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

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

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Communicated by George Klein, December 29, 1975

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 alloantisera. 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 the equivalent of four mouse interferon reference units. The specific activity of the interferon preparations was 1 × 10⁶ to 6 × 10⁶ units/mg of protein. Control preparations consisted of the concentrated supernatants from unoinculated suspension cultures.

Alloantisera. Labeling of Target Cells. Cytotoxicity Assay. Alloimmune sera were prepared in C57Bl/6 mice (H-2b) by six intraperitoneal (i.p.) injections of 5 × 10⁶ L1210 cells (H-2d) or in C57Bl/6 mice by six i.p. injections of 10⁶ Balb/c (H-2d) 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 target L1210 cells with radioactive chromium (⁵¹Cr) 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:

\[
\text{\% lysis} = \frac{\text{s}1\text{Cr release from target cells incubated with residual antiserum (after absorption on lymphocytes) and 1:10 dilution of rabbit serum}}{\text{s}1\text{Cr release from target cells incubated with 1:10 dilution of rabbit serum}} \times 100
\]

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 × 10⁶ splenic lymphocytes per ml or 40 × 10⁶ 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 × 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 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 ⁵¹Cr-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 ⁵¹Cr 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.
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 $1.6 \times 10^{-6}$ (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 $8 \times 10^{-5}$, 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 $1.6 \times 10^{-6}$ 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 preparation titering $1.3 \times 10^{-6}$ 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
(three mice in each group): (1) a mouse interferon preparation titering $1.6 \times 10^{-6}$; (2) the same preparation inactivated by incubation with crystalline trypsin (500 μg/ml at 37°C for 1 hr) and having a residual titer of $1 \times 10^{-1}$; (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 $1.6 \times 10^{-6}$ on human fibroblasts and $8 \times 10^{-6}$ 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 10^6) 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.

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 $1.6 \times 10^{-6}$. 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...
young (2–3 week old) and mature (6–8 week old) DBA/2 mice with potent mouse interferon preparations is accompanied by an enhanced expression of histocompatibility antigens on the surface of thymocytes and splenic lymphocytes as measured by an enhanced absorption of alloantiserum. Although pure interferon is not available, the evidence presented above and as discussed in our previous reports (1, 2, 14, 18, 21) strongly suggests that interferon is the factor responsible for this phenomenon.

Two possibilities may be suggested to explain the increased expression of H-2 antigens on thymocytes from interferon-treated mice. First: like cortisone treatment, interferon treatment might result in the loss of a thymic cell population 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 thymocytes, but did induce a marked enhancement in the expression of H-2 antigens (comparable to that observed for cortisone-treated mice—Fig. 6, Table 1), this explanation seems unlikely. Second: interferon treatment may result in a general increase in the expression of H-2 antigens on thymocytes. 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 expression of H-2 antigens but that this effect is most pronounced 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 antigens on splenic lymphocytes from young, but not from mature mice (18). We do not at present have a satisfactory explanation for this apparent difference between our present in vitro 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 enhances the expression of cell surface histocompatibility antigens in vitro 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 interferon did not affect the expression of the theta antigen on thymocytes and splenic lymphocytes (18), it will be of interest to determine its effect on the expression of those lymphocyte markers that vary with the stage of lymphocyte maturation, i.e., TL antigens (24). Despite our paucity of information, it is tempting to speculate that interferon may influence 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.

We are indebted to Dr. Kari Cantell (Helsinki) for his gift of human leukocyte interferon, to Mrs. Josiane Buywyd and Miss Jacqueline Begon-Lours for the preparation of mouse interferon and to Mrs. Marie-Thérèse Bandu for the assay of mouse and human interferon. This work was aided in part by grants from the Institut National de la Santé et de la Recherche Médicale (Contract no. 74-4-041-2 and Contract no. 74-4-426-36) and the Fondation Française pour la Recherche Médicale.

Table 1. Comparison of the effect of interferon and cortisone on total cell number in thymus and spleen

<table>
<thead>
<tr>
<th>Organ</th>
<th>Controls</th>
<th>Interferon-treated</th>
<th>Cortisone-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>91</td>
<td>101</td>
<td>3.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>92</td>
<td>79</td>
<td>18</td>
</tr>
</tbody>
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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 x 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.