Importance of Treatment Regimen of Interferon as an Antitumor Agent

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ABSTRACT

A highly purified hybrid human leukocyte interferon, IFN-αAD, produced in Escherichia coli, has been used to define optimum treatment conditions for L1210 leukemia in mice. Treatments prior to tumor inoculation were ineffective. Treatments from the third day post-tumor inoculation were most effective, and treatments every third day were more effective than were regimens involving more frequent treatments. IFN-αAD was effective in vivo against tumors formed from a line of L1210 cells resistant to IFN-αAD in cell cultures. These and other results indicate the importance and nature of indirect mechanisms of action for efficacy of interferons against tumors.

INTRODUCTION

Antitumor activities of interferons have been attributed to a number of effects observed in cell cultures. These include direct antiproliferative activity as well as indirect activities such as stimulation of NK cell activity, antibody synthesis, and antibody-mediated cell cytotoxicity (2, 5–7, 10–13, 15, 27). However, antitumor effects have been observed in mice bearing tumor cells resistant to antiproliferative effects of interferon (14). Although stimulation of NK cell activity has been observed in clinical studies (3), a recent report indicates decreased rather than increased NK cell activity after interferon treatment (17). The contribution of mechanisms assayed in cell cultures to in vivo antitumor effects is further confounded by use of impure interferon preparations and observations that opposing effects may occur depending on the interferon concentration used and the timing of treatments (4, 18). Generally, mechanisms of action have been assumed from in vitro assays without regard to how the parameters measured are affected in optimum treatment regimens.

Cloning of human interferon genes into bacteria has led to production of highly purified materials, such as the human leukocyte interferon subtypes, interferon-αA and interferon-αD (previously designated LeIF-A and -D) (8, 9).

The presence of common restriction enzyme sites in the various leukocyte interferon genes has allowed production of novel molecular hybrid interferons, such as IFN-αAD (28), which consists of the 61 NH2-terminal amino acids of interferon-αA and the 104- amino acid COOH-terminal portion of interferon-αD. IFN-αAD is remarkable for its high activity on mouse cells as well as human cells (16, 29), and this interferon also shows antiviral activity in encephalomyocarditis virus-infected mice (29). IFN-αAD appears to have essentially the same specificity on mouse cells as purified mouse interferon preparations.3 In the present study, we have investigated various treatment regimens with highly purified IFN-αAD in L1210 tumor-bearing mice with the objective of defining optimum regimens so that significant mechanisms of action may be identified.

MATERIALS AND METHODS

Interferon. The preparation of IFN-αAD has been reported elsewhere (28). IFN-αAD was purified from Escherichia coli lysates by a combination of methods described previously (26, 30). The purified material was homogeneous by polyacrylamide gel electrophoresis and had a specific activity of 1.9 x 108 units/mg protein when titrated on HeLa cells against the NIH human leukocyte interferon standard (G-023-901-527). All interferon titers are given here in terms of the IU.

Mice. Adult (8 to 10 weeks old) female C57BL X DBA/2 F1, (hereafter called BD2F1) mice used for all experiments were purchased from Charles River Laboratories, Wilmington, Mass. Twenty mice/group were inoculated with L1210 cells i.p. for all experiments.

Cells. L1210 cells were serially passed and maintained by weekly inoculation of 1 x 106 cells into the peritoneal cavity of DBA/2 mice (Charles River Laboratories).

