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Immunology

Exogenous expression of mouse interferon γ cDNA in mouse neuroblastoma C1300 cells results in reduced tumorigenicity by augmented anti-tumor immunity

(retroviral vector/H-2 class I gene expression)

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ABSTRACT **To examine the influence of interferon γ (IFN-γ) on tumorigenicity, we established constitutively IFN-γ-producing cell lines from a malignant mouse neuroblastoma, C1300, by retroviral transfer of a mouse IFN-γ cDNA. The gene-transferred cells generally showed an enhanced high-level expression of the major histocompatibility complex class I antigens at the cell surface and the transcription levels, irrespective of their IFN-γ-producing potential. Although *in vitro* cell growth of these cells was unaffected by the IFN-γ production, their s.c. tumor growth in syngeneic A/J mice was dependent upon levels of IFN-γ production; tumors induced by a low-producer line grew well at a rate similar to those induced by the parental one, but tumor growth of a high-producer line was strongly suppressed. This apparent tumor suppression was abolished by simultaneous i.p. injection of anti-Lyt2.2 and/or anti-IFN-γ monoclonal antibodies, and subsequently large tumors of the high producer were generated. Anti-asialoganglioside GM_1 antibodies allowed the high-producer line to induce a substantial but only transient tumor growth, whereas other antibodies, such as anti-Lyt2.1, anti-IFN-β, and anti-activated macrophage, had no such effect. The mice immunized with the high-producer line were resistant to tumor growth of the parental cells but permitted another kind of A/J tumor line, Sa-1, to induce remarkable tumors. These results indicate that the reduced tumorigenicity of the IFN-γ high-producer line was due to the augmented specific anti-tumor immunity, in which cytotoxic T lymphocytes seemed to play a decisive role, probably as a result of the immunomodulatory effects of the IFN-γ derived from the tumor.**

Interferon γ (IFN-γ) plays important roles in the immune response (1) such as regulation of the induction of cytotoxic T lymphocytes (2). Moreover, IFN-γ increases the killing activity of natural killer (NK) cells (3), enhances the antigen-presenting functions of macrophages (4), and also promotes the tumoricidal activity of macrophages (5). Based on these diverse, immunomodulatory activities of IFN-γ, clinical trials as well as animal model experiments for anti-tumor IFN-γ therapy have been developed (6). Repeated administration or continuous perfusion of IFN-γ is required to sustain its anti-tumor effects, due to its short half-life *in vivo* (7). However, there are technical problems involved in such a treatment procedure that might be still insufficient to maintain the administered IFN-γ in close proximity to the tumor for a long time. Therefore, to more effectively expose tumor cells to IFN-γ, we constructed two cell lines that constitutively produce IFN-γ from a malignant mouse neuroblastoma line, C1300 (8), using a retroviral vector carrying mouse IFN-γ cDNA (9). In this work, the major histocompatibility complex (MHC) class I antigen expression of these cell lines and their tumorigenicity are investigated.

MATERIALS AND METHODS

Mouse IFN-γ Gene-Transferred C1300 Cells. Construction of the ψ2(Muγ)8 cell that produces an IFN-γ expression retrovirus, pSVX(MuγΔAs) (Fig. 1), has been described (9). We also obtained the line ψ2(*neo*)1 carrying the vector pZIP.NEO.SV(X)1 (10) DNA without gene insertion (Fig. 1), which produces a retrovirus only for the expression of the *neo* gene, conferring G418 resistance to mammalian cells (11). C1300 cells were infected with the virus supernatant of the ψ2(Muγ)8 or the ψ2(*neo*)1 cells in the presence of 8 μg of Polybrene per ml (Sigma); this was followed by selection and cloning in the medium containing G418 (Geneticin; Sigma) at 800 μg/ml. Out of several G418-resistant C1300 sublines obtained, two clones, C1300(Muγ)3 and C1300(Muγ)12, were picked up as low- and high-IFN-γ producers, respectively. The IFN-γ yields of the low and high producers in the confluent culture fluid were 5 international units (IU)/ml or less and about 50 IU/ml, respectively. Alternatively, we obtained a G418-resistant C1300 line, termed C1300(*neo*), as a control for gene-transferred cells. The C1300(*neo*) as well as the parental C1300 cells did not secrete any IFN activity in the culture medium (not shown).

