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Accelerated tumor development in interferon-treated B6.C-*Hyal-1*^{a} mice

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ABSTRACT **The *Hyal-1* locus, which we have previously described and mapped to mouse chromosome 9, influences the serum levels and molecular weight forms of hyaluronidase. We have also shown that the growth of two transplantable tumors, the 3LL carcinoma and the B16F10 melanoma, is influenced by the alleles at *Hyal-1*, in that the tumors develop more slowly in congenic B6.C-*Hyal-1*^{a} (also called HW23) mice than in the parental *Hyal-1*^{b} C57BL/6 mice. Here we present evidence that tumor development is stimulated and mortality is accelerated in B6.C-*Hyal-1*^{a} mice grafted with 3LL carcinoma cells when treated with α/β interferon (IFN-α/β) or with IFN-β, whereas in IFN-treated C57BL/6 mice 3LL tumor growth is inhibited. Likewise, in B6.C-*Hyal-1*^{a} mice grafted with B16F10 melanoma cells, IFN-α/β treatment results in stimulation of tumor growth, whereas in IFN-treated C57BL/6 mice tumor growth is inhibited and mortality delayed. Thus, IFN-α/β treatment of B6.C-*Hyal-1*^{a} mice results in stimulation of tumor development and sometimes in accelerated mortality. This is the opposite of the usually described effect of IFN treatment in mice, which is inhibition of tumor development and delayed mortality, as was indeed observed in the C57BL/6 mice in the present experiments. These results provide the first indication that host genes can up- or down-regulate the antitumor activity of IFN and that, on some genetic backgrounds, IFN treatment enhances rather than inhibits tumor development. This may help to explain the apparent discordance between mouse model studies, which hitherto have consistently reported inhibition of tumor formation by IFN, and the clinical trials, in which only a limited percentage of individuals show tumor regression while others have no beneficial effect or even have progression of disease in spite of the IFN treatment.**

Because of their manifold interesting biologic activities, an increasing number of cytokines are used currently in the clinic or are being considered for such use. Most cytokines act on a great variety of cells and can up- or down-regulate the synthesis of many different proteins, and, probably as a result of this complex activity, pronounced individual variations are observed in humans treated with these substances (1). Based on their antitumor activities, the first cytokines to have found large-scale use in the clinic were the interferons (IFNs), and human IFN-α and -β are currently administered to a great variety of patients with various tumor conditions for which IFN treatment has shown some effectiveness—for example, metastatic melanoma, different forms of carcinoma, myeloma, and ovarian cancers (2). Tumor regression and clinical improvement are observed only in a limited percentage of treated individuals, whereas in others, in spite of identical treatment dosage and regimen and tumors of apparently comparable histological type, there is tumor progression (3). It would be of obvious interest to understand the differences in the individual responses to IFN treatment in particular and to cytokine treatment in general, and our efforts have been directed toward identifying genes that influence the response to cytokines. To this purpose we are studying the effect of mouse genotype on cytokine activity; in the present paper we report a marked effect of a specific locus, *Hyal-1*, or a locus closely linked to *Hyal-1*, on the antitumor activity of mouse IFN-α/β (mIFN-α/β) and of mouse IFN-β (mIFN-β). The *Hyal-1* locus is situated on the distal part of mouse chromosome 9 and influences the levels and polymorphism of serum hyaluronidase (4, 5). In C57BL/6 mice, which have the *Hyal-1*^{b} allele, the major form of hyaluronidase in the circulation is a 60-kDa form, whereas in BALB/c mice, which have the *Hyal-1*^{a} allele, the major forms are 60, 120, and 140 kDa, representing 3 times as much serum enzyme activity as in C57BL/6 mice. We have previously shown that the alleles at *Hyal-1* influence the growth rate of two transplantable murine tumors, the 3LL lung carcinoma and the B16F10 melanoma. This conclusion was reached with the aid of the congenic B6.C-*Hyal-1*^{a} strain, which carries on a C57BL/6 background a small region of chromosome 9 that is of BALB/c origin and contains the *H7*, *Fv2*, and *Hyal-1* loci. B6.C-*Hyal-1*^{a} and C57BL/6 strains are otherwise genetically identical and therefore are well suited to compare the effect of the *Hyal-1*^{a} and *Hyal-1*^{b} alleles on tumor development. Intrafootpad inoculation of tumor cells into mice of both strains results in a slower development of 3LL lung carcinoma and of B16F10 melanoma tumors in the congenic *Hyal-1*^{a} strain, as witnessed by a slower rate of increase in tumor size and by a longer survival time (6). Based on the hypothesis that natural variations in the rate of tumor development might influence the efficacy of IFN treatment, and given the slower rate of tumor progression in B6.C-*Hyal-1*^{a} mice, we decided to test the possibility of an enhanced antitumor effect of IFN in such animals. In fact, we found exactly the opposite, in that IFN treatment of B6.C-*Hyal-1*^{a} mice does not inhibit but stimulates tumor growth and in some instances accelerates the mortality of the tumor-bearing animals. The evidence leading to this conclusion is presented here.

