Int. J. Cancer: **51,** 956–961 (1992)

Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

INTERFERON-α/β IN VIRUS-INDUCED MOUSE MAMMARY CARCINOGENESIS: EFFECTS ON THE SPONTANEOUS PROCESS AND ON THE PROGRESSION OF TRANSPLANTED PRE-NEOPLASTIC LESIONS

F. BASOLO[1], G. FONTANINI[1], C. SERRA[4], A. DOLEI[2], E. PROIETTI[3], F. BELARDELLI[3], P.G. CONALDI[4], M. BISTOCCHI[1], F. SQUARTINI[1] and A. TONIOLO[4,5]

[1]*Institute of Pathological Anatomy, University of Pisa;* [2]*Institute of Microbiology, University of Sassari;* [3]*Laboratory of Virology, Istituto Superiore di Sanità, Roma; and* [4]*Department of Biomedicine, University of Pisa, Italy.*

Low levels of anti-viral activity, mainly interferon α/β (IFN-α/β), are regularly found in lymphoid tissues of BALB/c mice infected with the C3H strain of mammary tumor virus. At the time of tumor development, significant amounts of anti-viral activity were detected in homogenates of spleen and mammary tumors, but not in blood and normal mammary glands. This activity is pH2-resistant and neutralized by antibody to IFN/α-β. The pathogenetic role of IFN in mammary carcinogenesis was investigated in 2 ways: (a) by treating virus-infected newborn mice with antibody to IFN-α/β, and (b) by giving either the latter antibody or IFN-α/β to virus-free animals transplanted with pre-neoplastic lesions. Mice were treated only for 2 months, starting either 1 week after birth or immediately after tumor transplant. In case (a), treatment with antibody to IFN-α/β shortened the incubation period of mammary carcinomas and decreased the mean survival time. In case (b), anti-IFN antibody did not significantly affect the development of mammary tumors. However, exogenous IFN-α/β markedly reduced both tumor incidence and mortality rate. These results indicate that endogenous IFN-α/β plays a crucial role in the *in vivo* restriction of the early infectious phase of spontaneous carcinogenesis and that relatively high doses of IFN-α/β may inhibit the progression of pre-neoplastic lesions.

The interferon (IFN) system has a wide range of biologic effects both *in vitro* and *in vivo,* including anti-tumor activities. Though many studies have been performed on the anti-tumor effects of IFN both in experimental animals (reviewed in Gresser, 1985) and in cancer patients (reviewed in Okita and Kaneko, 1990), little is known with regard to the IFN effects on mammary tumorigenesis. Original observations of Came and Moore (1971) showed that MTV-induced mammary tumorigenesis could be retarded by continuous treatment either with crude IFN preparations or with poly I:C, an IFN inducer. More recently, similar results were obtained by injecting purified IFN-alpha and -gamma into BALB/c mice already carrying spontaneous mammary tumors, but no complete remissions were obtained (De Clercq *et al.,* 1982). In particular, although some recent studies have reported the anti-tumor effects of IFN against transplantable (Balkwill *et al.,* 1986) or chemically-induced (Shah *et al.,* 1989) breast tumors, no data are still available on the role of endogenous IFN in the early stages of viral-induced mammary tumorigenesis and, more important, on the effects of the IFN system in controlling the progression of pre-neoplastic lesions to malignancy.

The natural history of MTV-induced murine mammary tumors includes the early phase of neonatal infection, the development of pre-neoplastic lesions (hyperplastic alveolar nodules, HANs), and the progression of these lesions to malignant neoplasia. We have previously shown that the neonatal infection of BALB/c mice with the C3H-variant of murine mammary tumor virus (MTV) induces chronic production of low levels of anti-viral activity in lymphoid organs (Basolo *et al.,* 1986). This activity was characterized mainly as interferon-α/β (IFN-α/β) and appeared to be responsible for the enhanced resistance of mice to the induction of tumors by leukemic retroviruses.

Since antibody to mouse IFN has proven useful in demonstrating the role of endogenous interferon in the pathogenesis of viral and neoplastic disease (Gresser, 1985; Jarpe *et al.,* 1989), we investigated how treatment with anti-IFN antibody could influence both the long-term outcome of neonatal MTV infection and the progression of transplanted pre-neoplastic lesions. The results show that the chronic activation of the IFN system *in vivo* confers resistance against the development of viral-induced mammary carcinomas, and suggest that relatively high doses of IFN-α/β may be effective in inhibiting tumor progression in this system.