Neither the parental nor the gene-transferred C1300 cells displayed production of ecotropic murine leukemia virus on XC plaque assay (12) (not shown).

DNA and RNA Blot Analysis. For DNA blot analysis, 10 μg of high molecular weight DNA samples, each extracted from cultured cells (13), was digested with the restriction enzyme *Xba* I (Takara-Shuzo, Kyoto), electrophoresed in a 1% agarose gel, and then transferred onto a nitrocellulose membrane according to Southern (14). For RNA blot analysis, 10 μg of denatured total cellular RNA samples isolated using guanidinium isothiocyanate as described (15) was electrophoresed in a 1.3% agarose gel containing 2.2 M formaldehyde (16) and then transferred onto a nitrocellulose membrane. The membranes were hybridized with appropriate DNA probes labeled with [α-^{32}P]dCTP (110 TBq/mmol = 3000 Ci/mmol; Amersham) by nick-translation (17). After

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Abbreviations: IFN, interferon; MHC, major histocompatibility complex; mAb, monoclonal antibody; IU, international units; NK, natural killer; CTL, cytotoxic T lymphocyte.

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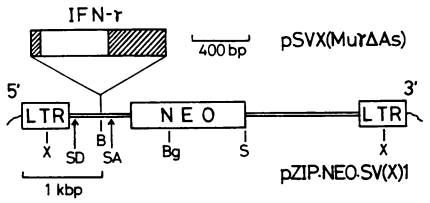

FIG. 1. Schematic structure of retrovirus expressing IFN-γ. The vectors were cloned in the plasmid pBR322 for replication in *Escherichia coli*. The coding regions of the mouse IFN-γ cDNA insert (0.92 kb) are shown open; untranslated regions are hatched. Long terminal repeats (LTRs) are derived from Moloney murine leukemia virus. Arrows SD and SA indicate splicing donor and acceptor sites, respectively, used for generating the subgenomic RNA encoding the *neo* gene. Restriction endonuclease sites are abbreviated as follows: B, *Bam*HI; Bg, *Bgl* II; S, *Sma* I; X, *Xba* I. kbp, Kilobase pairs.

hybridization, the membranes were washed and then exposed to x-ray film (AIF RX) (Fuji, Tokyo).

The DNA probes used were as follows: the 0.92-kilobase-pair (kb) *Sau*3AI–*Sma* I fragment from pBMγ-E3 (18) for the mouse IFN-γ gene, the 0.9-kb *Bgl* II–*Sma* I fragment from pZIP.NEO.SV(X)1 (10) for the *neo* gene, the 0.75-kb *Sac* II–*Pvu* II fragment from pH202 (19) for the H-2K gene, the 5.3-kb *Eco*RI fragment from the cosmid 34.2 (20) for the I-Aβ gene, and the 1.1-kb *Pst* I fragment from pAL41 (21) for the mouse β-actin gene.

Recombinant IFN-γ and IFN Assay. Pure mouse recombinant IFN-γ (1×10^7 IU/mg of protein) was donated by Shionogi Research Laboratories (Osaka). The antiviral activity of the culture supernatants was measured by the reduction of the cytopathic effect of vesicular stomatitis virus on L cells (22) and expressed in IU, as calibrated against the reference mouse IFN-α/β (NIH G002-904-511).

