ABSTRACT

We investigated the influence of glucocorticoids on two effects of interferons (IFNs) which are thought to relate to their antitumor actions: cytotoxic activity and induction of HLA antigen expression. We treated human myeloid cell lines (U-937, HL-60, THP-1, K-562, and KG-1a), and T- (MOLT-4) and B- (Daudi) lymphoblastic cell lines with concentrations of IFN-α, IFN-γ, and dexamethasone (Dex) which are commonly achieved in the circulation following therapeutic administration. The results show that for every cell line except Daudi, the greatest inhibition of cell growth occurred when IFN-γ and Dex treatments were combined. The advantage of combined IFN-γ and Dex treatment over treatment with either agent alone was most dramatic for the three cell lines U-937, HL-60, and THP-1 which have monocyte characteristics. There was also more growth inhibition by the combination of IFN-α and Dex than by either agent alone for all seven cell lines tested. The induction of HLA antigen expression by IFN-α and IFN-γ, an effect which could increase recognition of the tumor cells by the immune system, was as great or greater in the presence of Dex as in its absence. These results demonstrate that glucocorticoids do not inhibit, and in some cases enhance, two effects of IFNs that appear to be related to their antitumor actions: inhibition of tumor cell proliferation and enhancement of HLA antigen expression.

INTRODUCTION

Since Gresser and his colleagues demonstrated in 1967 that interferon had antitumor activity, IFNs have been shown to inhibit a variety of virus-induced, “spontaneous,” and transplantable tumors (reviewed in Ref. 1). Clinical studies have recently shown that IFNs may be efficacious for the treatment of certain types of leukemia (2-4). Although the mechanisms by which IFNs inhibit tumor cell growth are not completely resolved, both direct and indirect actions appear to be important. The direct antiproliferative actions of IFNs have been demonstrated with many tumor cells (5-8). However, even when tumor cells are resistant to interferon in cell culture, their growth in mice can be inhibited with interferon (9). This protection may be due to enhanced recognition of the tumor cells by the immune system and may be related to IFN enhancement of antigens encoded by the MHC (10-12).

Glucocorticoids are present continuously in virtually all patients receiving IFN therapy. Their levels may be elevated as a result of stress (13) or because they are administered therapeutically as lympholytic or palliative agents (14). Glucocorticoids might also inhibit antitumor effects of interferons, which could have important clinical implications. Since IFNs are used increasingly for the treatment of hematological malignancies (2-4), we have examined the individual and combined effects of glucocorticoids and IFN on leukocyte cell lines. The end points examined were cell proliferation, HLA antigen expression, and cell viability. We show that for the cell lines examined, Dex usually enhances the antiproliferative actions of IFN-α and -γ, and does not inhibit HLA antigen induction by these IFNs.

MATERIALS AND METHODS

Interferons and Dexamethasone. Human lymphoblastoid interferon-α (Wellferon) was generously supplied by Burroughs Wellcome, Inc. IFN-α units reported here were determined by the suppliers. Human recombinant interferon-γ (kindly provided by Dr. M. Shepard, Gentech, Inc.) was produced in bacterial cultures following cloning of the appropriate genes into Escherichia coli (16). The interferon-γ used in this study was >95% pure as determined by gel electrophoresis and silver staining (17) and its antiviral activity was 2-4 x 10⁴ units/mg protein. Dex was purchased from Steraloids, Inc. (Wilton, NH).

Cell Culture. U-937, MOLT-4, Daudi, HL-60, K-562, and THP-1 cell lines were obtained from the American Type Culture Collection (Rockville, MD). The KG-1a cell line was a gift of E. D. Ball, Dartmouth Medical School. Each cell line was cultured in RPMI 1640 medium (KC Biological, Inc., Lenexa, KS) supplemented with 10% fetal bovine serum (Sterile Systems, Inc., Logan, UT), 2 mM glutamine, and 50 μg/ml gentamycin (United State Biochemical Corp., Cleveland, OH) in a humidified atmosphere containing 5% CO₂. On day 0, cells were seeded at between 2 and 3 x 10⁴/ml in 6-well plates (Costar, Cambridge, MA). Experimental groups were treated with IFN-α (optimal concentration of 200 and suboptimal concentration of 1 unit/ml), IFN-γ (optimal concentration of 100 and suboptimal concentration of 1 unit/ml), Dex (200 nm), or IFN plus Dex. The optimal concentrations of IFNs and Dex used for these studies were those which are achievable therapeutically and would be expected to saturate their respective receptors.