MATERIAL AND METHODS

Animals

Inbred virgin female BALB/cfC3H mice (*i.e.,* BALB/c mice carrying the C3H variant of MTV) were used in studies of spontaneous tumorigenesis. Breeding female BALB/cfC3H mice, aged 9 to 12 months, were used as donors of HANs. Three- to four-week-old syngeneic female BALB/c mice (*i.e.,* mice free of exogenous MTV) were used as recipients of HANs, and as donors of normal pituitaries. All animals were obtained from our own mouse colony; mice were housed 3 to 4 per cage and given a standard maintenance diet and water *ad libitum.*

Interferon, antibody to interferon, interferon titration and characterization

The natural MuIFN-α/β used for animal treatment was obtained and purified as already reported (Balkwill and Proietti, 1986). The activity of the IFN-α/β preparations used in these experiments was 1×10^7 experimental units (EU)/mg. One of these units, as expressed in the text, is equivalent to 4 IFN reference units. Each dose contained approximately 1×10^5 EU and was diluted in PBS containing 400 μg/ml BSA (low-endotoxin grade; Pierce Rockford, IL). Control mice were injected with corresponding doses of PBS-BSA. The IgG fraction of sheep anti-MuIFN-α/β (batch R2/3B) were obtained from the serum of animals immunized with either purified MuIFN-α/β (Proietti *et al.,* 1986). Control IgG preparations were obtained from non-immunized animals. All sera were de-complemented and extensively absorbed on murine cells. Immunoglobulin fractions were separated by ammonium sulphate precipitation (protein concentration varied between 10 and 33 mg/ml). The neutralizing titer of sheep IgG against 4 to 8 EU of MuIFN-α/β was 2×10^5; this serum did not neutralize MuIFN-gamma. Control sheep IgG preparation failed to neutralize MuIFN-α/β (titer < 10). Neutralizing units (4×10^4) in 200 μl PBS were injected every 10 days for 8 weeks. Control groups received corresponding doses of control

[5]To whom correspondence and reprint requests should be addressed, at Department of Biomedicine, University of Pisa, 35-39, Via San Zeno, 56100 Pisa, Italy. Fax: +39-50-555.477.

Received January 29, 1992 and in revised form March 26, 1992.

IgG preparation. Purified rat monoclonal antibody (MAb) to murine IFN-gamma (clone AN-18) was kindly provided by S. Landolfo (University of Turin, Italy) as affinity-purified IgG derived from tissue-culture medium. This MAb neutralized murine recombinant IFN-γ (BioSource, Camarillo, CA) at a titer of approximately 3×10^3/ml; its titer against murine IFN-α/β was < 10.

IFN determinations were performed by the classic plaque-reduction assay with murine L-929 cells and vesicular stomatitis virus (VSV) as a challenge (Belardelli *et al.*, 1987). A reference sample of MuIFN-α/β (128 U/ml corresponding to 300 U/ml of a reference NIH standard) was used to monitor the assay. Five percent tissue extracts in tissue-culture medium were obtained from mouse tissues by sonication; homogenates were centrifuged at 250,000 *g* for 15 min before testing (Basolo *et al.*, 1986). Neutralization of IFN-α/β and IFN-gamma was performed as reported (Belardelli *et al.*, 1987). Distinction between acid-labile and acid-resistant IFN was made by assaying each sample before and after treatment at pH 2 at 4°C for 4 hr; acid-treated samples were neutralized with the appropriate amount of 1N NaOH before testing.

Experimental design

Spontaneous MTV-induced tumorigenesis. Treatment and control groups consisted of newborn female BALB/cfC3H mice aged 5 to 7 days. The animals were injected i.p. either with purified sheep antibody to IFN-α/β, or with a corresponding dose of control sheep IgG preparation. Mice were injected at 10-day intervals for 2 months with 200 μl of each preparation, except that only 50 μl were given at the first injection. At the end of treatment, 2 pituitary glands obtained from syngeneic BALB/c females were transplanted under the kidney capsule of each mouse (Basolo *et al.*, 1988). Pituitary transplantation was performed to accelerate the development of mammary tumors by hormone supplementation. All animals were examined weekly for tumor onset. When tumors developed, their growth rate was monitored by measuring tumor diameters every third day. Mice were either followed to the time of death to establish survival times, or killed one month after tumor onset to obtain samples for evaluating the anti-viral activity in different tissues and for histopathology. At autopsy, lung metastases and the number of primary tumors were also evaluated. The success of ectopic pituitary transplant was evaluated by examining histologic sections of the right kidney at the site of implantation. On this basis, only 4/220 mice had to be excluded from the study due to transplantation failure.