MATERIALS AND METHODS

Interferons. Two different preparations of mIFN were used. Natural mIFN-α/β, a mixture consisting of ≈80% mIFN-β and of 20% of different species of mIFN-α, the major species being mIFN-α2, was prepared in Newcastle disease virus (NDV)-induced C243 cells and purified as published (7, 8); the titer of the preparation used in our experiments was 500,000 units/0.5 ml with a specific activity of 7.3×10^8 units per mg of protein. Recombinant mIFN-β was produced by NIH 3T3 cells into which a construct had been introduced that contained the mIFN-β gene under the control of the promoter of the Moloney murine leukemia virus long terminal repeat. Since this is a new, high IFN-β-producing cell line, we will briefly describe its origin. This cell line was derived from a ψ-2 packaging cell line (9) transformed with the pMPZen-

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Abbreviations: IFN, interferon; mIFN, mouse IFN.

IFNβ construct. Plasmid pMPZen-IFNβ was derived from pMPZen (provided by S. Cory, Melbourne, Australia), which was linearized by digestion with *Hpa* I. A 0.7-kilobase (kb) *Pst* I–*Pst* I fragment from pMβ_3 (10) containing the murine cDNA IFN-β sequence was blunt-ended with T4 DNA polymerase and ligated with the pMPZen vector to generate pMPZen-IFNβ. The pMPZen-IFNβ cell line was obtained by calcium phosphate transfection of the ψ-2 packaging cell line with a mixture of the pMPZen-IFNβ retroviral plasmid and the *neo* gene-encoding pAG60 plasmid DNA (11) at a ratio of 10:1 (wt/wt), followed by selection in G418 (400 μg/ml). The pMPZen-IFNβ cell line obtained in this manner was grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (50 μg/ml). This clone produced constitutively 10^5 units of IFN-β per 10^5 cells per 48 h. The preparation used for our experiments contained 300,000 units/0.5 ml and was free of retroviral particles ($<10^2$ colony-forming units/ml).

In all the experiments, IFN was administered i.p., 0.5 ml per inoculation.

Animals. C57BL/6 and B6.C-*Hyal-1*^{a} (also called HW23 or B6.C-*H7*^{a}) mice were originally obtained from D. Bailey and L. Mobraaten (The Jackson Laboratory) and have been maintained as an inbred colony at Orsay. Mice of these two strains are genetically identical except for a small region of chromosome 9 that is of BALB/c origin in B6.C-*Hyal-1*^{a} mice. This strain was developed as a congenic strain on a C57BL/6 background by selecting for the BALB/c allele at the *H7* locus during the backcrossing (12). As a result of the close linkage of *H7* and *Hyal-1*, the *Hyal-1*^{a} allele was introduced as a passenger gene. The two mouse strains are identical at most loci, including the major histocompatibility complex locus and therefore can be used to compare the effect of the *Hyal-1* alleles on the development of transplantable tumors.

