# Cross-Talk between T Cells and Innate Immune Cells Is Crucial for IFN-γ-Dependent Tumor Rejection[1]

**Zhiguang Li,* Felicia Pradera,† Thomas Kammertoens,† Bing Li,* Shubai Liu,* and Zhihai Qin[2]*†**

**Though the importance of IFN-γ in tumor immunity has been well-demonstrated, little is known about its source and how it is induced. By using various bone marrow chimeric mice, we show here that IFN-γ essential for tumor immunity is solely produced by hemopoietic cells. Surprisingly, IFN-γ derived from T cells was not necessary for tumor immunity in this model. In the immunized mice, in which only innate immune cells have the IFN-γ-producing potential, tumors were efficiently rejected. The innate immune cells, such as NK1.1$^+$ cells and CD11b$^+$ cells, can provide sufficient amounts of IFN-γ which requires, however, the help of T cells. The close cooperation between T cells and innate immune cells during tumor regression is likely mediated by IL-2. Together, our results clearly illustrate how T cells cooperate with innate immune cells for IFN-γ-mediated tumor rejection and this may have important indications for clinical trials of tumor immunotherapy.** *The Journal of Immunology,* **2007, 179: 1568–1576.**

The crucial role of IFN-γ in tumor immunity has been demonstrated in different models (1–3). IFN-γ knockout or IFN-γ receptor knockout mice exhibit severely impaired antitumor capability (4, 5). Tumor cells genetically modified to secrete IFN-γ are often rejected (6, 7). Neutralization of IFN-γ by mAb leads to tumor outgrowth (8, 9). Plenty of studies have been conducted to dissect the mechanism by which IFN-γ mediates tumor rejection. For example, IFN-γ can act on various immune cells, such as NK cells, macrophages, neutrophils, and T lymphocytes, increasing their tumoricidal activities and promoting the cytokine release from these cells (1, 2). IFN-γ can also act on the vasculature which is crucial for tumor growth, either inhibiting tumor-induced angiogenesis (5, 10–12) or destroying established tumor-associated blood vessels (13). Moreover, IFN-γ can act directly on tumor cells, up-regulating the MHC class I expression and thereby increasing tumor cell recognition and killing by CTL, and in some cases promoting the antiproliferative and proapoptotic effects on tumor cells (1). In contrast to extensive studies of the mechanism by which IFN-γ acts for tumor immunity, little is known about its source and how it is induced during tumor rejection.

Activated T cells are often considered an important and necessary IFN-γ producer for tumor immunity (1). The evidence comes mainly from adoptive T cell transfer experiments. T cells with IFN-γ-producing ability, detected in vitro upon stimulation with specific tumor Ags, can mediate tumor regression when adoptively transferred into tumor-bearing mice. In contrast, T cells exhibiting poor IFN-γ-producing ability cannot mediate tumor regression (14, 15). This concept, however, has been challenged recently by other experiments showing that IFN-γ-deficient T cells could specifically eradicate established pulmonary metastasis when transferred into tumor-bearing mice (16–18). Gattinoni et al. (19) have even reported that more differentiated T cells with greater IFN-γ production ability were less effective for tumor treatment. Except for the discrepancy, due possibly to the tumor or animal models used, all of the results are based on adoptive transfer experiments which have several obvious defects: T cells are usually activated in vitro, infused in an unreasonably large number (often $>10^7$) into the tumor-bearing mice; the establishment of the balance between tumor cells and their environment cannot be as ideal as it was in reality; and more seriously, the contribution of IFN-γ produced by endogenous T cells of recipient mice cannot be excluded. Therefore, the role of T cell-derived IFN-γ in tumor immunity remains unclear at present, though the IFN-γ-secreting ability is often used as one of the important criteria to select T cells for adoptive immunotherapy of tumors (20).

