Comparison of Combinations of Interferons with Tumor Specific and Nonspecific Monoclonal Antibodies as Therapy for Murine B- and T-Cell Lymphomas

Teresa Y. Basham, Michael A. Palladino, Christopher C. Badger, Irwin D. Bernstein, Ronald Levy, and Thomas C. Merigan

INTRODUCTION

We have previously shown that recombinant human hybrid rHIFN-α A/D will cooperate synergistically with syngeneic monoclonal antibody therapy. All three interferon preparations have direct in vitro antigrowth activity for 38C13 and SL2. All three interferons have direct antiproliferative activity in vivo for 38C13 lymphoma at high doses; however, none of these interferons has independent antitumor activity for SL2 in vivo. These data indicate that there is no relationship between in vitro growth cytoplasia and in vivo antitumor response. All three interferon preparations will potentiate both tumor specific and nonspecific monoclonal antibody therapy. Natural MIFN-α/β and recombinant human hybrid interferon-α A/D, which should share a common cell surface receptor, had similar antitumor activity in both models. Combining recombinant human hybrid interferon-α A/D and rMIFN-γ therapy was not additive for 38C13 lymphoma and a three-way combination with antidiotype was not significantly more effective than combination therapy with one interferon type. In general, rMIFN-γ was more effective in in vivo combination therapy against the s.c. T-cell lymphoma than against the i.p. B-cell lymphoma and was more synergistic with anti-Thy.1.1 than with antidiotype.

MATERIALS AND METHODS

Mice. C3H/HeN strain mice were purchased from Simonsen Laboratory, Gilroy, CA. AKR/J strain mice were purchased from The Jackson Laboratory, Bar Harbor, ME. Mice were between 8 and 10 weeks of age when used in therapy experiments.

Other Methods. A carcinogen [dimethylbenz(a)anthracene] induced immature B-cell lymphoma, 38C13, was produced in a C3H/HeB mouse depleted of T-cells (16, 17). This tumor and its in vitro adapted cell line express IgM (α) with a unique idiotype to which a panel of monoclonal antibodies have been produced and described (18). These monoclonal antibodies have been previously used to treat this tumor in vivo (19) and in combination with recombinant human hybrid rHIFN-α A/D (1). SL2 was derived from a spontaneous thymoma in AKR/J mice (20). In vivo and in vitro SL2 expresses high levels of Thy 1.1 antigen on its cell surface. Production of IgG2a monoclonal anti-Thy 1.1 and its therapeutic effect on SL2 in vivo have been previously described (21, 22).
Interferons. Pure preparations of rHIFN-α A/D (specific activity, 7 \times 10^{7} \text{ units/mg}) was generously supplied by Dr. Michael Brunda, Hoffmann-La Roche, Nutley, NJ. This interferon has antiviral activity similar to native mouse IFN. Natural murine IFN-α/β (specific activity, 1.2 \times 10^{7} \text{ units/mg}), produced from mouse fibroblast cultures induced with Newcastle disease virus (Lee Biomolecular Research Laboratories, Inc., San Diego, CA) was provided by the American Cancer Society. This interferon preparation contains \pm 15\% IFN-α and \pm 85\% IFN-β by antibody neutralization assay and electrophoretic profile (provided by the manufacturer). rMIFN-γ (specific activity, 1.3 \times 10^{7} \text{ units/mg}) was supplied by Dr. Michael Palladino, Genentech, Inc., South San Francisco, CA.

\textbf{In Vitro} Proliferation Studies. Growth rates for 38C13 and SL2 were measured by viable cell counts. For cell counts, 10^{6} \text{ cells/ml} were cultured in 25-cm² flasks (10 ml/flask) and samples were counted daily for 3 days with trypan blue exclusion. Interferon concentrations from 10–10,000 units/ml were assayed.

\textbf{In Vivo} Studies. In general, groups of 10 C3H/HeN mice were given injections of 10^{4} 38C13 i.p. and therapy was begun 48 h later. Monoclonal antiidiotype antibody, SIC5 (10 μg), was given as a single i.p. injection. An irrelevant IgG2a monoclonal antibody (10 μg) was used as a control. Interferons-α A/D and natural α/β (10⁵–10^{6} \text{ units/mouse/day}) were injected i.p. and continued on a 3 times weekly schedule for 3 weeks. Recombinant rMIFN-γ (10^{5}–10^{6} \text{ units/mouse/day}) was injected i.p. daily for 9 days.