Preliminary studies showed that the optimal concentrations for inhibition of U-937 cell growth were 200 units/ml IFN-α and 100 units/ml IFN-γ. These concentrations also cause maximum induction of Fc receptors on HL-60 and U-937 cell lines (18) and saturate IFN receptors in other cell types (19). Also, Dex concentration of 200 nm was chosen since human monocytes have ~9,000 glucocorticoid binding sites/cell and a K_d for Dex of ~7 nm (20). The cells from patients with hematological cancers (acute and chronic lymphocytic leukemia, lymphoma, acute nonlymphocytic leukemia, etc.) have 400-14,000 glucocorticoid binding sites/cell (21), and many immunosuppressive effects of glucocorticoids reach a maximum at a concentration of 100 nm (reviewed in Ref. 22).

Cell Growth and Viability. Cells were harvested after being cultured for 1, 3, or 5 days and counted on a model ZB Coulter Counter (Coulter Electronics, Inc., Hialeah, FL). Cell viability was examined by trypan blue dye exclusion.

Indirect Immunofluorescence Assay. The method for indirect immunofluorescence assay was described previously (23). The specificities of the primary antibodies used in this study are listed in Table 1. P3 and LPC-1 were used as negative controls since their binding specificity is unknown, and they do not bind to human leukocytes.

Data Analysis. For each cell line, a total of nine separate cultures were analyzed. Figures show the mean ± SD for these nine cultures.
RESULTS

Growth Inhibitory Activity. Although both glucocorticoids and IFNs are known to have cytotoxic activity, the combined effect of glucocorticoids and IFN has not been reported. We first examined the individual and combined effects of Dex and IFN on tumor cell proliferation. Cells were harvested and counted after treatment with different agents for 1, 3, and 5 days. The culture vessels were examined microscopically to ensure that all of the cells were recovered. Fig. 1 shows the effect of optimal concentrations (as defined in "Materials and Methods") of IFNs and Dex on growth of three cell lines after a 5-day treatment. Effects on days 1 and 3 were similar but less pronounced. Proliferation of U-937 cells was inhibited by IFN-α, and more dramatically by IFN-γ. Although Dex did not affect the growth of U-937 when used alone, it did significantly enhance the action of IFN-α and −γ (P < 0.01) after a 5-day treatment. The growth of MOLT-4 cells was inhibited by IFN-α, −γ, and Dex (P < 0.01), and the combined effects between each IFN and Dex were additive. With Daudi cells, IFN-α caused dramatic inhibition of growth. Dex also inhibited the growth of Daudi cells when used alone (P < 0.01), but did not alter the effect of IFN-α. Viability of U-937 and MOLT-4 cells was >95% after treatment with all agents. The viability of Daudi cells was dramatically decreased by IFN-α (92% viable cells on day 1, 79% on day 3, and 60% on day 5), slightly decreased by Dex (90%), and not affected by IFN-γ.

While serum concentrations of IFN and Dex as high as those used above are achieved therapeutically, it is unlikely that these levels can be maintained continuously in vivo without unacceptable toxicity. It was therefore of interest to determine whether lower concentrations had additive or synergistic effects. Fig. 2 shows the effect of Dex plus suboptimal concentrations of IFN on growth of three cell lines which have monocytoid characteristics. In all three cases, the greatest inhibition occurred when IFN-γ and Dex were combined, and these agents had synergistic effects on cell growth. IFN-α and Dex caused additive inhibition of HL-60 and THP-1 growth, and synergistic inhibition of U-937 cell growth. Fig. 3 shows the effect of suboptimal concentrations of IFN on the growth of four nonmonocytoid lines. These cell lines were different from the monocytoid lines shown in Fig. 2 in two ways: Dex was consistently the most effective single agent, and the effects of IFN-γ and Dex were only additive (MOLT-4, K-562) or less than additive (Daudi, KG-1a). For Daudi cells, IFN-α plus Dex was the most effective treatment tested, while IFN-γ plus Dex was equal to or better than the other treatments for the three remaining cell lines.