Besides conventional αβ T lymphocytes, γδ T cells (21), NK cells (1), B cells (22), macrophages (23), dendritic cells (24), and even nonhemopoietic fibroblasts (25, 26) have been shown to be able to produce IFN-γ in vitro upon proper stimulation. The contribution of IFN-γ from these cells in the generation of tumor immunity has been implied in several studies. Rag2$^{-/-}$STAT1$^{-/-}$ mice, compared with Rag2$^{-/-}$ mice, were more susceptible to methylcholanthrene-induced tumorigenesis, suggesting that IFN-γ derived from innate immune cells contributes to the inhibition of tumor incidence (27). Recently, Gao et al. (28) showed that γδ T cells could be a crucial early source of IFN-γ in a primary carcinogenesis model. NK cells and NKT cells were shown to play important roles in α-galactosylceramide-mediated inhibition of tumor growth by secreting copious amounts of IFN-γ (29). A recently identified dendritic cell subset which is B220$^+$ NK1.1$^+$ and can secrete high levels of IFN-γ upon contact with a variety of

*National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; and †Institute of Immunology, Campus Benjamin Franklin, Charite, Berlin, Germany

Received for publication November 8, 2006. Accepted for publication February 16, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants of the Chinese Academy of Sciences (to Z.Q.), the National Natural Science Foundation of China (30471571, 30530330), the Ministry of Science and Technology of China (863-2006AA02Z4B9, 973-2006CB504304, and 2006CB910901), and Deutsche Forschungsgemeinschaft (SFB506, R1).

[2] Address correspondence and reprint requests to Dr. Zhihai Qin, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China. E-mail address: zhihai@ibp.ac.cn

Table I. *BM chimeric mice used in the study*[a]

| Designation | Donor 1 | Donor 2 | Recipient | Description |
|---|---|---|---|---|
| +>+ | IFN-$\gamma^{+/-}$ | | IFN-$\gamma^{+/-}$ | All cells are IFN-$\gamma$ competent |
| −>+ | IFN-$\gamma^{-/-}$ | | IFN-$\gamma^{+/-}$ | Hemopoietic cells are IFN-$\gamma$ deficient |
| +>− | IFN-$\gamma^{+/-}$ | | IFN-$\gamma^{-/-}$ | Nonhemopoietic cells are IFN-$\gamma$ deficient |
| −>− | IFN-$\gamma^{-/-}$ | | IFN-$\gamma^{-/-}$ | All cells are IFN-$\gamma$ deficient |
| −R>+ | IFN-$\gamma^{-/-}$ | Rag-$1^{-/-}$ | IFN-$\gamma^{+/-}$ | Innate immune cells and non-BM cells are IFN-$\gamma$ competent |
| −R>− | IFN-$\gamma^{-/-}$ | Rag-$1^{-/-}$ | IFN-$\gamma^{-/-}$ | Innate immune cells are IFN-$\gamma$ competent |

[a] IFN-$\gamma^{+/-}$ or IFN-$\gamma^{-/-}$ mice were lethally irradiated and were reconstituted with BM cells from IFN-$\gamma^{+/-}$, IFN-$\gamma^{-/-}$, or with a 1:1 BM mixture of IFN-$\gamma^{-/-}$ and Rag-$1^{-/-}$ mice.

tumor cells was found to be sufficient in preventing tumor outgrowth when adoptively transferred into tumor-bearing Rag$2^{-/-}$ IL2rg$^{-/-}$ mice (30). In a previous study investigating the mechanism underlying cyclophosphamide-mediated tumor regression, we found that cyclophosphamide treatment switched the cytokine production of tumor-associated macrophages from IL-10 to IFN-$\gamma$ and this switch was essential for tumor regression (13).

Tumor regression is a complicated and dynamic process which relies on the efficient functioning of each cell and the close cooperation between various cells. Dissecting the exact role of each cell and how cooperation is coordinated between these cells will greatly extend our understanding of this process and will provide new chances to develop cancer treatment strategies. In this study, by using various IFN-$\gamma$-deficient bone marrow (BM)[3] chimera, we demonstrate that the major task of T lymphocytes in tumor immunity is not the production, but the induction, of IFN-$\gamma$. Innate immune cells, e.g., non-T/B BM-derived cells, with the help of T lymphocytes, can provide sufficient amounts of IFN-$\gamma$ for tumor immunity.