Antibodies. Rat monoclonal antibodies (mAbs) against mouse IFN-γ, R4-6A2 (IgG1) (23), and mouse IFN-β, 7F-D3 (IgG1) (24), and a sheep anti-mouse IFN-α/β antiserum (NIH G024-501-568) (25, 26) were used in IFN neutralization experiments. Anti-Lyt2.1 (IgG2b) and anti-Lyt2.2 (IgG2a) mAbs have been described (27). Purified anti-activated mouse macrophage mAb, AcM1 (IgG2c), was prepared as described (28). A rabbit anti-asialoganglioside GM_1 antibody (IgG fraction) (29) was purchased from Wako Pure Chemical (Osaka, Japan); a normal rabbit serum was used as a control. Monomorphic anti-MHC class I mAb, M1/42 (IgG2a), has been described (30).

***In Vitro* Cell Growth.** Cells (1×10^4) in 1 ml of culture medium of C1300 sublines were plated in triplicate wells of a 24-well tissue culture plate and incubated at 37°C in 5% CO_2 in air. At the indicated times, the viable cell number in each well was counted using a hemocytometer slide following trypan-blue staining of cells.

Tumor Induction. Female A/J mice, 6–8 weeks old, were obtained from the Facility of Experimental Animals, Faculty of Medicine, Kyoto University, and the Shizuoka Laboratory Animal Center (Shizuoka, Japan). Mice were s.c. implanted once in the right or left flank with $1–3 \times 10^6$ cells in 100 μl of phosphate-buffered saline (PBS). Tumor formation was confirmed by palpation. Tumor size, assessed by the diameter (mm), was measured at regular intervals.

i.p. Injection of Antibody. Mice were i.p. injected once with 250 μg of anti-Lyt2.1, anti-Lyt2.2, anti-IFN-γ (R4-6A2), anti-IFN-β (7F-D3), or anti-activated macrophage (AcM1) mAb in 0.5 ml of PBS or with about 1 mg of rabbit anti-asialoganglioside GM_1 immunoglobulin antibodies in 0.5 ml of PBS just after s.c. tumor cell inoculation.

Flow Cytofluorometric Analysis. Single-cell suspensions were incubated with the antibody M1/42; this was followed by incubation with fluorescein isothiocyanate-coupled rabbit anti-mouse immunoglobulin antibodies. Incubation was carried out at 4°C for 30 min. Cell-surface staining was analyzed with a flow cytometer (Spectrum III; Ortho Diagnostics) gated to exclude nonviable cells, as described (31). Data were expressed as arbitrarily normalized fluorescence histograms—i.e., relative number of cells vs. logarithm of fluorescence intensity.

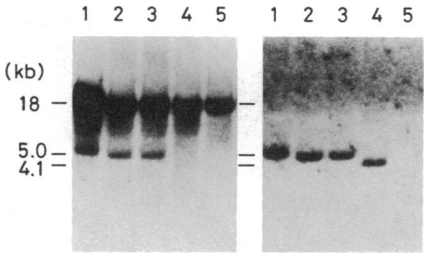

FIG. 2. Southern analysis of cellular DNAs from C1300 sublines. Cell lines were ψ2(Muγ)8 (lanes 1), C1300(Muγ)3 (lanes 2), C1300(Muγ)12 (lanes 3), C1300(*neo*) (lanes 4), and C1300 (lanes 5). IFN-γ (*Left*) and *neo* (*Right*) probes were used. Sizes (in kb) were calibrated by comparison with the *Hin*dIII digest of λ phage DNA.

RESULTS

Southern and Northern Analyses. In Fig. 2, we demonstrate that C1300(Muγ)3 and C1300(Muγ)12 cells carried the 5.0-kb *Xba* I DNA fragment hybridizable with either of the IFN-γ or the *neo* probe, as well as the 18-kb genomic IFN-γ gene fragment, whereas C1300 and C1300(*neo*) cells carried only the genomic gene. The *neo* gene-specific *Xba* I fragment of the C1300(*neo*) cell DNA was 4.1 kb in length. These observations indicate that the integrated proviral genomes were intact.