Regulation of HLA Expression. Another action of IFNs which may be related to their tumoricidal effects is the enhancement

Table 1 Monoclonal antibodies used in this study

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Isotype</th>
<th>Specificity</th>
<th>Source</th>
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<td>lgG2a</td>
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<td>29</td>
</tr>
</tbody>
</table>

⁴Unpublished observation: MoAb 67.8 was produced by R. Graziano, M. W. Fanger, and E. D. Ball, Dartmouth Medical School.

Data were analyzed by analysis of variance using Newman-Keuls test (30). P < 0.05 was considered significant.
of HLA antigen expression. Therefore, cells from the same experiments shown in Fig. 1 were examined by immunofluorescence assay to determine the individual and combined effect of Dex and IFN on HLA expression. Fig. 4 shows the results for three cell lines tested. In the U-937 cell line, IFN-γ enhanced HLA class I expression 2.5-fold on day 5 while IFN-α caused a 2-fold increase. Although Dex did not affect HLA class I expression, it slightly increased the effect of IFN-α and IFN-γ. This apparent enhancement was not statistically significant. The results for MOLT-4 cells were nearly identical; IFN-γ caused a 7-fold increase of HLA class I antigen expression on day 5, and IFN-α was about one-half as effective as IFN-γ, causing a 4-fold increase. Dex was again without effect. With Daudi cells, all three agents significantly increased HLA class II antigen expression (P < 0.01), and combination of IFN-α with Dex was additive. Daudi cells do not express class I antigens on their surface, and these antigens were not induced by any of the three agents used (data not shown). These results show that amplification of HLA antigen expression by IFN-α and -γ is either enhanced or unaffected by Dex.

Regulation of Other Cell Surface Antigens. Apparent increases in HLA antigen expression could have a trivial explanation, such as a generalized enhancement of protein synthesis or a change in cell size. To rule this out, we used the flow cytometer to examine forward angle light scatter, which is proportional to the cross-sectional area of the cell (31). None of the treatments caused a significant change in this parameter. Furthermore, we examined the effect of IFN and Dex on the expression of an additional surface antigen for each of the cell lines.

Fig. 5 shows that FcR expression on U-937 cells was increased more than 7-fold by IFN-γ, and unlike HLA expression, was inhibited by Dex (P < 0.01). IFN-α caused a slight increase in FcR expression (P < 0.05), and this too was inhibited by Dex (P < 0.01). The binding of MoAb OKT8 to MOLT-4 cells was also examined. OKT8 binding was increased by Dex (P < 0.01) and increased further by combined treatment with IFN-γ and Dex (P < 0.05), although IFN-γ alone did not have a significant effect on OKT8 binding. IFN-α slightly inhibited OKT8 binding (P < 0.05). With Daudi cells, the relative amount of slg was monitored using MoAb 67.8. IFN-α caused a dramatic decrease of slg on day 5, while Dex and IFN-γ did not significantly alter slg expression.

**DISCUSSION**

The major findings in this report are (a) combined treatment with IFN-α and Dex resulted in an additive or more than additive antiproliferative effect on all seven cell lines tested after a 5-day treatment, (b) IFN-γ and Dex were synergistic for inhibition of growth of three cell lines (U-937, HL-60, and THP-1) and additive for inhibition of growth of two other cell lines (MOLT-4 and K-562), (c) Dex did not inhibit the IFN-α or -γ enhancement of HLA-classes I and II antigen expression, (d) Dex increased HLA class II antigen expression on Daudi cells, and (e) Dex and IFN-γ plus Dex increased the binding of MoAb OKT8 to MOLT-4 cells.

Glucocorticoids have been used to treat leukemia and lymphoma for more than 30 years. As single agents, glucocorticoids are most active in childhood acute lymphoblastic leukemia (14). One mechanism for the antitumor effect of glucocorticoids is direct lysis of malignant lymphocytes (14, 32). Our results show that Dex acts directly on several cell lines (Daudi, MOLT-4, K-562, and KG-1a) to cause substantial inhibition of growth. These findings are consistent with the studies by others (33).

Glucocorticoids are less effective in myeloid leukemia than in acute lymphoblastic leukemia (14). Our results indicate that glucocorticoids did not affect the growth of U-937 cells and decreased the proliferation of HL-60 and THP-1 cells only slightly. The reason for this lack of cytostatic effect is unclear, but absence or insensitivity of glucocorticoid receptors can be ruled out, because Dex inhibits FcR expression in each of these cells (Fig. 5, Ref. 15).