## Materials and Methods

### *Cell lines*

MCA205 fibrosarcoma cells are of C57BL/6 origin (31). J558L plasmacytoma (32) and TS/A adenocarcinoma cells (33) are of BALB/c origin. J558L-mOva and TS/A-mOva were derived from J558L and TS/A, respectively, by transfection with pcDNA3-TfR-OVA plasmid expressing membrane-bound OVA protein under the CMV promoter (34). The expression and function of OVA in J558L-mOva and TS/A-mOva cells have been confirmed by RT-PCR, Western-blotting, and a MTT-based proliferation assay (data not shown).

### *Mice*

C57BL/6 and BALB/c mice were purchased from Charles River Laboratories and Weitonglihua. DO11.10 TCR-transgenic mice with CD4$^+$ T lymphocytes specifically recognizing OVA peptide 323–339 and IFN-$\gamma$-deficient mice were obtained from The Jackson Laboratory. To generate IFN-$\gamma$-deficient DO11.10-transgenic mice, DO11.10 mice were paired with IFN-$\gamma^{-/-}$ mice and the intercross between their offspring led to the birth of DO11.10/IFN-$\gamma^+$ and DO11.10/IFN-$\gamma^-$ mice. To generate BM chimeric mice, BM was harvested from the femurs of IFN-$\gamma^{+/-}$, IFN-$\gamma^{-/-}$, or Rag-$1^{-/-}$ mice and washed twice in Dulbecco's PBS (D-PBS). A total of $5 \times 10^6$ BM cells either from a single donor or as a 1:1 mixture of two different donors were injected i.v. into lethally irradiated (10.2 Gy) IFN-$\gamma^{+/-}$ and IFN-$\gamma^{-/-}$ recipient mice within 2 h. Together, six different BM chimeras were generated (Table I). The chimerism of the *IFN-$\gamma$* gene in mice was confirmed by PCR and flow cytometry (data not shown). For PCR, genomic DNA from both blood and tail was prepared and typed with the following primers: 5′-AgA AgT AAg Tgg AAg ggC CCA gAA g-3′ and 5′-Agg gAA ACT ggg AgA ggA gAA ATA T-3′ for the *IFN-$\gamma$* gene, 5′-TCA gCg CAg ggg CgC CCg gTT CTT T-3′ and 5′-ATC gAC AAg ACC ggC TTC CAT CCg-3′ for the neo$^R$ gene. For flow cytometry, single-cell suspensions were prepared from spleens of the indicated mice and cultured for 16 h at $2 \times 10^6$/ml with or without 10 ng/ml PMA (Sigma-Aldrich) and 50 ng/ml ionomycin (Sigma-Aldrich) in supplemented RPMI 1640. Cells were then incubated with anti-CD3-FITC (17A2; BD Biosciences) and anti-IFN-$\gamma$-PE (XMG1.2; BD Biosciences). Data were collected on a BD Biosciences FACSCalibur flow cytometer and analyzed using CellQuest software. Mice were bred in the Forschungseinrichtung fuer Experimentelle Medizin of Free University of Berlin and the animal facilities of Beijing University. In all experiments described here, sex- and age-matched mice were used. Animal study was conducted after the approval of the corresponding authorities.

### *In vivo experiments*

Exponentially growing tumor cells were harvested and washed with D-PBS and s.c. injected in 0.2 ml of D-PBS into the abdominal region of mice in numbers as indicated. Tumor growth was monitored every 2–3 days. Mice bearing a tumor >10 mm in diameter were recorded as tumor positive in the present study, because we never observed the regression of a tumor >10 mm in diameter. To generate protective immunity, mice were immunized with $5 \times 10^5$ irradiated (100 Gy) MCA205 tumor cells. Two weeks later, mice were contralaterally challenged with viable tumor cells in number as indicated.

For adoptive T cell transfer experiments, tumor cells were s.c. inoculated at both flanks of BALB/c mice, with $2.5 \times 10^6$ J558L or $1.0 \times 10^5$ TS/A cells on the left and $2.5 \times 10^6$ J558L-mOva or $1.0 \times 10^5$ TS/A-mOva cells on the right flank region. Spleen cells from DO11.10/IFN-$\gamma^+$ or DO11.10/IFN-$\gamma^-$ mice were isolated and stimulated in vitro with irradiated tumor cells (40:1) and 10 U/ml recombinant human IL-2 (BD Biosciences) for 3 days. For mice bearing J558L and J558L-mOva, $1.0 \times 10^7$ splenocytes were i.v. injected via tail veil into each mouse 24 h after tumor inoculation. For mice bearing TS/A and TS/A-mOva, cyclophosphamide was i.p. administrated into each mouse at a dose of 15 mg/kg 10 days later after tumor inoculation. And 36 h after cyclophosphamide administration, $1.0 \times 10^7$ splenocytes were i.v. injected via tail veil into each mouse.