Mice were age- and sex-matched, with the age ranging between 2 and 4 months. The animals were fed autoclaved food and water ad libitum.

Tumor Cells. Two transplantable tumor lines were used: the 3LL lung cell carcinoma (13) and the B16F10 melanoma (14). Both of these tumors have previously been shown to be sensitive to the antitumor effect of murine IFN-α/β in C57BL/6 mice (15). The cells were maintained *in vitro* in RPMI 1640 medium (GIBCO/BRL) supplemented with 10% fetal bovine serum, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. Tumor cells suspended in 40 μl of culture medium without serum were inoculated into the left footpad. The cell concentration used was either 20,000 or 200,000 cells per mouse and is indicated for each individual experiment. The footpad was chosen as the site for the inoculation of the tumor cells because this allows for easy measurement of tumor nodules, since, before metastasizing, the tumors develop locally for at least two weeks. Diameters of growing tumors were measured with a spring-loaded caliper (Dial Thickness Gage 0.01–10 mm; Mitutoyo, Tokyo) and were expressed in 10^{-2} mm units. Statistical analyses of the differences in tumor sizes and survival were performed by using the nonparametric rank-sum test (16).

Histological Preparations. Tissues were fixed in formalin, and, when appropriate, bone was demineralized by exposure to a 5% solution of trichloroacetic acid (TCA) in formalin. From each specimen, 5-μm paraffin cross-sections were prepared and stained with hematoxylin and eosin.

RESULTS

Effect of IFN on the Development of 3LL Tumors in C57BL/6 and B6.C-*Hyal-1*^{a} Mice. The effect of IFN administration on the development of the 3LL lung carcinoma in B6.C-*Hyal-1*^{a} and C57BL/6 mice was examined in three experiments, two with IFN-α/β (Figs. 1 and 2) and one with IFN-β (Fig. 3), giving concordant results. The IFN-α/β administrations were started 3 or 4 days after inoculation of the carcinoma cells, at a time when the subcutaneous nodules became palpable. In general, IFN was administered i.p. every other day, 500,000 units in 0.5-ml, and was maintained till the onset of mortality. Footpad tumor size was monitored at different intervals, and the animals were autopsied and examined for metastases at death. IFN treatment of B6.C-*Hyal-1*^{a} mice resulted in accelerated tumor development, as indicated by an increase of tumor size as compared to untreated animals (Fig. 1*B*). Anatomo-pathological examination of tumor tissue derived from IFN-treated and untreated animals showed that the larger tumor sizes in the IFN-treated mice were not the result of edematous swelling of the tumors but corresponded to a greater number of tumor cells. In C57BL/6 mice, on the contrary, the administration of IFN-α/β resulted in a slight retardation of tumor development (Fig. 1*A*). The stimulation of tumor growth in IFN-treated B6.C-*Hyal-1*^{a} mice was accompanied by an accelerated mortality as compared with the tumor-bearing, untreated controls, whereas the time of mortality of the IFN-treated C57BL/6 mice was slightly delayed. The combined survival curves of the two experiments in which mIFN-α/β was administered are graphically represented in Fig. 2. In both experiments, autopsy of the mice revealed massive invasion of the lungs by pulmonary metastases in all groups.