Transplantation of HANs. To evaluate the effects of exogenous IFN or of antibody to IFN on the progression of pre-neoplastic mammary lesions induced by MTV, HANs obtained from syngeneic BALB/cfC3H were transplanted into MTV-free BALB/c females aged 2 months. To this end, donor females were treated for 3 days with a mixture of hydrocortisone, sheep prolactin, and rat GH to make HANs visible (Basolo *et al.*, 1988). HANs were then picked up and transplanted into the fat pad of an abdominal mammary gland, which had been previously prepared by removal of the glandular tree. Treatment with IFN-α/β or antibody to IFN-α/β was initiated the day before transplantation. In short, 4 groups of transplanted mice were injected i.p. with: (i) natural purified IFN-α/β (10^5 units in 200 μl of PBS containing 0.1 mg of BSA, every other day for 2 months); (ii) control BSA (0.1 mg in 200 μl of PBS; same schedule as in first group); (iii) purified sheep IgG to murine IFN-α/β (0.1 mg containing approximately 2×10^4 neutralizing units; given in 200 μl of PBS every 10 days for 2 months); (iv) control sheep IgG preparation (a corresponding dose with the same schedule as in third group). At the end of treatment, all groups underwent ectopic pituitary transplantation as already described; follow-up was as reported in the preceding section.

Measurement of HANs outgrowth. IFN-treated mice and a control group were killed 8 weeks after the HANs transplant, *i.e.*, at the end of IFN treatment. Mammary fat pads with nodule outgrowths were removed and prepared as whole mounts. The percentage of filled fat pad was measured at low magnification by using a calibrated ocular micrometer, as reported by Basolo *et al.* (1988).

Histopathology

Samples from each tumor were fixed in formalin, embedded in paraffin, cut into 4-μm sections, and stained with hematoxylin and eosin. According to the criteria used in previous investigations, tumors were classified as either type A (acinous) or type B (cystic). The mitotic index was evaluated by counting the mitoses in 5 different fields of the same tumor.

Statistical analysis was performed with the PC software Statgraphics (STSC, Rockville, MD).

RESULTS

Spontaneous tumorigenesis: effect of anti-IFN-α/β on mice survival and tumor incidence

Treatment with antibody to IFN-α/β resulted in an accelerated mortality rate as compared with mice given control IgG preparation (Fig. 1). All the treated mice died within 285 days, at which time 45% of controls were still alive. Statistical analysis by U Mann-Whitney test showed a significant difference between the survival curves ($p < 0.002$). Tumor incidence at 250 days of age was significantly increased by treatment with antibody to IFN-α/β (Table I). At this time, in fact, 100% of treated mice (compared with 55% of controls given normal sheep IgG) had developed palpable tumors. However, no significant differences were found in frequency of lung metastases at the time of death, or in mean number of tumors per mouse, or in tumor growth rate as determined by size measurement. This was confirmed by the finding of equivalent mitotic indices in both groups, and by the fact that no histopathological differences were detected between the 2 groups. All tumors, in fact, were adenocarcinomas type A.

Transplant of HANs: effects of anti-IFN-α/β and IFN α/β on tumor incidence and growth rate

Treatment of HANs-transplanted mice with anti-IFN-α/β or control IgG preparation showed that the tumor incidence

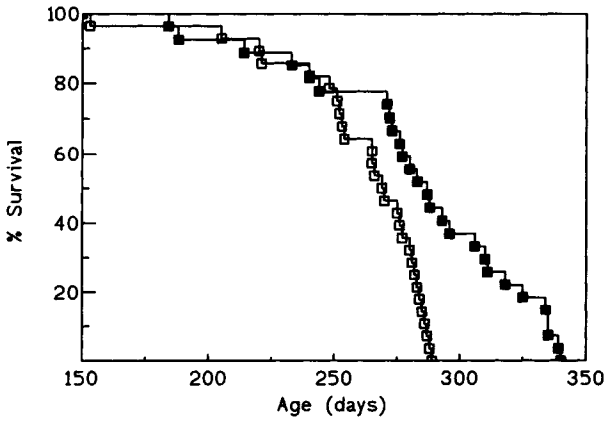

FIGURE 1 – Spontaneous tumorigenesis model: survival curves of mice treated at birth with neutralizing antibody against IFN-α/β. Controls treated with normal sheep IgG [■] (n = 26), or mice given anti-IFN-α/β antibody [□] (n = 29; $p < 0.002$). Treatment: i.p. injection of 2×10^4 IFN-neutralizing units every 10 days for 2 months.

TABLE I – SPONTANEOUS MAMMARY TUMORIGENESIS: EFFECTS OF NEONATAL TREATMENT OF BALB/cfC3H MICE WITH ANTI-IFN-α/β ANTIBODY

Parameter	Treatment		p
	Normal sheep IgG	Anti-IFN α/β	
Tumor incidence at 250 days of age[1]	15/27 (55.5%)	28/28 (100%)	<0.001
Lung metastases	5/27 (18.5%)	8/28 (28.6%)	NS
Number of tumors/mouse[2]	2.10 ± 0.23	1.90 ± 0.21	NS
Tumor growth rate (mm/day)	0.411 ± 0.041	0.423 ± 0.047	NS

[1]Positives/total; percent positives in brackets.–[2]Mean ± S.E.