To neutralize IFN-$\gamma$ or to deplete NK cells, mice were i.p. injected with 400 $\mu$g of R46A2 (anti-mouse IFN-$\gamma$) or PK136 (anti-NK1.1) 2 days before and 8 days after the challenge of tumor cells. NK cell depletion was verified by measuring the disappearance of the DX5$^+$ population. The Ab treatment led to depletion of ~90% of NK cells (data not shown). For the depletion of T cell subsets, immunized C57BL/6 mice or different BM chimeric mice were i.p. injected with 400 $\mu$g of rat anti-mouse mAb GK1.5 (anti-CD4) or 2.43 (anti-CD8) in 0.5 ml of D-PBS. Depletion of the respective T cell subpopulation was confirmed by flow cytometric analysis of peripheral blood cells using PE-labeled anti-CD4 (RM4-5; BD Biosciences) and anti-CD8 mAbs (53-6.7; BD Biosciences). The complete depletion of the respective T cell subsets lasted for at least 3 wk.

### *Cytokine detection*

For the detection of mouse IFN-$\gamma$ and IL-2 in cell culture supernatant, ELISA kits (BD Biosciences) were used. The spleen or draining lymph node cells from different BM chimeric mice were isolated. Single-cell suspensions were prepared and cultured at a concentration of $2 \times 10^6$ immune cells/ml in RPMI 1640 medium with or without stimulation of inactivated MCA205 tumor cells (40:1) or various mitogens, such as Con A (20 $\mu$g/ml), LPS (1 $\mu$g/ml), or poly I:C (25 $\mu$g/ml; Sigma-Aldrich). Neutralization of IL-2 activity was done by the addition of 1 $\mu$g/ml mAb (JES6-1A12; BD Biosciences) in the draining lymph node cell culture. Supernatant were collected after 3–5 days and cytokines were determined. The IFN-$\gamma$ level in the serum was determined using the mouse IFN-$\gamma$ in vivo capture assay kit (BD Biosciences) according to the manufacturer's instruction.

### *Immunohistochemistry*

The BM chimeric mice were left untreated or immunized with $5 \times 10^5$ irradiated MCA205 tumor cells. Two weeks after immunization, mice were challenged with $1 \times 10^6$ viable MCA205 cells. Tumors were isolated 4, 7,

[3] Abbreviations used in this paper: BM, bone marrow; D-PBS, Dulbecco's PBS.

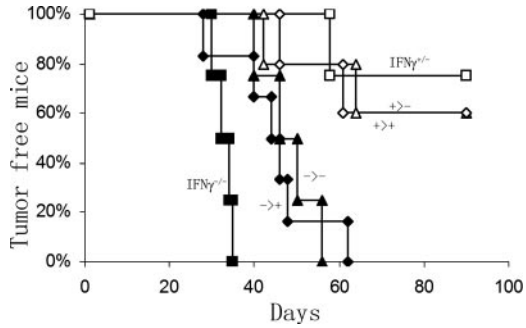


**FIGURE 1.** IFN-γ produced by BM-derived cells is necessary for effective tumor immunity. Lethally irradiated IFN-$\gamma^{+/-}$ and IFN-$\gamma^{-/-}$ mice (8–12 mice/group) were reconstituted with IFN-$\gamma^{+/-}$ (◇ and △) or IFN-$\gamma^{-/-}$ BM cells (◆ and ▲). Ten weeks after reconstitution, mice were immunized with $5 \times 10^5$ irradiated MCA205 cells and challenged 2 wk later with $1 \times 10^5$ MCA205 cells. Immunized nonreconstituted IFN-$\gamma^{+/-}$ (□) and IFN-$\gamma^{-/-}$ mice (■) served as control. Tumor growth was monitored for >90 days. Mice with a tumor larger than 10 mm in diameter were recorded as tumor positive. Shown is the percentage of tumor-free mice on different days after tumor cell challenge. Two experiments were done with similar results.