AKR mice (10/group) were given injections s.c. of 10^{5} SL2 cells and therapy was begun 48 h later. Monoclonal IgG2a anti-Thy 1.1 (100 μg) was given as a single injection i.p. or i.v. Interferons (10^{5}–10^{10} \text{ units/day}) were given i.p. daily for 7–9 days. Mice were monitored for time of survival. Results were analyzed by standard normal Z test based on a binomial distribution for synergy between therapeutics.

\section*{RESULTS}

Comparison of 3 IFN Preparations for Direct Antiproliferative Activity against 38C13. The effect of IFN on the growth rate of 38C13 was measured by viable cell counts (Fig. 1A). Results showed that rHIFN-α A/D had growth inhibitory activity which is time and dose dependent. Maximum inhibition was achieved with 10^{3} \text{ units/ml} by day 3. MIFN-α/β, which is \pm 85\% IFN-β had similar antiproliferative activity for 38C13 in \textit{vitro} at the higher concentrations (10^{4}–10^{6} \text{ units/ml}) and effectively eliminated the cultures by day 3, but was less effective at 10^{5} \text{ units/ml}. While rMIFN-γ inhibits cell growth by approximately 30\% as compared to control cultures there was no time or dose effect as seen with the other 2 IFN preparations. Similar results were obtained by measuring uptake of \textsuperscript{3}H\textsuperscript{}thymidine after treatment with these interferons (data not shown).

Comparison of 3 IFNs as Single Agents and in Combination Therapy with Monoclonal Antiidiotype Antibody for 38C13 B-Cell Lymphoma. Previous studies showed that optimum responses were observed when recombinant rHIFN-α A/D was administered i.p. 3 times weekly for 3 weeks (9 days) against 10^{3} 38C13 cells transplanted i.p. and therapy begun 48 h later (1, 23). Initial studies comparing i.v., i.p., and s.c. administration daily versus 3 times weekly indicated that MIFN-α/β therapy could also be administered by this regimen for maximum antitumor response. A dose range of 10^{7}–10^{6} \text{ units/mouse/day} was compared.

Previous \textit{in vivo} therapy experience with rMIFN-γ against L1210/NCI leukemia and 38C13 lymphoma indicated that a daily schedule (9 days) would be more effective than a 3 times weekly schedule. Optimum route of IFN administration for an i.p. tumor was determined to be i.p. and maximum antitumor activity was achieved with 2 \times 10^{5} \text{ units/mouse/day} (data not shown). Therefore in these combination experiments a dose range of 10^{3}–10^{5} \text{ units/mouse/day} was compared. Animals treated with each of these 3 IFN preparations received equal total doses (9 days).

The composite results of 3 experiments (28–30 animals/group) comparing the direct antitumor effects of these 3 IFNs and their ability to potentiate monoclonal antiidiotype therapy for i.p. 38C13 lymphoma are shown in Table 1. The data are presented as percentage of survival and MST. \textit{In vivo} direct antitumor activity was observed at 10^{3} \text{ units/mouse/day} with \pm 7 survivors with rHIFN-α, 16\% survivors with rMIFN-γ, and an increase in MST of 8–9 days with all 3 IFN preparations as compared
to control animals. At 10^6 units, rHIFN-α and MIFN-α/β protected 20–22% of the animals, respectively, with an increase in MST of 11–12 days. A sufficient quantity of rMIFN-γ was not available to test 10^6 units.

In combination with 10 μg i.p. monoclonal antiidiotype antibody at the initiation of therapy (Table 1), 10^3–10^6 units of rHIFN-α/mouse/day increased survivors by 40–50% as compared to rHIFN-α and antiidiotype antibody alone. Maximum antitumor activity of the combination therapy under these experimental conditions resulted in 90% long-term survivors (greater than 60 days) at 10^6 units/mouse/day. These results indicate a synergistic response between rHIFN-α and antiidiotype similar to that previously reported (1). Natural mouse IFN-α/β had similar antitumor activity in combination with antiidiotype antibody over the same dose range, and maximum survival was 95% at 10^6 units/mouse/day.