RESULTS

A daily dose of 10⁵ units IFN-αAD given i.p. for the 5 days preceding administration of L1210 tumor cells to mice had no effect on survival of the mice. Similarly, treatments during the first 2 days after inoculation of tumor cells caused no significant effect on survival. However, if treatments were extended to later times after tumor inoculation, significant cure was observed. The data in Chart 1 show that daily treatments from the third day post-tumor inoculation appear to be more effective than treatments commencing 1, 5, 7, or 9 days post-tumor inoculation. These treatments were given daily until the 12th day post-tumor inoculation, and all the treatment regimens conferred significant protection (p < 0.05) except the one commencing at 9 days post-tumor inoculation. Logrank x² analysis (21) showed that the regimen commencing 3 days post-tumor inoculation was more effective (p < 0.05) than any of the other treatment regimens. The relatively poor effect of the regimen commencing 9 days post-tumor inoculation must be due in part to the fact that only 3 treatments were given in this regimen and some deaths occurred before treatment commenced. However, we conclude that treatments commencing on the third day post-tumor inoculation are most beneficial. These studies were extended to examination of the effects of frequency and duration of treatments. Treatments commencing 3 days post-tumor inoculation and then given daily, or every second, third, or fourth day, all conferred protection (Chart 2a) which was significant in each case when compared to the control group. Treatments every third day conferred the greatest protection, and this was significantly greater than that conferred by treatments given daily or every fourth day. The relative poor effect of treatments every fourth day may be due in part to the fact that only 4 treatments were administered before deaths occurred. However, 7 treat-

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2 The abbreviations used are: NK cell, natural killer cell; IFN-αAD, hybrid human leukocyte interferon composed of the 61 NH2-terminal residues of subtype IFN-αA and the 104 COOH-terminal residues of subtype IFN-αD.

3 C. Czarniecki, unpublished results.

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The effect of duration of treatments was examined by giving daily treatments from the third day post-tumor inoculation. The regimens examined were a single treatment and treatments for 3, 5, 7, 9, or 12 days. The results, in Chart 2b, show that prolongation of survival is proportional to the duration of treatment. Only the 2 shortest treatments failed to confer significant protection.

Measurement of blood concentrations of IFN-αAD after i.p. administration of 10⁶ units/mouse showed that the maximum titer was 2500 units/ml, and this occurred after 30 min. Clearance from the circulation occurred over 4 hr, at which time blood concentrations were no longer detectable. The circulating interferon must represent that injected into the animals and not induced mouse interferon because the assay was positive on human WISH cells as well as on mouse L-929 cells (28). In L1210 cultures, IFN-αAD at concentrations of 2,500 and 10,000 units/ml caused 40 and 50% inhibition of the rate of growth, respectively, measured by the number of viable L1210 cells after 3 days of incubation in the presence of IFN-αAD. These observations indicate that direct effects of IFN-αAD may play a role in antitumor effects.

In order to assess the importance, if any, of direct inhibition of L1210 cells by IFN-αAD in vivo, a line of the L1210 cells resistant to the growth-inhibitory effects of IFN-αAD was developed. This was achieved by continuous passage in culture in the presence of 10⁶ units/ml IFN-αAD over a period of 10 weeks. Surviving cells were cloned in agar, and one clone was selected in medium containing IFN-αAD (3 x 10⁴ units/ml) and resistance has now persisted for more than 10 passages after removal of the interferon. This interferon-resistant cell line causes death of BD2F₁ mice at 10⁶ cells/mouse, but the mean time of death is 30 to 35 days. However, mice treated 7 times with IFN-αAD at 48-hr intervals from 1 day post-tumor inoculation caused significant protection against the resistant line of L1210 cells (Chart 3).

Maximum inhibition of in vitro plaque-forming cells is known to occur when animals are given interferon before antigenic stimulation (sheep RBC), and enhancement occurs when interferon is given after 2 or 4 days (4). Thus, interferon treatments from the day of tumor inoculation or earlier could suppress development of an immune response, whereas treatments from the third day post-tumor inoculation could enhance antitumor antibody production and cure mice bearing the L1210 leukemia. If this is the case and antibody response is necessary for antitumor effects of IFN-αAD, then treatment of mice with IFN-αAD before tumor inoculation should abrogate the efficacy of treatments given after tumor inoculation. This was found to be the case. Treatment of BD2F₁ mice with IFN-αAD prior to tumor inoculation decreased the antitumor activity of IFN-αAD given post-tumor inoculation. Mice which received one injection of IFN-αAD (10⁵ units) 48 hr prior to tumor inoculation had a mean survival time significantly (p < 0.05) shorter than did mice which were not pretreated (Chart 4).