TABLE II – TREATMENT OF HANs-TRANSPLANTED BALB/c MICE WITH IFN OR ANTI-IFN ANTIBODY

Parameter	Control	Treatment	p
Treatment: anti-IFN-α/β			
Tumor incidence[1]	20/23 (86.9%)	13/16 (81.2%)[2]	NS
Tumor growth rate (mm/day)[2]	0.430 ± 0.069	0.262 ± 0.078	NS (0.08)
Lung metastases[1]	5/20 (25.0%)	1/13 (7.7%)	NS
Treatment: IFN-α/β			
Tumor incidence	31/39 (79.5%)	7/22 (31.8%)	<0.0001
Tumor growth rate (mm/day)	0.383 ± 0.0522	0.537 ± 0.086	NS (0.11)
Lung metastases	9/31 (29.0%)	4/7 (57.1%)	NS

[1]Positives/total 450 days post-transplantation. Percent positives in parentheses.–[2]Mean ± S.E.

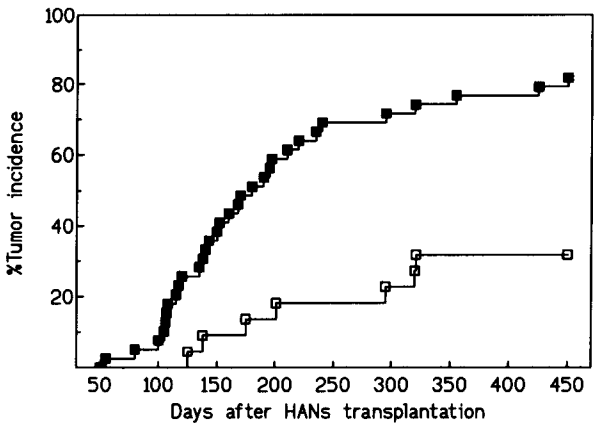

FIGURE 2 – HANs transplant model. Tumor incidence in mice treated every other day for 2 months after transplant with: control BSA [■] (n = 39), or IFN-α/β [□] (n = 22; $p < 0.01$). Treatment: i.p. injection of 10^5 experimental units every other day for 2 months.

curve was barely retarded by anti-IFN antibody (data not shown) and that tumors developing in treated animals tended to have a slower growth rate, but statistical significance was not reached ($p = 0.08$; Table II). Only 1 out of 13 animals in this group developed lung metastases.

Parallel experiments carried out in mice given IFN-α/β at the time of transplant revealed a significant reduction of tumor incidence as compared with the control group receiving carrier BSA ($p < 0.01$). This is shown both by tumor incidence at 450 days after transplant (Table II) and by the incidence curve (Fig. 2). The incidence of tumors in IFN-treated mice failed to increase after day 325 post-transplant, showing that this treatment was effective in restraining tumor development. Unexpectedly, the tumors arising in IFN-treated animals were characterized by an increased growth rate, although statistical significance was not reached ($p = 0.11$).

Morphometric analysis of the growth of pre-neoplastic lesions immediately at the end of IFN treatment (*i.e.*, 8 weeks after HANs transplant) showed that IFN-α/β caused a significant reduction of the fat pad area occupied by the outgrowth of the hyperplastic glandular tree: 46.9% ± 8.5 in control mice *vs.* 29.4% ± 6.3 in the IFN-treated group (mean ± SD, $p < 0.001$; Fig. 3,*a,b*). This result shows that pre-neoplastic lesions are susceptible to growth inhibition by IFN-α/β.

IFN levels in tissues of tumor-bearing mice

The questions as to whether mammary tumorigenesis was accompanied by the appearance of measurable amounts of IFN-like activity in host's tissues, and whether this activity could be modulated by short-term treatment with IFN-α/β or anti-IFN-α/β were addressed by measuring IFN-like anti-viral activity in tissues of treated and control mice. Thus, 1 month after the onset of tumors, groups of animals were killed to obtain samples of blood, normal mammary gland, spleen and mammary tumor. Each group of treated or control mice consisted of 7 to 18 mice. Samples for IFN determinations were prepared by centrifugation (plasma), or tissue homogenization (spleen, normal and tumorous mammary gland). Table III shows that no anti-viral activity could be detected in the plasma or normal breast tissue of tumor-bearing mice, whereas significant levels of anti-viral activity were present both in spleen and in mammary tumors of all tested mice. That spleen homogenates of normal BALB/c mice (*i.e.*, mice free of exogenous MTV) were consistently devoid of IFN-like activity was ascertained in control experiments using animals of various ages (data not shown). Thus it appears that viral mammary tumorigenesis in mice is accompanied by the specific appearance of IFN-like activity in spleen and tumor tissues, as suggested by Basolo *et al.* (1986). Statistical analysis of all data revealed that, among the different groups, only mice given antibody to IFN-α/β exhibited a marginal but consistent decrease of mean IFN-like levels in spleen and mammary tumors. This occurred both in the spontaneous tumorigenesis model and in HANs-transplanted mice, but the reduction was statistically significant only in the mammary tumors developing in the latter group. It appears, therefore, that a 2-month treatment with IFN-neutralizing antibody in the progressive pathogenetic phase of mammary tumorigenesis is capable of producing long-term effects lasting for several months after the end of treatment.