Combination therapy with rMIFN-γ at 10^6–10^7 units and IgG2a antiidiotype resulted in only a 20% increase in long-term survivors as compared to the individual agents (28–30 mice/group). A sufficient quantity of rMIFN-γ was not available for testing higher doses. By standard 2-sample normal Z test statistical analysis, rHIFN-α was more synergistic with IgG2a monoclonal antiidiotype than was rMIFN-γ (P = 0.001) at 10^3, 10^4, and 10^5 units.

In our previous studies, rHIFN-α (10^4 units) also synergistically (P = 0.003) increased the median survival time by approximately 2 weeks of those mice that were treated with a less active monoclonal antiidiotype of the IgG2b isotype (1). We examined the effect of rMIFN-γ in combination with IgG2b antiidiotype (100 μg/i.p. administration) in this model as a measure of synergy versus additivity. Recombinant MIFN-γ (10^5 units) was administered i.p. daily for 9 days. Survival results (Fig. 2) show that rMIFN-γ alone increased MST by 6 days with 1 of 10 surviving and IgG2b antiidiotype alone increased MST by 3 days with 1 of 10 surviving as compared with control irrelevant IgG2b treated mice. The combination treated animals had an increase in MST of 17 days as compared with controls and 4 of 10 long-term survivors. Compared with the individual effects, this is an increase of 8 days and 2 long-term survivors in the combination therapy group, which is synergistic at a significance level of P = 0.01.

Combination Therapy with rHIFN-α, rMIFN-γ, and Mono-

clonal Antiidiotype Antibody to 38C13 Lymphoma. Because IFN-γ and IFN-α are very different in their structure, receptor, inducer, and cellular sources, we were interested to see if they would cooperate for increased antitumor action in vivo against 38C13 lymphoma. Groups of 10 C3H/HeN mice received 10^3 38C13 cells i.p. and therapy was begun 48 h later. Recombinant rHIFN-γ (10^3 units/mouse/day) was administered i.p. daily and rMIFN-γ (10^5 units/mouse/day) was administered i.p. 3 times weekly for 3 weeks. A single i.p. injection of monoclonal antiidiotype antibody (10 μg) was given at the onset of therapy. Control and IFN treated animals received an irrelevant IgG2a (10 μg) monoclonal antibody. Survival time was measured and the results from one experiment are shown in Fig. 3. Control animals had a MST of 22 days. The rHIFN-α treated group had no survivors and MST of 26 days. Recombinant rMIFN-γ produced 2 of 10 survivors with a MST of 26 days. Combination IFN therapy was less effective than rMIFN-γ alone and resulted in 0 of 10 survivors with a MST of 30 days.

Four of 10 monoclonal antiidiotype antibody treated animals survived with a MST of 33 days. Combination therapy with antiidiotype and rHIFN-α (10^6 units) resulted in 8 of 10 survivors (40% increase) and combination therapy with rMIFN-γ (10^5 units) resulted in 8 of 10 survivors, a 20% increase in survivors as compared to the individual agents. The 3-way combination therapy of antiidiotype, rHIFN-α, and rMIFN-γ protected 9 of 10 animals similar to either IFN combination alone. This experiment was repeated with MIFN-α/γ and with a lower dose of rMIFN-γ (10^4 units/mouse/day) included, with similar results (data not shown).

Combination IFN Therapy with a Tumor Nonspecific Monoclonal Antibody to SL2 T-Cell Lymphoma. Next we were interested in determining whether a T-cell lymphoma would respond to the 3 IFN types in a way similar to the B-cell lymphoma and to determine whether potentiation of monoclonal antibody therapy requires tumor specific antibodies. For these studies we used AKR/J SL2 lymphoma and anti-Thy 1.1 monoclonal antibody.

