystalline trypsin (500 μg/ml at 37° for 1 hr) and having a residual titer of 1×10^{-1}; (*3*) a mock interferon preparation demonstrating no antiviral activity at a 1:5 dilution; (*4*) the same mock interferon preparation pretreated with trypsin (as in group 2); (*5*) a human leukocyte interferon preparation having a titer of 1.6×10^{-6} on human fibroblasts and 8×10^{-1} on mouse L cells; and (*6*) no treatment.

As can be seen in Fig. 4, only splenic lymphocytes from mice in group (*1*), treated with potent mouse interferon, demonstrated an enhanced absorption of alloantiserum.

Use of an Inducer of Endogenous Interferon—Newcastle Disease Virus. Six 7-week-old female DBA/2 mice were inoculated intravenously (i.v.) with 0.25 ml (mean tissue culture infective dose, $TCID_{50}$, of 10^9) of NDV. Six hours thereafter, two mice were sacrificed and the serum interferon titer of each mouse was 1:51,200. As can be seen in Fig. 5, splenic lymphocytes and thymocytes from mice inoculated with NDV 24 hr previously demonstrated an enhanced absorption of alloantiserum compared to cells from control mice.

In other experiments mice were injected with 10-fold dilutions of NDV and the serum interferon levels were determined for each group. A slight but still significant increase in the absorption of alloantiserum was observed for splenic lymphocytes and thymocytes from mice injected with 10^6 $TCID_{50}$ of NDV. The serum interferon titer in these mice was 1:320 at 6 hr.
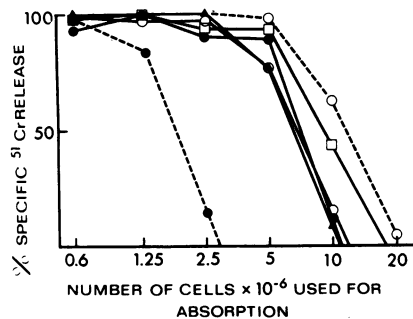

FIG. 4. Quantitative absorption of a C57Bl/6 mouse anti-L1210 cell serum by various numbers of splenic cells from 14-day-old DBA/2 mice inoculated with mouse interferon (● - - - ●); mouse interferon treated with trypsin (●——●); a control preparation (O - - - O); a control preparation treated with trypsin (O——O); human leukocyte interferon (▲——▲); or left untreated (□——□).
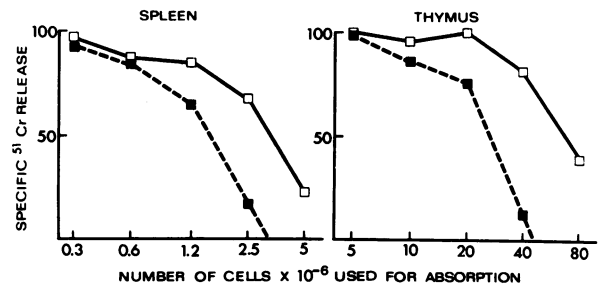

FIG. 5. Quantitative absorption of a C57Bl/6 mouse anti-L1210 cell serum by various numbers of thymocytes and splenic cells from 7-week-old DBA/2 mice inoculated with NDV (■ - - - ■), or left untreated (□——□).

A comparison of the effect of cortisone and interferon on the expression of histocompatibility antigens on splenic lymphocytes and thymocytes

It has been shown that cortisone treatment of mice is accompanied by a marked loss of thymocytes and the preservation within the thymus of a cell population which expresses histocompatibility antigens strongly (22, 23). Therefore, it was considered of interest to compare the effect of interferon on the expression of histocompatibility antigens on lymphoid cells with that induced by cortisone.

Four 7-week-old DBA/2 male and female mice were injected i.p. four times at 12 hr intervals with 0.25 ml of an interferon preparation titering 1.6×10^{-6}. At the time of the first interferon injection another group of nine mice was injected once i.p. with cortisone acetate (375 mg/kg of body weight). It was necessary to inject nine mice with cortisone to obtain sufficient number of cells from the thymuses. Four mice were left untreated. Forty-eight hours after the first injection all mice were sacrificed and the spleens or thymuses from mice in each group were pooled.