Fig. 3 shows that C1300(Muγ)3 and C1300(Muγ)12 contained transcripts (5.6 kb) homologous to the IFN-γ cDNA and/or the *neo* gene probes, corresponding to the proviral-derived full-length ones [the genomic IFN-γ gene-derived mRNA is about 1.2 kb in length (32)]. The quantity of the transcripts was apparently similar in the two IFN-γ gene-transferred lines despite their different IFN-γ production levels (see *Materials and Methods*), although the reason for this difference is not clear. C1300(*neo*) cells had transcripts (4.3 kb) detectable by the *neo* but not IFN-γ probe, whereas C1300 cells had no such transcripts.

***In Vitro* Tumor Cell Growth.** The C1300 cell used in this work was highly resistant to the anti-cell growth effect of IFN-γ, since cell growth rate was not altered in the presence of 1×10^4 IU of recombinant IFN-γ per ml (Fig. 4 *Inset*). Correspondingly, IFN-γ gene-transferred cells as well as C1300(*neo*) cells exhibited a cell growth kinetics similar to that of the C1300 cell (Fig. 4).

MHC Antigen Gene Expression of the C1300 Sublines. IFN-γ is capable of augmenting the expression of the MHC class I gene and, in some cell types, inducing the class II gene expression (33–35). Consequently, the MHC gene expression of the IFN-γ gene-transferred cells should be enhanced by the action of their own IFN-γ (9). To verify this, RNA blot hybridization was performed using the H-2K and I-Aβ

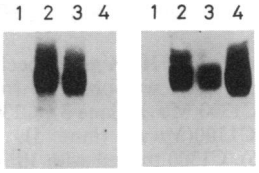

FIG. 3. Blotting analysis of transcripts of C1300 sublines. RNAs used were prepared from C1300 (lanes 1), C1300(Muγ)3 (lanes 2), C1300(Muγ)12 (lanes 3), and C1300(*neo*) (lanes 4). They were hybridized with IFN-γ (*Left*) and *neo* (*Right*) probes.

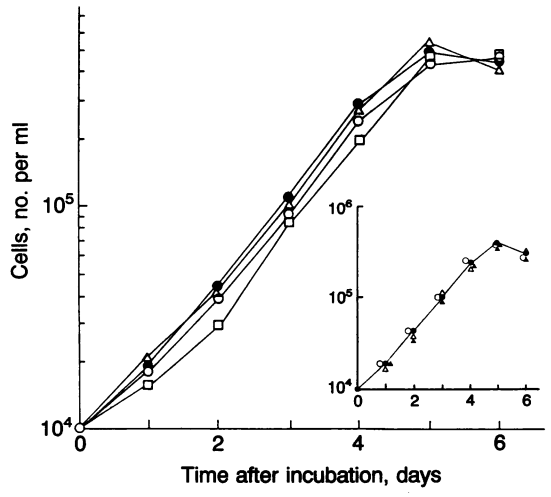

FIG. 4. *In vitro* cell growth of C1300 sublines. ●, C1300; □, C1300(*neo*); ○, C1300(Muγ)3; △, C1300(Muγ)12. (*Inset*) Growth of C1300 in the absence (●) or presence of IFN-γ at 10^2 IU/ml (○), 10^3 IU/ml (▲), or 10^4 IU/ml (△).

probes. Simultaneously, β-actin-specific transcripts were probed as a control. Fig. 5 shows a marked increase in the steady-state level of H-2K-specific transcripts in both of the IFN-γ gene-transferred sublines (lanes 3 and 4) compared with the parental C1300 and C1300(*neo*) cells (lanes 1 and 2), irrespective of the IFN-γ production level. IFN-γ treatment augmented the H-2K gene expression of the C1300 cells in a dose-dependent manner (lanes 8–11), but the expression level was still lower than those of the IFN-γ gene-transferred sublines. Moreover, the augmentation was abrogated by the exogenous addition of anti-IFN-γ antibody into the cell culture (lane 7). In contrast, the enhanced expression of the H-2K gene of the IFN-γ gene-transferred sublines was not altered by the treatment of a sufficient amount of anti-IFN-γ antibody to neutralize 10^3 IU of IFN-γ per ml (lanes 5 and 6), in harmony with a previous report (9). On the other hand, no class II gene expression was detected by RNA blot analysis in any of the C1300 sublines tested in this work (data not shown). The steady-state level of β-actin mRNA was roughly constant in all the RNA samples (Fig. 5 *Lower*).