Characterization of anti-viral activity found in tissues of tumor-bearing mice

Samples of spleen and tumor homogenates containing medium to high titers of anti-viral activity were tested after

low-pH treatment or incubation with neutralizing antibodies specific to murine IFN-α/β, or IFN-γ. As shown in Table IV, treatment for 4 hr at pH 2 failed to alter significantly the titer of the anti-viral activity measured by the classical VSV plaque-reduction assay with murine cells. In contrast, IFN-like activity was significantly reduced by pre-incubation with sheep anti-mouse IFN-α/β, and was only marginally neutralized by MAb to mouse IFN-γ. Both antibody preparations were used at dilutions capable of neutralizing over 300 units of the relevant IFN in this test (see "Material and Methods"). Thus, the anti-viral activity found in spleen and mammary tumor homogenates could be ascribed, with a reasonable degree of certainty, to a mixture containing IFN-α/β together with minor amounts of IFN-γ.

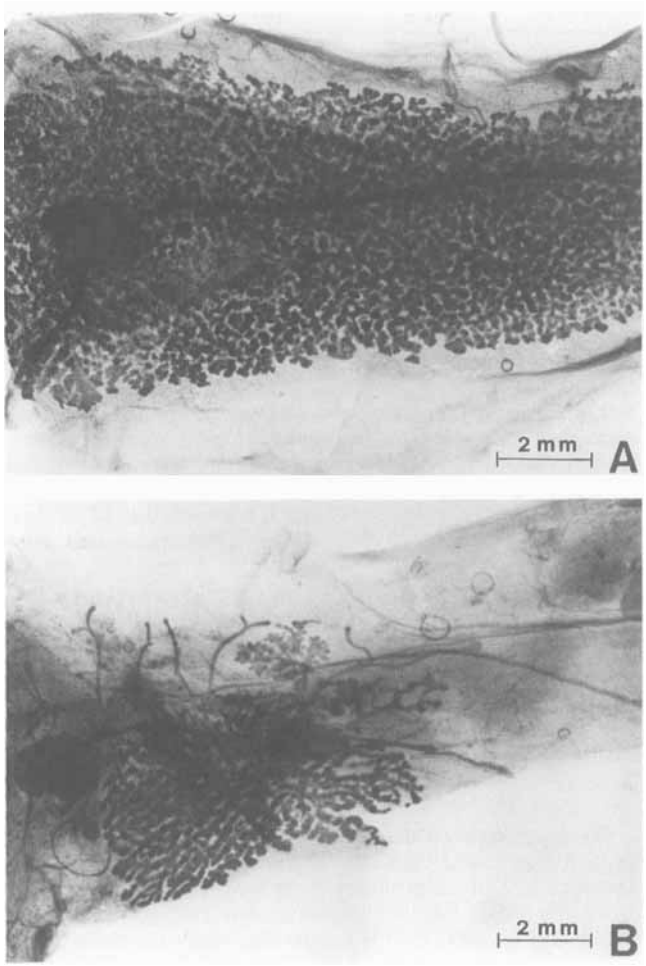

FIGURE 3 – HANs outgrowth in the abdominal mammary fat pad of syngeneic mice 2 months post-transplantation; whole-mount preparation. Mice were treated either with carrier BSA (*a;* n = 23), or with IFN-α/β (*b;* n = 12). Treatment: i.p. injection of 10^5 experimental units every other day for 2 months, starting the day before transplant. Statistical analysis showed that the area of filled fat pad was significantly reduced in treated mice as compared with the control group ($p < 0.0001$).