As can be seen from Fig. 6, thymocytes from both interferon- and cortisone-treated mice exhibited an enhanced absorption of alloantiserum. Splenic lymphocytes from interferon-treated mice showed an enhanced absorption of alloantiserum, whereas no significant effect was observed for splenic lymphocytes from cortisone-treated mice. (In a second experiment splenic lymphocytes from cortisone-treated mice appeared to exhibit a slightly decreased absorbing capacity.)

Also, interferon treatment of mice did not significantly affect the total cell number in thymus and spleen, whereas cortisone treatment was accompanied by a marked loss of cells in both organs (Table 1).

DISCUSSION

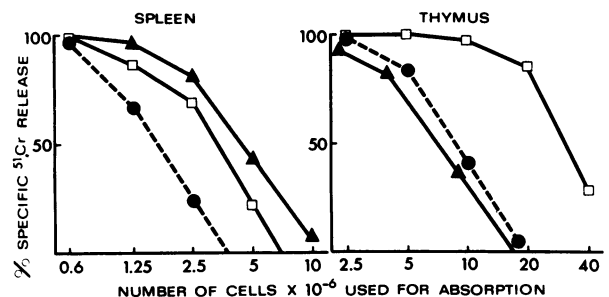The results presented herein indicate that treatment of

FIG. 6. Quantitative absorption of a C57Bl/6 mouse anti-L1210 cell serum by various numbers of thymocytes and splenic cells from 7-week-old DBA/2 mice injected with interferon, (● - - - ●) or cortisone (▲——▲), or left untreated (□——□).

Immunology: Lindahl et al.

Proc. Nat. Acad. Sci. USA 73 (1976)   1287

| Organ | Number of viable cells ×10⁻⁶ per organ | | |
	Controls	Interferon-treated	Cortisone-treated
Thymus	91	101	3.5
Spleen	92	79	18

Four 6-week-old DBA/2 mice were injected i.p. four times at 12
hr intervals with 0.25 ml of an interferon preparation titering 1.6 ×
10^{-6}. Four mice were left untreated and four mice were injected
once i.p. with cortisone acetate (375 mg/kg of body weight). All
mice were sacrificed 48 hr after the first injection and cell counts
were performed on cell suspensions from thymus and spleen by the
trypan blue dye exclusion method.

young (2–3 week old) and mature (6–8 week old) DBA/2
mice with potent mouse interferon preparations is accompa-
nied by an enhanced expression of histocompatibility anti-
gens on the surface of thymocytes and splenic lymphocytes
as measured by an enhanced absorption of alloantiserum.
Although pure interferon is not available, the evidence pre-
sented above and as discussed in our previous reports (1, 2,
14, 18, 21) strongly suggests that interferon is the factor re-
sponsible for this phenomenon.

Two possibilities may be suggested to explain the in-
creased expression of H-2 antigens on thymocytes from in-
terferon-treated mice. First: like cortisone treatment, inter-
feron treatment might result in the loss of a thymic cell pop-
ulation which expresses H-2 antigen poorly. The remaining
cells might, like cortisone-resistant thymocytes, express H-2
antigens strongly. However, since interferon treatment did
not result in a significant decrease in the number of thymo-
cytes, but did induce a marked enhancement in the expres-
sion of H-2 antigens (comparable to that observed for corti-
sone-treated mice—Fig. 6, Table 1), this explanation seems
unlikely. Second: interferon treatment may result in a gen-
eral increase in the expression of H-2 antigens on thymo-
cytes. Since our previous results from in vitro experiments
suggested that the effect of interferon on the expression of
H-2 antigens on cortisone-resistant thymocytes was not very
pronounced (18), we suggest that interferon treatment of
mice is accompanied by a general enhancement of the ex-
pression of H-2 antigens but that this effect is most pro-
nounced on those thymic cells which normally express H-2
antigens poorly. These cells are considered to represent the
majority of "immature" thymocytes (23).

In contrast to cortisone, interferon treatment of young and
adult mice also resulted in an increased expression of H-2
antigens on splenic lymphocytes (Fig. 6). We had previously
observed that interferon treatment of spleen cells in vitro
was accompanied by an enhanced expression of H-2 anti-
gens on splenic lymphocytes from young, but not from ma-
ture mice (18). We do not at present have a satisfactory ex-
planation for this apparent difference between our present
in vivo and previous in vitro results as pertains to the effect
of interferon on splenic lymphocytes from mature mice.