We first determined the effects of the 3 different IFN preparations on the growth rate of AKR/J SL2 in vitro by analyzing the number of viable cells in cultures containing 10^6–10^7 units/ml of IFN. The average of 3 results of 3 separate experiments (Fig. 1B) shows that recombinant HIFN-α A/D has less anti-
proliferative activity for SL2 than for 38C13 but does inhibit approximately 80% of cell growth at 10^5 units/ml on day 3. Natural fibroblast MIFN-α/β was slightly less effective, inhibiting approximately 60% of SL2 growth at 10^5 units/ml by day 3. Interestingly, rMIFN-γ was much more effective in inhibiting SL2 growth than 38C13. Recombinant MIFN-γ induced a time dependent response that resulted in approximately 65% inhibition by day 3 at 10^5-10^6 units/ml.

Previous studies have shown that i.v. infusion, 2 h after s.c. transplantation of 1-3 x 10^5 AKR/J SL2 cells, of 1.5 mg anti-Thy 1.1 monoclonal antibody of the IgG2a isotype can cure 70-100% of the animals (22). For the purpose of examining IFN potentiation of monoclonal anti-Thy 1.1 therapy, 10^4 SL2 cells were transplanted s.c. and therapy was begun 48 h later with a single injection i.p. or i.v. of 100 μg of antibody, which resulted in 2-3 cures/10 animals.

Initial in vivo IFN studies in SL2 compared daily versus 3 times weekly i.p., i.v., or s.c. administration of interferon at doses of 10^5-10^6 units/mouse/day. While 38C13 transplanted s.c. responds to high dose daily interferons of all types regardless of route of administration, SL2 showed no antitumor response to any of these interferons. Even at the highest dose (10^6 units daily) no animals were cured and median survival time was extended only 3-5 days (data not shown). For the combination IFN and anti-Thy 1.1 therapy studies, all 3 IFN preparations were administered i.p. at 10^4-10^5 units/mouse daily for 7-9 days. The composite results from 3 combination therapy experiments (20-30 animals/group) are shown in Table 2. The data are presented as percentage of survival and MST. As stated before, none of the 3 IFN preparations had direct antitumor activity in this model. Monoclonal anti-Thy 1.1 antibody therapy alone consistently cured approximately 25% of the mice. Combination therapy with 10^4 units of IFN (all 3 types) had little or no protective effect over anti-Thy 1.1 therapy alone. Doses of 5 x 10^4-10^5 units/mouse/day of all 3 IFN preparations potentiated anti-Thy 1.1 therapy for SL2 lymphoma with 30-50% increases in long-term survivors (greater than 60 days).

**DISCUSSION**

We have used 2 murine models, C3H 38C13 B-cell and AKR/J SL2 T-cell lymphomas to study the antitumor effects of 3 different interferon preparations alone and in combination with tumor specific and nonspecific monoclonal antibody therapy. Previously we have shown that combination therapy with syngeneic antitumor antibody and recombinant rHIFN-α A/D synergistically increased median survival time in mice challenged with a lethal dose of 38C13 tumor cells compared with the sum of the median survival times of the 2 individual treatments (1). In this study we have determined that tumor specific monoclonal antibodies are not required to maintain synergism in the combination therapy and that s.c. SL2 lymphoma is also responsive to this type of therapy.

There are 3 distinct types of IFN available to be tested clinically. α interferons (there are at least 13) are derived primarily from leucocytes (i.e., B- and natural killer cells) and they share about 40% sequence homology with IFN-β which is derived primarily from fibroblasts and epithelial and endothelial cells. The α and β interferons are induced by viruses and share a common receptor (24). IFN-γ, which has little structural homology with the other 2 types, has a separate receptor and is derived from T-lymphocytes and natural killer cells stimulated by mitogens and antigens (25).

As a source of IFN-α we used a human hybrid recombinant rHIFN-α A/D, which is potentially antigenic in mice; however, this was not a problem because it was the most effective of these 3 IFN preparations in potentiation of monoclonal antiidiotype therapy (40-50% increase in survivors over the individual agents). Anti-Thy 1.1 therapy for s.c. SL2 was also increased by 30-50% by rHIFN-α, as compared to the individual agents. *In vitro* antiproliferative studies indicate that rHIFN-α has direct antiproliferative activity for SL2 as well as 38C13, which was time and dose dependent.