Although glucocorticoids are not effective in the treatment of myelogenous leukemia, IFNs have recently shown promise for the chronic form of this disease (3, 4). It is noteworthy that in our study Dex enhances the antiproliferative effect of both IFNs at a concentration easily achieved therapeutically. IFN-γ and Dex appear to have a synergistic effect in inhibiting the growth of U-937, HL-60, and THP-1 cell lines. One mechanism by which Dex could cause IFN-γ to have an enhanced antiproliferative effect is through induction of a higher level of IFN-γ receptors. This possibility is supported by the studies of Strickland et al. (34) who reported that Dex increases the number of IFN-γ receptors on normal human monocytes. It will be of interest to determine whether Dex increases the expression of
either IFN-α or γ receptors on the cell lines used for our studies. It is striking that IFN-γ and Dex had the greatest interactive effect on the three cell lines which are reported to be either monocyte-like (U-937 and THP-1) or myelomonocytic (HL-60). These results suggest that combined use of IFN-γ and Dex could be a useful treatment of monocytic leukemia.

The inhibition of cell growth reported in this study is most likely due to hormonal effects of the IFNs and Dex, and may therefore also occur in vivo: (a) we used culture medium containing 10% fetal bovine serum, which favors vigorous and rapid growth of all the cell lines used for this study; (b) these effects occurred at concentrations of IFN and Dex which cause 50–95% saturation of their respective receptors (19, 20) and which are commonly achieved therapeutically; (c) we have obtained similar effects using the HL-60 cell line grown in serum-free defined medium (35).

Our data show that IFNs enhance HLA class I antigen expression on U-937 and MOLT-4 cells, and that all three agents (Dex and IFN-α and γ) increase HLA class II antigen expression on Daudi cells. Enhanced HLA classes I and II antigen expression by IFNs have been widely reported (36–40). On the other hand, glucocorticoid induction of HLA class II antigen expression on a B-lymphocyte cell line has not been shown previously, although a similar effect has been reported for human monocytes (41).

The effectiveness of the host's immune system to recognize and destroy tumor cells depends not only on the magnitude of the immune response but also on the capacity of tumor cells to evade destruction (42). It has recently become clear that T-cells recognize foreign antigens in association with homologous MHC products (43). Class I antigen alterations have been observed on murine tumors (44, 45), and clinical observations have shown that in certain human malignant tumors, classes I and II antigen expression may be reduced (40, 42). Also, oncogenicity was markedly reduced following DNA-mediated transformation using MHC class I-specific DNA (11, 42). Enhanced HLA expression may therefore be another mechanism for the antitumor function of IFNs and may explain the effectiveness of IFNs in vivo against a tumor cell line resistant to the in vitro antiproliferative effect of IFN (9). Our results suggest that combining glucocorticoids with IFN therapy should not decrease HLA induction and may enhance cytotoxic mechanisms.

In conclusion, our study shows that for all five myeloid cell lines and the T-cell line tested, the greatest inhibition of cell growth occurred when IFN-γ and Dex treatments were combined. In every case, the combination of IFN-α plus Dex caused greater growth inhibition than either IFN-α or Dex used alone. The induction of HLA antigen expression by both IFN-α and γ in the presence of Dex was equal to or greater than HLA induction in the absence of Dex. Thus, potential antitumor effects of interferons are not decreased by Dex at therapeutic concentrations but are sometimes enhanced. Our results suggest that combined therapy with IFN plus glucocorticoid could be more effective than IFN alone, even in glucocorticoid unresponsive malignancies.

ACKNOWLEDGMENT

We wish to thank Drs. E. D. Ball, A. Munck, A. L. Howell, and M. Fanger for their helpful suggestions. We also thank Genentech, Inc. and Burroughs Wellcome, Inc. for use of their interferons.

REFERENCES

11. Hui, K., Grosveld, F., and Fanger, M. W. Direct stimulation of ADCC by interferon has been shown in certain human malignant tumors, classes I and II antigen expression may be reduced (40, 42). Also, onecogenicity was markedly decreased following DNA-mediated transformation using MHC class I-specific DNA (11, 42). Enhanced HLA expression may therefore be another mechanism for the antitumor function of IFNs and may explain the effectiveness of IFNs in vivo against a tumor cell line resistant to the in vitro antiproliferative effect of IFN (9). Our results suggest that combining glucocorticoids with IFN therapy should not decrease HLA induction and may enhance cytotoxic mechanisms.

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REFERENCES


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