The stimulation of tumor development and the accelerated mortality of the IFN-treated B6.C-*Hyal-1*^{a} mice were quite

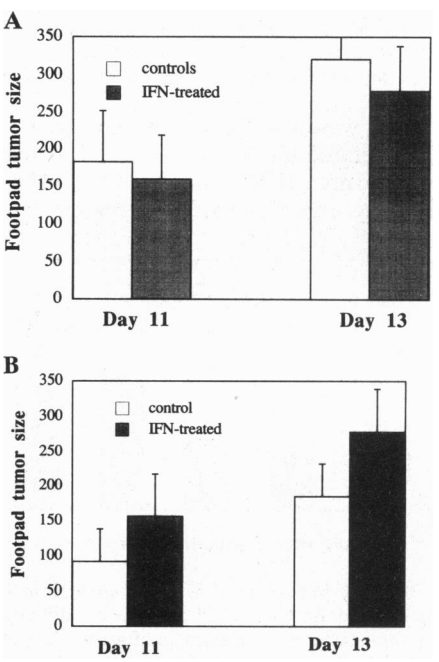

FIG. 1. Influence of IFN-α/β on 3LL tumor development. Twenty-five female B6.C-*Hyal-1*^{a} (*B*) and 25 female C57BL/6 (*A*) mice received an intrafootpad inoculation of 20,000 3LL cells. Four days later, when the footpad tumor nodules became visible, IFN-α/β was administered to 12 mice of each strain, and the 13 remaining mice were kept as untreated controls. The IFN inoculations were continued every other day for 2 weeks, making a total of 7 inoculations. The size of the footpad tumor nodules, expressed in 10^{-2} mm units, was measured in the IFN-treated and control groups 11 and 13 days after onset of the experiment, and the mean value ($\pm$ SD) is represented. Statistical analysis using the nonparametric Mann–Whitney rank-sum test showed that the difference between the IFN-treated and control B6.C-*Hyal-1*^{a} mice (Fig. 1*B*) was significant at the 1% level both on day 11 and 13, whereas the difference between the IFN-treated and the control C57BL/6 mice (Fig. 1*A*) was not significant on day 11 and was significant at the 5% level on day 13.

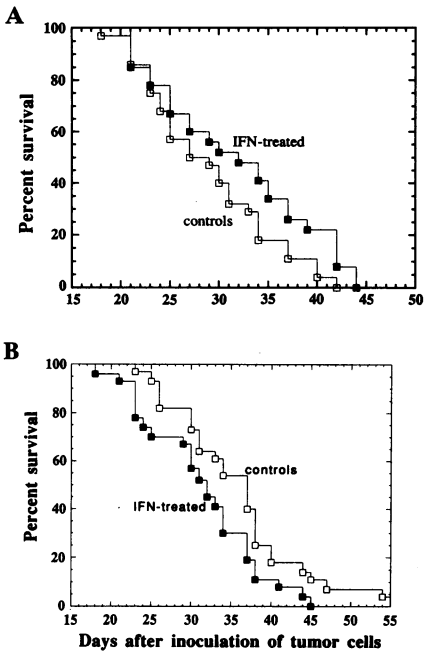

FIG. 2. Effect of IFN-α/β on survival after inoculation of 3LL cells. The combined cumulative survival curves of the IFN-treated and control C57BL/6 mice (*A*) and B6.C-*Hyal-1*^{a} mice (*B*) of the experiment summarized in the legend to Fig. 1 and of a second experiment, also carried out in female mice, comprising 15 mice in each control group and 15 in each IFN-treated group are shown. For each strain, the combined total number of animals represented is therefore 28 for the control groups and 27 for the IFN-treated groups. The difference between IFN-treated and control groups is significant at the 1% level.