Natural murine virus induced fibroblast MIFN-α/β was used as a source of IFN-β (≥85%) in these studies. As MIFN-α/β and rHIFN-α share a common receptor, it is not surprising that these 2 IFN types elicit a similar antitumor response. In fact the natural MIFN-α/β inhibited the cell growth of both 38C13 and SL2 in a similar time and dose dependent manner as did rHIFN-α and had similar direct antitumor activity alone against 38C13 at 10^6 units/mouse/day. Also, MIFN-α/β potentiated monoclonal antidiotype therapy and anti-Thy 1.1 therapy over a similar dose range as rHIFN-α. However, in both murine models, these *in vivo* and *in vitro* effects were consistently slightly lower than those achieved with recombinant rHIFNα. The reason(s) for this is not clear but may be related to small differences in specific activity per milligram of protein and/or affinity for the receptor.

Murine rMIFN-γ, which is a T-cell derived product and thought to be more potent as an immunomodulating agent, was not as active *in vitro* as a direct antiproliferative agent for 38C13 as were rHIFN-α and MIFN-α/β but was a very effective growth inhibitory agent for SL2. In contrast, rMIFN-γ did have antitumor activity at 10^5 units for i.p. 38C13 but not for s.c. SL2. There was no apparent relationship between *in vivo* sensitivity to interferon and antitumor activity in these 2 models for any of the 3 interferon preparations.

Recombinant MIFN-γ was able to potentiate antidiotype therapy with both the active IgG2a and inactive IgG2b isotypes. Statistical analysis of composite results from 3 individual experiments with each interferon type indicates that while rMIFN-γ combination therapy achieved similar overall antitumor responses as with rHIFN-α at 10^6 units, it was not as synergistic. In contrast, in the SL2 model, combination therapy with rMIFN-γ and anti-Thy1.1 was very synergistic (70-80% long-term survivors as compared with 25% for antibody alone) and equally or more effective than rHIFN-α or MIFN-α/β combination therapy.

As structural and biological differences exist between IFN-γ and IFN-α, we were also interested to see if combination IFN

**Table 2 Comparison of 3 interferons in combination therapy with anti-Thy 1.1 for AKR/J SL2 T-cell lymphoma**

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* IgG2a anti-Thy 1.1 (100 μg) i.p. at start of therapy. Interferons were administered i.p. daily for 9 days.

* Composite data from 3 experiments (20-30 mice/group).
therapy would be useful in treating B-cell lymphomas and if their potentiation of monoclonal antiidiotype therapy would be additive. The results indicate that their antitumor effects were not additive; in fact, they were lower than with rMIFN-γ alone at 10^4 units. In addition using concentrations of the individual IFNs that would potentiate antiidiotype therapy, the 3-way combination therapy was not different in antitumor effect from the combination of each IFN and was not able to cure 100% of the animals.

Studies in the 38C13 model of the mechanism of the synergistic response achieved with interferon and monoclonal antiidiotype antibody indicate that the effect of interferon is mediated by host effects rather than direct effects. An interferon resistant subclone of 38C13 is equally sensitive to antitumor effects of combination therapy. Neither IFN-α nor IFN-γ increase tumor idiotype on 38C13 although they both enhance H-2. Interferons enhance antibody dependent tumor cell cytoly sis, probably via increased Fcγ receptor expression on the effector cell populations.

It remains to be determined how useful animal model data will be in designing clinical protocols. Further studies in humans are required in order to determine whether the variables described in this study (tumor type, site and/or stage of disease, and IFN type) are important clinically. However, there is already some indication that different types of lymphoma/leukemia show a differential response to these IFN types. In addition, it is clinically useful to know that a synergistic antitumor response can be maintained with combination therapy involving monoclonal antibodies to certain normal differentiation antigens as well as with tumor specific monoclonal antibodies. Combining more than one IFN type with antibody was not more effective for 38C13 B-cell lymphoma but may be useful for other types of tumors. The antitumor responses achieved in this study are certainly encouraging with respect to the possibilities for therapeutic combinations of the natural biological response modifiers in management of human disease.

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