DISCUSSION

Infection with the mammotropic MTV represents a natural model of carcinogenesis in which 3 phases can be easily distinguished: the initial stimulus (viral infection), the appearance of hyperplastic lesions, and the progression of these pre-neoplastic lesions to malignancy. This multi-step process is rather prolonged and is susceptible to control by hormones and growth factors (Squartini *et al.,* 1983). Extensive cellular interactions and interactions of soluble factors with mammary cells are also required for the development of mammary tumors (Squartini *et al.,* 1983; Kidwell *et al.,* 1987). Thus it appeared that studies on the cytokines involved in this multi-step process could provide considerable insights into defining new strategies of chemopreventive intervention. The data presented in this article provide the first evidence concerning the role of endogenous IFN-α/β in the pathogenesis of MTV-induced carcinogenesis. In fact, a natural role for endogenous IFN in surveillance against mammary tumors is suggested by the finding that injection of neutralizing antibody to IFN-α/β for a short time immediately after neonatal infection expedited both tumor onset and mortality rate. Further support to the idea that endogenous IFN may contrast the growth of transformed mammary cells *in vivo* derives from direct measurements of IFN-like anti-viral activity in different tissues of tumor-bearing mice. No IFN-like activity was detected in plasma and in normal breast tissue, whereas significant amounts were consistently found both in the spleen and in mammary tumors of virtually all mice. Equivalent amounts were present in tissues from both the spontaneous tumorigenesis and the HANs transplant models. Neutralization and pH2 resistance studies showed that the anti-viral activity was mainly due to IFN-α/β. This shows that the intra-tumoral and splenic IFN response is part of the physiological reaction of mice to mammary tumorigenesis.

IFN production can be induced by contact of virus-infected or virus-transformed cells with leukocytes both *in vitro* and *in vivo* (Trinchieri *et al.,* 1978), and it is known that MTV replicates in a variety of cells, including sub-sets of splenic B lymphocytes (Lopez *et al.,* 1985). The interferon system has a host of anti-viral activities, which include inhibitory effects on the expression of different retroviruses (Barré-Sinoussi *et al.,*

TABLE III – LEVELS OF ANTI-VIRAL ACTIVITY IN TISSUE SAMPLES OF CONTROL AND TREATED TUMOR-BEARING MICE[1]

Group	Interferon (units/50 mg)[2]			
	Plasma	Normal breast	Spleen	Mammary tumor
Spontaneous tumorigenesis				
Control normal sheep IgG	<10	<10	102.5 ± 14.7	445.0 ± 81.2
Sheep IgG anti-IFN-α/β	<10	<10	72.5 ± 12.7	345.2 ± 51.5
HANs transplant				
Control normal sheep IgG	<10	<10	189.7 ± 22.8	266.1 ± 25.5
Sheep IgG anti-IFN-α/β[3]	<10	<10	107.5 ± 12.7*	109.3 ± 12.2**
Control (BSA)	<10	<10	156.7 ± 9.7	304.8 ± 32.6
IFN-α/β	<10	<10	111.4 ± 4.9	387.5 ± 37.9

[1]Samples obtained one month after the onset of tumor: centrifuged plasma or 5% tissue homogenates.–[2]Geometric mean ± S.D. (each group consisted of 7 to 18 samples).–[3]Significantly different from the relevant control group: *, $p < 0.05$; **$p < 0.02$.

TABLE IV – CHARACTERIZATION OF INTERFERON SPECIES IN SPLEEN AND TUMOR HOMOGENATES OF UNTREATED MICE[1]

Sample	None	Interferon (units/50mg) using treatment[2]			
		pH2	Normal sheep IgG	Anti-IFN-α/β	Anti-IFN-γ
Spleen	144 ± 36	130 ± 41	152 ± 43	30 ± 12	86 ± 22
Tumor	310 ± 51	290 ± 38	280 ± 47	24 ± 8	192 ± 43

[1]Samples obtained one month after the onset of tumor.–[2]Geometric mean ± S.D. (each group consisted of 4 to 5 samples). pH2 treatment: 4 hr at 4°C. Neutralization: 2 hr at 4°C with 1:100 sheep antibody to murine IFN-α/β, or undiluted rat MAb to murine IFN-γ. An equivalent amount of normal sheep IgG preparation was used as control.

1979; Sen and Sarkar, 1980; Wells *et al.,* 1991). Moreover, the growth of both normal and malignant cells, including breast cancer cells of human and murine origin (De Clercq *et al.,* 1982; Chen *et al.,* 1988), is inhibited *in vitro* and *in vivo.* Although antibodies to IFN-α/β were capable of accelerating MTV-induced mammary carcinogenesis, the same antibody preparation failed to produce significant effects in mice in which pre-neoplastic lesions had been transplanted. It is worth recalling that in other murine models the injection of antibodies to IFN-α/β resulted in an increased tumor take after transplant with syngeneic tumors (Gresser, 1985). Our results indicate, therefore, that the endogenous IFN-α/β inhibits MTV-induced carcinogenesis mainly by acting on the early stages of the tumorigenic process.

Although the data obtained with antibodies in mice transplanted with pre-neoplastic lesions suggest that the low levels of endogenous IFN were ineffective in inhibiting tumor progression in this system, the injection of relatively high doses of IFN-α/β resulted in a marked inhibition of tumor incidence. That the growth of pre-neoplastic lesions is indeed susceptible *in vivo* to inhibition by IFN-α/β was shown by the finding that the outgrowth of the transplanted pre-neoplastic glandular tree was markedly reduced by systemic treatment with this cytokine.