unexpected and were the opposite of the various degrees of inhibition or retardation of tumor development that are usually observed after IFN treatment of tumor-bearing mice. In the preceding experiments, IFN treatment was started several days after the inoculation of the tumor cells, when the footpad tumor nodules had developed enough to become visible. However, in many experimental murine systems in which inhibition of tumor formation by IFN has been reported, the treatment was initiated either the day before or at the same time as the administration of the tumor cells. The effect of IFN-β treatment on 3LL tumor development in B6.C-*Hyal-1*^{a} mice was therefore examined in an experiment in which the IFN administration was started 1 day before the tumor cells were inoculated and was continued on the day of inoculation and every other day thereafter until day 21, when the first mice started dying. In a second group of mice, the treatment with IFN-β was only started on day 11, at a time when the footpad tumor nodules were already well developed in the untreated control group, and IFN inoculations were continued every other day, also until day 21. In spite of the different treatment schedules, significant stimulation of tumor growth was observed in both treatment groups, with the largest tumors developing in the animals in which IFN treatment had been started on the day before the inoculation of the cells (Fig. 3). Apparently, the acceleration of tumor growth by IFN was not dependent on the timing of initiation of treatment but occurred regardless of whether IFN administration was concurrent with tumor cell inoculation or was initiated later. Furthermore, the IFN-treated group displayed a slightly accelerated onset of mortality as compared with the control group (Fig. 4*A*). In contrast, even when 10 times as many tumor cells—i.e., 200,000 instead of 20,000—had been administered, IFN treatment of C57BL/6 mice resulted in retardation of 3LL tumor development, accompanied by a delay in mortality (Fig. 4*B*).

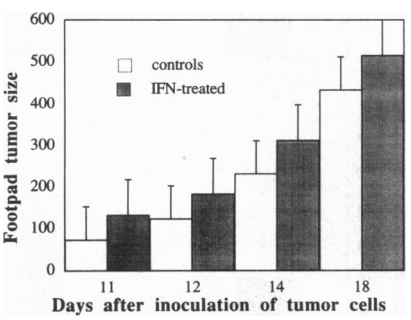

FIG. 3. Effect of IFN-β on 3LL tumor growth in B6.C-*Hyal-1* mice. Thirty female B6.C-*Hyal-1*^{a} mice were divided into three groups of 10 animals each. The animals of group A received an i.p. inoculation of IFN-β (300,000 units in 0.5 ml) on day -1. On day 0, all mice received an intrafootpad inoculation of 20,000 3LL cells, and the mice of group A (■) received another inoculation of IFN-β. From then on, the mice of this group received an i.p. inoculation of IFN-β every other day, amounting to a total of 12 inoculations. For the mice of group B (not shown), the IFN-β inoculations were started 11 days after the inoculation of the cells, when the footpad tumor nodules were palpable, amounting to a total of 6 IFN inoculations, and group C (□) served as the non-IFN-treated controls. Mean sizes of the footpad tumor nodules, expressed in mm units, of group A (IFN-treated) and group C (controls) were measured on days 11, 12, 14, and 18. Statistical analysis of the individual values using the nonparametric rank-sum Mann–Whitney test showed that the difference between the IFN-treated and the control groups was significant at the 1% level. The footpad tumor sizes of group B (not shown) were intermediate between those of group A and group C. The cumulative survival of the mice belonging to groups A and C is given in Fig. 4*A*.

Effect of IFN on the Development of B16F10 Melanomas. In view of the unexpected nature of these observations, pointing to stimulation instead of inhibition of 3LL tumor growth by IFNs in B6.C-*Hyal-1*^{a} mice, the experiments were repeated

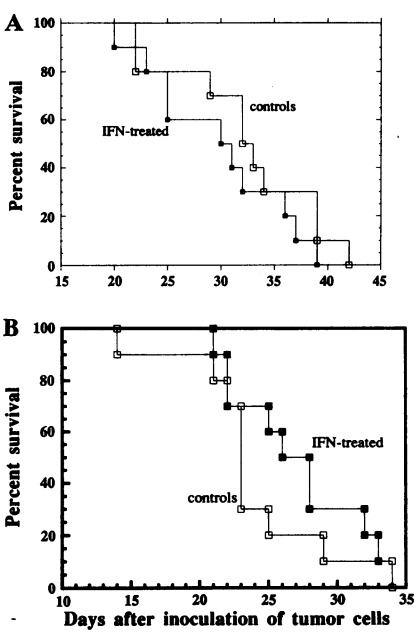

FIG. 4. Effect of IFN-β on survival after inoculation of 3LL cells. (*A*) Cumulative survival curves of the B6.C-*Hyal-1*^{a} mice of group A (IFN-β treatment started on day -1) and of group C (non-IFN-β-treated controls) of the experiment summarized in the legend to Fig. 3. (*B*) Contrary to B6.C-*Hyal-1*^{a} mice, C57BL/6 mice, even when inoculated with 10 times more 3LL cells—i.e., 200,000 instead of 20,000 per mouse—have a prolonged survival after treatment with IFN-β. In this experiment, IFN-β treatment was started 6 days after inoculation of the tumor cells and was continued every other day for 10 days. There were 10 mice per group. The difference between the control and the IFN-treated groups was significant at the 5% level.