The enhanced MHC class I gene expression in the IFN-γ gene-transferred cells was also confirmed by flow cytofluorometry of cell-surface antigen of the cultured cells, as shown

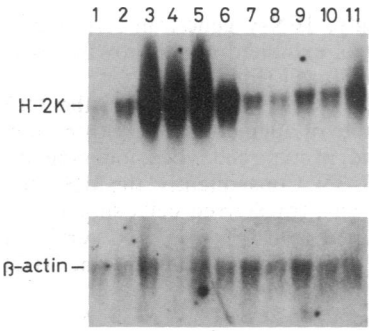

FIG. 5. Blotting analysis of H-2K gene-specific mRNA of C1300 sublines. Total cellular RNA was obtained from C1300 (lane 1), C1300(*neo*) (lane 2), C1300(Muγ)3 (lane 3), C1300(Muγ)12 (lane 4), anti-IFN-γ-treated C1300(Muγ)3 (lane 5), anti-IFN-γ-treated C1300(Muγ)12 (lane 6), C1300 treated with 10^3 IU of IFN-γ per ml and anti-IFN-γ (lane 7), and C1300 treated with 1, 10, 10^2, and 10^3 IU of IFN-γ per ml (lanes 8–11, respectively). They were hybridized with the H-2K (*Upper*) or the β-actin (*Lower*) probe. IFN-γ and/or anti-IFN-γ treatment was performed at 37°C for 24 hr prior to RNA preparation.

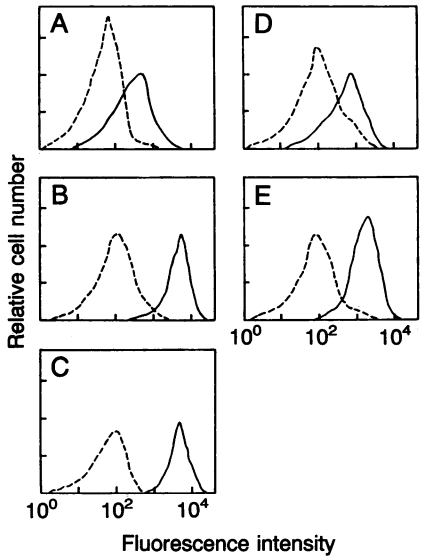

FIG. 6. Flow cytofluorometric analysis of MHC class I antigen expression of C1300 sublines. Analyzed sublines are shown in each panel: C1300 (*A*), C1300(Muγ)3 (*B*), C1300(Muγ)12 (*C*), C1300(*neo*) (*D*), C1300 + IFN-γ (*E*). IFN-γ treatment of C1300 was carried out at 37°C for 24 hr prior to analysis with 10^3 IU of IFN-γ per ml. Staining by M1/42 antibody is indicated by solid lines; unstained controls are indicated by broken lines.

in Fig. 6. The augmented H-2K antigen expression was sustained in the s.c. tumors as examined by immunofluorescence analysis of tumor sections (data not shown).

Tumorigenicity of the C1300 Sublines. The parental C1300 cell as well as the gene-transferred cells were tested for tumorigenicity by s.c. transplantation into the flank of syngeneic A/J mice. At cell doses ranging from 1×10^6 to 3×10^6, the tumor formation rate was >90% for C1300, C1300(*neo*), and low-producer C1300(Muγ)3 cells (for these lines, 36, 12, and 24 mice were used, respectively) but was almost nil (≤5%) for high-producer C1300(Muγ)12 (in this case, 53 mice were used).