**DISCUSSION**

It should be noted that the initial strain of L1210 cells used in this study (22) results in a mean survival time at 10⁶ cells/mouse of about 10 days. At lower tumor doses (5 x 10³ cells/mouse), the mean survival time of control groups is 15 to 20 days, and...
the majority of mice are cured by the IFN-αAD treatment regimens described here. The larger tumor dose was used in the present studies in order to assess the comparative efficacy of various treatment regimens. It is possible that the nature of the tumor cell line and the greater mean survival time that occurs with this L1210 strain in BDF2 mice may influence the effects observed.

Although more effective treatment regimens might be devised, the data presented here indicate that efficacy of IFN-αAD against the L1210 leukemia is greatest with treatments given every second or third day commencing after tumor inoculation. The doubling time of the L1210 cells used was approximately 12 hr for the initial cell line and 16 hr for the line resistant to IFN-αAD. A lag time of about 24 hr was observed in vivo for both cell lines. A tumor cell kill of at least 90% is expected to cause a delay in death of only 2 days (25). Much greater delays in mean survival time were observed in the present studies with an agent, IFN-αAD, which causes less than 50% inhibition of growth of the tumor cell in culture at the blood concentrations achieved after i.p. treatment of mice with 10⁶ units. Moreover, the line of L1210 cells resistant to growth-inhibitory effects of IFN-αAD caused tumors in mice which still responded to IFN-αAD treatment. To assure the resistant phenotype of the L1210 cells after in vivo passages, the growth-inhibitory effect of IFN-αAD was tested against these cells after 3 in vivo passages. The result of this experiment showed that these cells retained complete resistancy at 3 × 10⁶ units of IFN-αAD per ml. Thus, in vivo instability of the resistant phenotype of the L1210 cells is unlikely to be a factor affecting efficacy of IFN-αAD. Therefore, in the system described here, indirect effects seem to be of primary importance, although direct cytoxic effects cannot be ruled out. These results confirm the earlier studies of Gresser et al. (14) using impure mouse interferon and extend the number of effects in mice and mouse cells shared by mouse interferon and the hybrid human interferon, IFN-αAD.

IFN-αAD has been shown to stimulate human peripheral blood NK cell activity against K562 cells (16). However, the L1210 cells used in the present studies were not found to be targets for NK cell activity in mouse splenocytes. The lysis of L1210 cells by NK cell activity of BDF2 mice splenocytes did not exceed 5% at an effector/target cell ratio of 100/1 during the first 4 days of IFN-αAD treatment. No significant difference in NK cell activity between IFN-αAD-treated and untreated control mice was observed. The antitumor effects are most pronounced with treatments from the third day after tumor inoculation, which indicates the need for development of some response to the tumor. This could be an immune response to the tumor. However, any circulating antibodies mediating such effects are likely to be short lived or require other factors for efficacy because cured mice were found to be susceptible to reinoculation of L1210 tumor cells (data not shown). Several papers have been reported in the literature that interferons also enhance antibody-dependent cytotoxic cell activity (1, 19) and activate macrophage tumoricidal activity (23, 24). Since both of these effector functions require circulating antibody for maximal tumoricidal activity (20), it is conceivable that antitumor activity is the result of antibody-dependent cell-mediated cytotoxicity enhanced by IFN-αAD. Further investigation is necessary to determine the importance and extent of involvement of these effector functions. If specific immune responses play a role in controlling tumor development, then the effects of interferons on antibody responses could be important. For effective clinical use, optimized treatment regimens now need to be investigated further to determine mechanisms of primary importance for antitumor effects.

REFERENCES


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