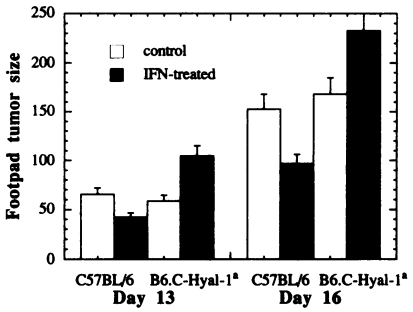

FIG. 5. Differential effect of IFN-α/β treatment on B16 melanoma development in C57BL/6 and B6.C-*Hyal-1*^{a} mice. Twenty-nine female C57BL/6 and 29 female B6.C-*Hyal-1*^{a} mice received an intrafootpad inoculation of 200,000 B16F10 melanoma cells on day 0. On day 5, when the black tumor nodules were plainly visible, IFN-α/β inoculations, 500,000 units in 0.5 ml, were started in 14 mice of each strain, whereas the 15 remaining mice were kept as untreated controls. The IFN-α/β inoculations were continued every other day, amounting to a total of 11 inoculations. Shown are mean footpad tumor sizes in IFN-treated and control mice on days 13 and 16 after grafting of the tumor cells, a time corresponding to 5 and 7 IFN inoculations, respectively. Statistical analysis of the individual footpad tumor values showed that the difference between the IFN-treated and the control groups was significant at the 1% level.

with another transplantable tumor, the B16F10 melanoma. The experiments with this tumor were performed with a dose of 200,000 cells inoculated into the left footpad. Intraperitoneal administration of IFN-α/β, 500,000 units in 0.5 ml, was initiated 5 or 6 days after the inoculation of the tumor cells, when the black footpad tumor nodules became visible, and was continued every other day until day 19 for one experiment and day 26 for the second experiment, corresponding to

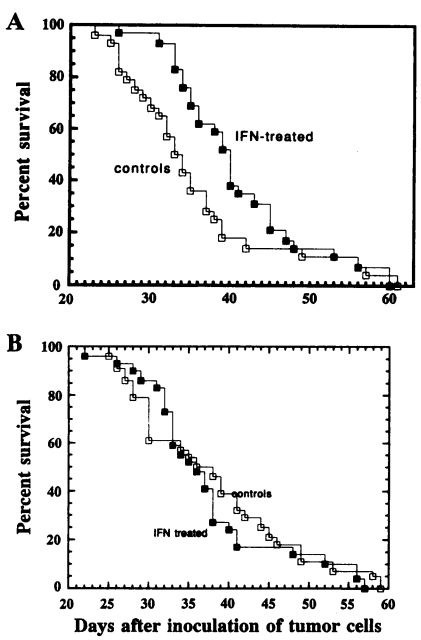

FIG. 6. Effect of IFN-α/β on survival after inoculation of B16F10 cells. Combined cumulative survival curves are shown of two different experiments, one carried out in female (see Fig. 5) and one in male mice. All mice received an intrafootpad inoculation of 200,000 B16F10 melanoma cells, and IFN treatment was started 5 (experiment using females) or 6 (experiment using males) days after the inoculation of the tumor cells. Both for the B6.C-*Hyal-1*^{a} (*B*) and C57BL/6 (*A*) mice, the combined total is 29 animals in the IFN-treated groups and 28 in the control groups. For the C57BL/6 mice, the difference between the IFN-treated and control group was significant at the 0.1% level.