The C1300 and the C1300(*neo*) tumors as well as the C1300(Muγ)3 tumors increased gradually in size as shown in Fig. 7*A*. The C1300(Muγ)12 tumors were almost always rejected during the course of tumor formation: small C1300(Muγ)12 tumors were usually formed during the first 2

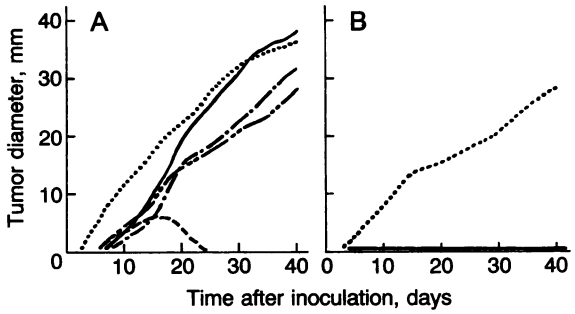

FIG. 7. (*A*) Tumor growth induced by C1300 sublines and line Sa-1. Following s.c. injection of C1300 (—), C1300(*neo*) (—·—), C1300(Muγ)3 (— - — -), C1300(Muγ)12 (---), and Sa-1 (·····) cells at a dose of 1×10^6 cells, the tumor size (mean diameter; SD ≤ 15%) in each tumor-bearing mouse was measured and expressed for 5–10 mice. (*B*) Tumor growth of C1300 and Sa-1 cells in the C1300(Muγ)-12-rejected mice. Mice were first s.c. implanted with $1–3 \times 10^6$ C1300(Muγ)12 cells. Four to 6 weeks after the cell inoculation, the mice that confirmed tumor rejection were s.c. reinjected with 1×10^6 cells of C1300 (—) or Sa-1 (·····). Mean tumor sizes of five mice (SD ≤ 10%) were plotted. No C1300 tumor growth was detected.

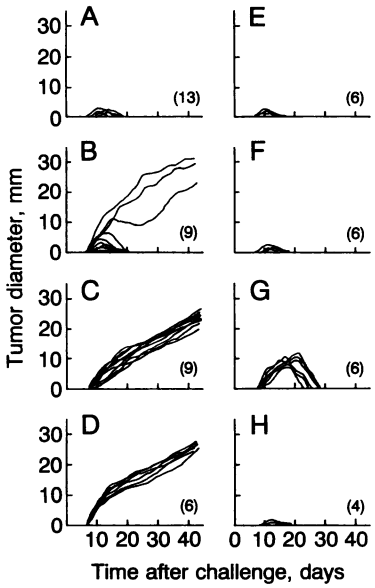

FIG. 8. Effect of *in vivo* administration of various antibodies on C1300(Muγ)12 rejection. Soon after s.c. immunization with 1×10^6 C1300(Muγ)12 cells, the mice were i.p. injected with the following antibodies: No antibody (*A*), anti-IFN-γ (*B*), anti-Lyt2.2 (*C*), anti-Lyt2.2 and anti-IFN-γ (*D*), anti-IFN-β (*E*), anti-Lyt2.1 (*F*), anti-asialoganglioside GM_1 (*G*), and anti-activated macrophage (*H*). Each line represents tumor growth in a single mouse. Numbers in parentheses represent the total number of mice used for each antibody.

weeks after the cell inoculation but disappeared within the third week (Fig. 8*A*). This characteristic profile of tumor formation and rejection is likely the result of the host anti-tumor immunity. Thus, we examined the effect of simultaneous i.p. injection of various antibodies, including ones specific for immunocompetent cells such as cytotoxic T lymphocytes, NK cells, and macrophages, on the s.c. growth of the C1300(Muγ)12 cells to ascertain which types of immunocompetent cells were involved in the tumor suppression.