Since we are not aware of any other substance that en-
hances the expression of cell surface histocompatibility anti-
gens in vivo and since the biologic role of these antigens
themselves is unknown, it is difficult to speculate at present
on the significance of our observations. Furthermore, we do
not yet know whether interferon can modify the expression

of other surface antigens on lymphoid cells. Although our
previous results from in vitro experiments showed that in-
terferon did not affect the expression of the theta antigen on
thymocytes and splenic lymphocytes (18), it will be of inter-
est to determine its effect on the expression of those lympho-
cyte markers that vary with the stage of lymphocyte matu-
ration, i.e., TL antigens (24). Despite our paucity of infor-
mation, it is tempting to speculate that interferon may influ-
ence lymphocyte maturation and that the modifications of
the cell surface on lymphocytes from interferon-treated
mice are related to the effects of interferon on lymphocyte
division and function.

1. Lindahl-Magnusson, P., Leary, P. & Gresser, I. (1972) Nature
New Biol. 237, 120–121.
2. Cerottini, J. C., Brunner, K. T., Lindahl, P. & Gresser, I.
(1973) Nature New Biol. 242, 152–153.
3. Rozee, K. R., Lee, S. H. S. & Ngan, J. (1973) Nature New Biol.
245, 16–18.
4. Blomgren, J., Strander, H. & Cantell, K. (1974) Scand. J. Im-
munol. 3, 697–705.
5. Thorbecke, G. J., Friedman-Kien, A. E. & Vilcek, J. (1974)
Cell. Immunol. 12, 290–295.
6. Braun, W. & Levy, H. B. (1972) Proc. Soc. Exp. Biol. Med.
141, 769–773.
7. Chester, T. J., Paucker, K. & Merigan, T. C. (1973) Nature
246, 92–94.
8. Gisler, R., Lindahl, P. & Gresser, I. (1974) J. Immunol. 113,
438–444.
9. Brodeur, B. R. & Merigan, T. C. (1974) J. Immunol. 113,
1319–1325.
10. Johnson, H. M., Smith, B. G. & Baron, S. (1975) J. Immunol.
114, 403–409.
11. Brodeur, B. R. & Merigan, T. C. (1975) J. Immunol. 114,
1323–1327.
12. Svet-Moldavsky, G. J. & Chernyakhovskaya, I. Ju. (1967) Na-
ture 215, 1299–1300.
13. Chernyakhovskaya, I. Ju., Slavina, E. G. & Svet-Moldavsky, G.
J. (1970) Nature 228, 71–72.
14. Lindahl, P., Leary, P. & Gresser, I. (1972) Proc. Nat. Acad.
Sci. USA 69, 721–725.
15. Hirsch, M. S., Ellis, D. A., Black, P. H., Monaco, A. P. &
Wood, M. L. (1973) Transplantation 17, 234–236.
16. De Maeyer, E., Mobraaten, L. & De Maeyer-Guignard, J.
(1973) C.R. Hebd. Seances Acad. Sci. Paris 277, 2101–2103.
17. Skurkovich, S. V., Klinova, E. G., Aleksandrovskaya, I. M.,
Levina, N. V., Arkhipova, N. A. & Bulicheva, T. I. (1973) Im-
munology 25, 317–322.
18. Lindahl, P., Leary, P. & Gresser, I. (1974) Eur. J. Immunol. 4,
779–784.
19. Tovey, M., Begon-Lours, J. & Gresser, I. (1974) Proc. Soc.
Exp. Biol. Med. 146, 809–815.
20. Oie, M. K., Gazdar, A. F., Buckler, C. E. & Baron, S. (1972) J.
Gen. Virol. 17, 107–109.
21. Lindahl, P., Leary, P. & Gresser, I. (1973) Proc. Nat. Acad.
Sci. USA 70, 2785–2788.
22. Cerottini, J. C. & Brunner, K. T. (1967) Immunology 13,
395–403.
23. Blomgren, H. & Svedmyr, E. (1971) Cell. Immunol. 2, 285–
299.
24. Komuro, K., Boyse, E. & Old, L. J. (1973) J. Exp. Med. 137,
533–536.