The mechanisms by which the IFN system inhibits tumor growth are poorly understood (Gresser, 1985). IFNs exert their direct anti-proliferative effects primarily by cytostatic mechanisms affecting the length of cell cycle (Baron *et al.,* 1991), but also by modulating the expression of receptors for growth factors and hormones. Growth inhibition occurs in mammary cells exposed to exogenous IFNs (Balkwill *et al.,* 1986) and, in these cells, IFNs have been shown to modulate also the expression of receptors for estrogen and progesterone, as well as for IL-6 and EGF (Shulman and Chen, 1990; Chakravarthy *et al.,* 1991). On the other hand, it is known that treatment of mice with rIFN-α or with inducers of IFN-α/β is followed by a sustained increase of class-I MHC antigens in several tissues (Halloran *et al.,* 1989), and that all 3 IFN types are capable of enhancing the expression of tumor-associated antigens in human adenocarcinoma cells freshly isolated from patients (Guadagni *et al.,* 1989). Thus, it is likely that phenomena of this type take place also in mice carrying transformed mammary cells and that they contribute to immune surveillance. In addition, NK cells play a pathogenetic role in murine mammary tumorigenesis through the release of a vast array of lymphokines (Wei *et al.,* 1989) and it is known that IFNs exert profound influences on NK activity (Robertson and Ritz, 1990).

In conclusion, it appears that the IFN system does play a role in virus-induced mammary tumorigenesis, both by interfering with the early infectious phase of the disease and, possibly at higher doses, by inhibiting the progression of pre-neoplastic lesions. With regard to therapy, these 2 phases seem to represent sensitive targets for modulation either by exogenous IFN or by means capable of altering the endogenous response.

ACKNOWLEDGEMENTS

We are grateful to Mr. R. Marsili, Mr. E. Manca and Mrs. A. Ruiu for technical help. We thank Dr. S. Landolfo for the generous gift of an antibody to IFN-γ. This work was supported by funds from the Associazione Italiana Ricerca sul Cancro (grant 1991 to F.B.), from the Ministero della Sanità (ISS; AIDS grant 1991, 6208-020) and from ENR (P.F. FATHA).

REFERENCES

BALKWILL, F.R., LEE, A., ALDAM, G., MOODIE, E., THOMAS, J.A. and FIERS, W., Human tumor xenografts treated with recombinant human tumor necrosis factor alone or in combinations with interferons. *Cancer Res.,* **46,** 3990–3994 (1986).

BALKWILL, F.R. and PROIETTI, E., Effects of mouse IFN on human tumor xenografts in the nude mouse host. *Int. J. Cancer,* **38,** 375–380 (1986).

BARON, S., TYRING, S.K., FLEISCHMANN, R.W., COPPENHAVER, D.H., NIESEL, D.W., KIMPEL, G.R., STANTON, J. and HUGHES, T.K., The interferons. Mechanisms of action and clinical applications. *J. Amer. med. Ass.,* **266,** 1375–1383 (1991).

BARRÉ-SINOUSSI, F., MONTAGNIER, L., LIDEREAU, R., SISMAN, J., WOOD, J. and CHERMAN, J.C., Enhancement of retrovirus production by anti-interferon serum. *Ann. Microbiol. (Paris),* **130B,** 349–362 (1979).

BASOLO, F., FONTANINI, G. and SQUARTINI, F., Differences in the progression of BALB/cfRIII and BALB/cfC3H mammary hyperplastic alveolar nodules transplanted into the gland-free fat pads of BALB/c mice. *Cancer Res.,* **48,** 3197–3202 (1988).

BASOLO, F., TONIOLO, A., BISTOCCHI, M., FONTANINI, G. and SQUARTINI, F., Reciprocal interference between milk-transmitted mammary tumor virus and Friend leukemia virus in mice: possible role of the interferon system. *Cancer Res.,* **46,** 4064–4070 (1986).

BELARDELLI, F., GESSANI, S., PROIETTI, E., LOCARDI, C., BORGHI, P., WATANABE, Y., KAWADE, Y. and GRESSER, I., Studies on the expression of spontaneous and induced interferons in mouse peritoneal macrophages by means of monoclonal antibodies to mouse interferons. *J. gen. Virol.,* **68,** 2203–2212 (1987).

CAME, P.E. and MOORE, D.H., Inhibition of spontaneous mammary carcinomas of mice by treatment with interferon and poly I:C. *Proc. Soc. exp. biol. Med.,* **137,** 304–305 (1971).