Administration of anti-Lyt2.2, but not anti-Lyt2.1, promoted remarkable tumor growth of the C1300(Muγ)12 cells in all the nine mice tested (Fig. 8 *C* and *F*). The tumor promotion by anti-Lyt2.2 was also slightly enhanced by simultaneous administration of anti-IFN-γ (Fig. 8*D*). Administration of anti-IFN-γ antibody alone also promoted large tumor growth in three of nine mice examined (Fig. 8*B*), whereas anti-IFN-β had no such effect (Fig. 8*E*). Anti-asialoganglioside GM_1 antibody administration resulted in only a moderate enhancement of tumor growth; the initial substantial tumor growth was observed during the first 2 weeks after cell injection, but the small tumors (at the most about 10 mm in diameter) disappeared within the third week (Fig. 8*G*). As a control, a normal rabbit serum displayed no effect (data not shown). An anti-activated macrophage antibody (AcM1) exhibited no effect on the tumor rejection (Fig. 8*H*). The results shown in Fig. 8 suggest that the observed tumor formation and growth patterns of C1300(Muγ)12 cells were determined by counteracting anti-Lyt2.2-sensitive effector cells—i.e., cytotoxic T lymphocytes (CTLs) (27).

In the mice having rejected C1300(Muγ)12 cells, s.c. reinjection of the parental cells could no longer induce any tumor, but injection of a different A/J strain tumor, a fibrosarcoma Sa-1, could do so (Fig. 7*B*). Therefore, the anti-tumor immunity in the mice is specific for the cell type of the immunized tumor cell.

DISCUSSION

We have shown that the tumorigenicity of a malignant neuroblastoma, C1300, in syngeneic A/J mice dramatically decreased after producing a relatively high titer of IFN-γ (about 50 IU/ml) but not producing a low titer of it ($\leq$5 IU/ml) by IFN-γ gene transfer: the high-IFN-γ producer C1300(Muγ)12 tumors almost completely regressed but not in the case of the low-producer C1300(Muγ)3. Nevertheless, it is unlikely that the apparent IFN-γ production-level dependence of the tumor suppression was caused by the direct anti-cell proliferative effect of IFN-γ produced by the gene-transferred cell itself, because the C1300 cell used in this work was insensitive to such effect and the *in vitro* cell growth was not altered after the gene transfer.

In contrast, the MHC class I gene expression was highly inducible by IFN-γ, and the IFN-γ gene-transferred sublines generally exhibited a high-level expression of the class I gene, considered to be caused by the action of their own products (9). The high-level expression of the class I antigen thus may be primarily important for the reduced tumorigenicity of the C1300(Muγ)12 cell; it is widely accepted that enhanced expression of class I antigens on tumor cells by IFN-γ results, though not always, in the reduction of tumorigenicity (36–38) by facilitating immune recognition of the tumor cells by MHC-restricted killer T cells (2), although the increase of MHC class I antigen expression can result in resistance to NK cell-mediated tumor cell lysis (39, 40). However, this could not be the sole reason for the decreased tumorigenicity of C1300(Muγ)12, because the low-producer C1300(Muγ)3 was still highly tumorigenic, as described above, in spite of its enhanced expression of the class I antigens. This suggests that the C1300(Muγ)12 cell might be devoid of tumorigenicity itself by chance. This possibility also is, however, excluded, because a substantial tumor growth of the high producer was seen in the BALB/c *nu/nu* mouse, as in the cases of other C1300 sublines used in this work (not shown).

Since the syngeneic mice after rejection of the high-producer tumor became resistant to tumor induction by the malignant parental cells without exerting any significant effect on the tumor growth of a different kind of A/J strain tumor line (a fibrosarcoma Sa-1), the tumor suppression seems to be due to host-mediated defense mechanisms specific for the cell type of challenged tumor cells. This was clearly demonstrated by administration of specific antibodies.