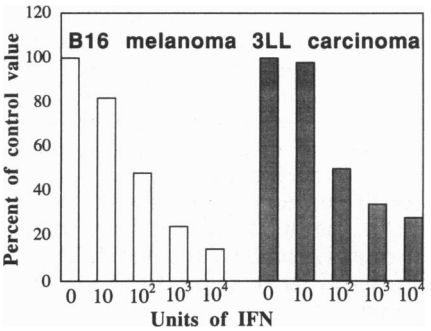

FIG. 7. Sensitivity of the B16F10 melanoma and the 3LL carcinoma cells to the direct antiproliferative effect of IFN-α/β. B16 melanoma and 3LL carcinoma cells were cultured for 4 days in the presence of 10-fold increasing amounts of IFN-α/β, expressed as units per culture, with two cultures per IFN concentration. On day 4, the cells were trypsinized and counted. The average number of cells derived from two cultures is shown, expressed as percent of the control value obtained from the two non-IFN-treated control cultures. When IFN-β instead of IFN-α/β was used, a comparable result was obtained.

the time when the first mice started dying in each experiment. IFN treatment resulted in stimulation of tumor growth in B6.C-*Hyal-1*^{a} mice and retardation of tumor growth and prolonged survival in C57BL/6 mice (Figs. 5 and 6). Thus, also when the tumor consisted of melanoma cells did we observe opposite effects of IFN administration, depending on the genotype of the mice. This suggests that the IFN-induced stimulation of tumor development observed in B6.C-*Hyal-1*^{a} mice is most probably a general phenomenon, not limited to a particular type of tumor.

Sensitivity of the Two Tumor Cell Lines to the Direct Antiproliferative Action of IFN. The mechanism of the antitumor activity of IFN-α and of IFN-β is complex and only partially understood. It involves, among others, stimulation of natural killer cells and of cytotoxic T-cell activity, the latter in part through the enhanced expression of MHC class I antigens at the surface of tumor cells. Inhibition of tumor growth through the direct action of IFN on tumor cells is a rare occurrence *in vivo*, but, in view of the stimulatory effect observed in B6.C-*Hyal-1*^{a} mice, it was important to determine the sensitivity of the two tumor cell lines to the direct antiproliferative effect of IFN-α/β. To this purpose, we measured the effect of different concentrations of IFN-α/β and of IFN-β, ranging from 10 to 10,000 units, on the replication of 3LL and B16F10 melanoma cells. Both cell lines were found to be sensitive to the antiproliferative effect of IFN, with the melanoma cells displaying a slightly greater sensitivity (Fig. 7).

DISCUSSION

We have previously presented evidence indicating that the presence of the *a* allele at *Hyal-1* is responsible for the slower tumor development and the delayed mortality of B6.C-*Hyal-1*^{a} mice as compared with C57BL/6 mice, and the most likely explanation for this enhanced resistance is provided by the higher hyaluronidase levels in the circulation of B6.C-*Hyal-1*^{a} mice (6). It seemed logical to think that the slower tumor growth in these animals would provide an advantage for IFN treatment and that the sum of the two factors, slower tumor development on the one hand and the antitumor activity of IFNs on the other, would lead to an enhanced protective effect. Yet, exactly the contrary was observed, since in the B6.C-*Hyal-1*^{a} mice IFN-α/β and IFN-β consistently stimulated tumor development, and, in the animals inoculated with the 3LL lung carcinoma, accelerated the

mortality. This is the opposite of the usually described effect of IFN-α and IFN-β on tumor-bearing mice, which is inhibition of various degrees and duration, depending on the type of tumor involved and on the timing and dosage of IFN administration (3, 17). In the experiments reported here, this type of response was indeed obtained in the IFN-treated C57BL/6 mice, inoculated with the 3LL lung carcinoma or with the B16F10 melanoma cells. This shows that, given the right conditions, the two tumor cell lines employed for our studies were sensitive to the antitumor effects of IFN *in vivo* and that sufficient IFN was used to obtain some degree of tumor inhibition.