CHAKRAVARTHY, A., CHEN, L.C., MEHTA, D. and HAMBURGER, A.W., Modulation of epidermal-growth-factor receptors by gamma interferon in a breast cancer cell line. *Anticancer Res.,* **11,** 347–352 (1991).

CHEN, L., MORY, Y., ZILBERSTEIN, A. and REVEL, M., Growth inhibition of human breast carcinoma and leukemia/lymphoma cell lines by recombinant interferon-beta 2. *Proc. nat. Acad. Sci. (Wash.),* **85,** 8037–8041 (1988).

DE CLERCQ, E., ZHANG, Z-X., HUYGEN, K. and LEYTEN, R., Inhibitory effect of interferon on the growth of spontaneous mammary tumors in mice. *J. nat. Cancer Inst.,* **69,** 653–657 (1982).

GRESSER, I. (ed.), How does interferon inhibit tumor growth? *In: Interferon 6,* pp. 93–126, Academic Press, London (1985).

GUADAGNI, A.U., SCHLÖM, J., JOHNSTON, W.W., SZPAK, C.A., GOLDSTEIN, D., SMALLEY, R., SIMPSON, J.F., BORDON, E.C., PESTKA, S. and GREINER, J.W., Selective interferon-induced enhancement of tumor-

associated antigens on a spectrum of freshly isolated human adenocarcinoma cells. *J. nat. Cancer Inst.,* **81,** 502–512 (1989).

HALLORAN, P.F., URMSON, J., VAN DER MEIDE, P.H. and AUTENRIED, P., Regulation of MHC expression *in vivo:* II. IFN-alpha/beta inducers and recombinant IFN-alpha modulate MHC antigen expression in mouse tissues. *J. Immunol.,* **142,** 4241–4247 (1989).

JARPE, M.A., HAYES, M.P., RUSSEL, J.K., JOHNSON, H.M. and RUSSEL, S.W., Causal association of interferon-gamma with tumor regression. *J. Interferon Res.,* **9,** 239–244 (1989).

KIDWELL, W.R., MOHANAM, S. and SALOMON, D.S., Growth factor production by mammary tumor cells. *In:* D. Medina, W. Kidwell, G. Heppner and E. Anderson (eds.), *Cellular and molecular biology of mammary cancer,* pp. 239–252, Plenum, New York (1987).

LOPEZ, D.M., CHARYULU, V. and PAUL, R.D., B-cell sub-sets in spleen of BALB/c mice: identification and isolation of MMTV-expressing and MMTV-responding sub-populations. *J. Immunol.,* **134,** 603–607 (1985).

OKITA, K. and KANEKO, T., The potential of interferons in malignant disease. *Drugs,* **39,** 1–6 (1990).

PROIETTI, E., GESSANI, S., BELARDELLI, F. and GRESSER, I., Mouse peritoneal cells confer an anti-viral state on mouse cell monolayers: role of interferon. *J. Virol.,* **57,** 456–463 (1986).

ROBERTSON, M.J. and RITZ, J., Biology and clinical relevance of human natural killer cells. *Blood,* **76,** 2421–2438 (1990).

SEN, G.C. and SARKAR, N.H., Effect of interferon on the production of mammary tumor virus by mammary tumor cells in culture. *Virology,* **102,** 431–433 (1980).

SHAH, P., VAN DER MEIDE, P.H., BORMAN, T., SCHROEDER, N., BLISS, J.M. and COOMBES, R.C., Effect of human recombinant tumor necrosis factor and rat gamma interferon on nitrosomethylurea-induced mammary tumors. *Brit. J. Cancer,* **59,** 206–209 (1989).

SHULMAN, L.M. and CHEN, L., IL-6 receptors on human breast cancer cells: quantification and regulation of expression. *J. Interferon Res.,* **10S1,** 21 (1990).

SQUARTINI, F., BASOLO, F. and BISTOCCHI, M., Lobuloalveolar differentiation and tumorigenesis: two separate activities of mammary tumor virus. *Cancer Res.,* **43,** 5879–5882 (1983).

TRINCHIERI, G., SANTOLI, D., DEE, R.R. and KNOWLES, B., Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Identification of the anti-viral activity as interferon and characterization of the human effector lymphocyte sub-populations. *J. exp. Med.,* **147,** 1299–1306 (1978).

WEI, W.Z., FULTON, A., WINKELHAKE, J. and HEPPNER, G., Correlation of natural killer activity with tumorigenesis of a pre-neoplastic mouse mammary lesion. *Cancer Res.,* **49,** 2709–2715 (1989).

WELLS, D.E., CHATTERJEE, S., MULLIGAN, M.J. and COMPANS, R.W., Inhibition of human immunodeficiency-virus-type 1-induced cell fusion by recombinant human interferons. *J. Virol.,* **65,** 6325–6330 (1991).