The tumor growth of the high producer was promoted by the simultaneous i.p. injection of anti-Lyt2.2, similarly to that of the parental cell. Anti-IFN-γ administration also occasionally permitted the C1300(Muγ)12 to induce an obvious large tumor and somewhat augment the tumor growth promoted by the anti-Lyt2.2 administration. On the other hand, administration of anti-asialoganglioside GM_1 antibody caused only a weak promotion of initial transient tumor growth but not prolonged tumor development, and the administration of an anti-activated macrophage antibody (AcM1) (28) exhibited no effect whatsoever on the tumor growth. Therefore, the tumor suppression seen in the high producer can be attributed to the anti-Lyt2.2-sensitive effectors, CTLs (27), which will attack specific tumor cells directly. IFN-γ derived from the tumor might regulate CTL maturation (41) and enhance the immune reactions (42). Thus, repeated administration of anti-IFN-γ antibodies should indicate more clearly the contribution of IFN-γ in tumor suppression and rejection. In addition, IFN-γ might induce the expression of tumor-associated antigen(s) (43, 44) as well as MHC class I antigens, although the precise nature of the C1300 cell antigen is not yet known. Actually, preliminary experiments indicated that augmented CTL activity specific for the C1300 cell line was induced in the mice immunized with the high-producer cells but not in the mice bearing tumors induced by any other C1300 sublines. On the other hand, other effector cells, such as NK cells and activated macrophages, seemed to appar-

ently play only a minor role, if any, in the tumor suppression and rejection.

Preliminary histochemical studies of the section of s.c. tumors revealed that IFN-γ gene-transferred cell tumors (e.g., on 10-day tumor) were infiltrated by Thy-1$^+$ small lymphocytes, whereas the parental cell tumors were barely infiltrated (data not shown), also suggesting involvement of T lymphocytes in the tumor regression. Interestingly, apparently similar infiltration of small lymphocytes was seen in the low- and high-producer cell tumors. Rather low-level expression of IFN-γ from the tumor cells may be sufficient to promote small lymphocyte chemotaxis, but the level of the low-producer line may be still insufficient to activate so fully the effector cells to suppress the tumor development; this may be possible by the high-producer line.

In alternative experiments, C1300 cells mixed with 1×10^5 IU of IFN-γ just before s.c. inoculation induced a tumor growth similar to that of the C1300 cells alone (data not shown). This suggests that the constitutive IFN-γ production over a certain level from the tumor cells is important for the tumor suppression and rejection; the effect of IFN-γ may be remarkable in the vicinity of the producing cells, albeit the production level is rather low. The IFN-γ-producing tumor cells can always be stimulated by their own products, thereby enhancing the MHC class I gene expression, provided that the tumor cells possess functional IFN-γ receptors (9). In addition, the location of IFN-γ is confined to the producer or in the neighborhood of the tumor cells. Subsequently, local immune responses to the tumor cells appear to be augmented by the IFN-γ in the vicinity of the tumor, and IFN-γ is also capable of regulating inflammatory responses (45, 46). IFN-γ is, moreover, a potent inducer for various bioreactive reagents (reviewed in ref. 47), some of which are capable of modulating immune reactions synergistically with IFN-γ.

In conclusion, our results demonstrate that the induction of a large malignant tumor by an IFN-γ-producing cell derived from a malignant tumor line is strongly suppressed by augmented specific anti-tumor immunity, where CTLs seem to predominantly play an important role, probably as a result of the immunomodulatory effects of the IFN-γ secreted by the tumor. Of course, it is impossible from the results presented here to deny that other effector cells such as macrophages and NK cells may play a role in the primary events leading to a specific immune response to the immunized tumor cells or in rejection of very small tumors, since these effector cells are important in immunosurveillance for neoplasia (48, 49) and can be activated by IFN-γ (3, 50, 51). IFN-γ can also enhance the antigen-presenting function of macrophages (4). Our approach using IFN-γ-producing tumor cells may provide new insights into the role of IFN-γ in immune response against tumors.

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