In spite of the considerable efforts of many investigators, there is still uncertainty as to the mechanisms by which IFNs inhibit or delay tumor development. All available evidence suggests that it is a combination of multiple mechanisms, at least partly operative through the stimulation of effector cells of the immune system, such as macrophages, natural killer cells, and cytotoxic T cells (18). Exceptionally, IFN has direct effects on tumor cells by inducing differentiation or through its antiproliferative activity. Like the inhibition, the acceleration of tumor development described in the present paper must take place via some host mechanism because, *in vitro*, the two tumor cell lines used in our studies were both sensitive to the direct antiproliferative activity of IFN. The only difference between B6.C-*Hyal-1*^{a} and C57BL/6 mice lies in a region situated on the distal part of chromosome 9, that is of BALB/c origin in B6.C-*Hyal-1*^{a} mice. Evidently, the presence of the BALB/c instead of the C57BL/6 allele at *Hyal-1*, and maybe at other closely linked loci in this region, is responsible for the stimulating effect of IFN on tumor growth. Yet, IFN has been shown to prolong the survival of BALB/c mice inoculated with tumor cells of different origins (3, 17), which means that the presence of the BALB/c alleles in the region of the *Hyal-1* locus does not always result in tumor stimulation by IFN. Rather, it is the combination of the BALB/c alleles on an otherwise C57BL/6 genetic background that is involved in the tumor-stimulating effect that we observed in B6.C-*Hyal-1*^{a} mice. The chromosomal region that contains the *Hyal-1*, *H7*, and *Fv2* loci is syntenic with a conserved region of human chromosome 3, 3p21, that contains several as-yet-uncharacterized loci that have been implicated in tumor development, such as small-cell lung carcinomas and renal carcinomas (19, 20). The human homolog of *Hyal-1* has not yet been identified because we have not been able to detect polymorphism for hyaluronidase in the limited number of human sera that we have examined.

There have been a few reports in the literature describing stimulation of tumor growth in specific mouse models in which the usual result is inhibition because of very special conditions of timing and dosage of administration (21, 22), but a consistent tumor-stimulating effect, depending on host genotype and regardless of timing, dosage, and number of IFN administrations, has not been reported. Animal model studies using IFNs have been carried out only in a limited number of inbred mouse strains, the most frequently used being BALB/c, DBA/2, C3H, AKR, Swiss, and C57BL/6. From the genetic point of view, an inbred strain represents only one individual, which means that all the murine model studies combined represent far less genetic diversity than just one clinical trial. This is probably why the results of the clinical trials with IFN have not lived up to the expectations raised by the mouse model studies, and we believe that the findings reported here may help to explain the apparent discordance between the results from mouse model studies and those of the clinic, a problem that has intrigued many investigators in the past. The question was aptly posed some years ago by I. Gresser: "Why is IFN active only in some patients? Is it because a given tumor is never the same in two patients despite apparent histologic similarities?. . . Is it because patients do not always respond to a given IFN in the same way?" (23). If we extrapolate from the findings reported in this paper, the latter possibility now has become a serious working hypothesis, and we believe that the fact that, depending on the genetic make-up of the host, the administration of a cytokine such as IFN to tumor-bearing animals can lead to either delayed or accelerated tumor development is relevant to the clinical use of these compounds. IFNs are presently being used to treat many different types of tumors—for example, malignant melanoma, renal carcinoma, and small cell lung carcinoma. For every type of tumor that is treated, a given percentage of patients displays a favorable response of various degrees and duration, whereas in others no favorable clinical response is seen and the IFN treatment is without apparent effect on the tumor, which progresses. In the latter group, however, it is obviously not possible to ascertain whether IFN did in fact stimulate tumor growth, since the natural course of tumor development is variable and different for each individual.

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