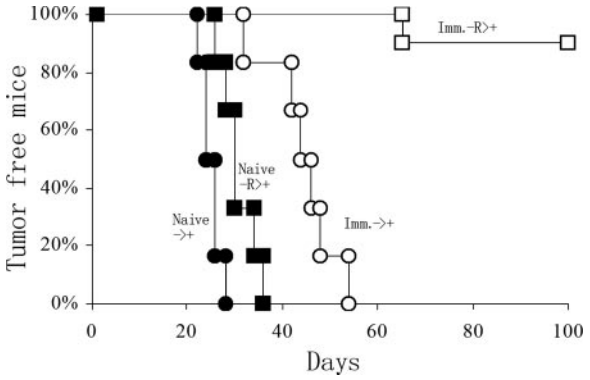


**FIGURE 2.** IFN-γ necessary for tumor immunity must not be produced by T and B lymphocytes. Lethally irradiated IFN-$\gamma^{+/-}$ mice (6–10 mice/group) were reconstituted with BM cells from IFN-$\gamma^{-/-}$ mice alone (−>+, circle) or together with BM cells from Rag-1 knockout mice (−R>+, square). The BM chimeric mice were left untreated (■ and ●) or immunized with $5 \times 10^5$ irradiated MCA205 cells (□ and ○) and challenged 2 wk later with $1 \times 10^5$ MCA205 cells. Tumor growth was monitored. Shown is one of three experiments with similar results.

and 10 days after the challenge. Preparation of cryostat tissue sections and alkaline phosphatase immunostaining were done as previously described (35). The mAbs used here were rat anti-mouse CD4 (Rm4-5), CD8 (53-6.7), CD31 (MEC13.3), and CD11b (M1/70; all from BD Biosciences). As secondary reagents, the alkaline phosphatase-conjugated goat anti-rat IgG was used (Jackson ImmunoResearch Laboratories). All sections were counterstained with Mayer's hematoxylin (Chroma Gesellschaft). Tissue sections of three mice per group were evaluated.

### *Flow cytometric analysis*

Single-cell suspensions of spleen, tumor, or draining lymph node were prepared as previously described (5). Cells were stained with PE-labeled anti-mouse IFNγ (XMG1.2), FITC-labeled anti-mouse CD11b (M1/70) and NK1.1 (PK136 mAbs; all from BD Biosciences). Data were collected on a BD Biosciences FACSCalibur flow cytometer and analyzed using CellQuest software, as described above.

To analyze the proliferation of NK cells in the presence or absence of IFN-γ-deficient $CD4^+$ T cells, BM chimeric mice were immunized and challenged as described above. Draining lymph node cells were isolated and incubated with CFDA-SE (5 μM; Molecular Probes) for 10 min at 37°C. After wash, cells were cultured with irradiated MCA205 cells (40:1) for 48 h, stained with PE-labeled PK136 (BD Biosciences), and analyzed on a FACSCalibur flow cytometer.

### *Statistic analysis*

Data were analyzed by the Wilcoxon signed-rank test, Student's *t* test, or one-way ANOVA and differences were considered significant at $p < 0.05$.

## Results

### *IFN-γ from BM-derived cells is crucial for tumor immunity*

It is well-known that IFN-γ is mainly produced by BM-derived cells. However, IFN-γ mRNA was detected in fibroblasts by several groups (25, 26). To investigate the role of different IFN-γ sources during tumor immunity, lethally irradiated IFN-$\gamma^{-/-}$ and IFN-$\gamma^{+/-}$ mice were successfully reconstituted with BM cells from either IFN-$\gamma^{+/-}$ or IFN-$\gamma^{-/-}$ mice (data not shown). The mice were then immunized and challenged with MCA205 cells. As shown in Fig. 1, all immunized IFN-$\gamma^{-/-}$ mice, as well as immunized −>− and −>+ BM chimeric mice, developed tumors before day 62. In contrast, >60% of immunized IFN-$\gamma^{+/-}$ mice, as well as immunized +>− and +>+ BM chimeric mice, remained tumor free during the observation period of 90 days. Without immunization, all IFN-$\gamma^{+/-}$ and IFN-$\gamma^{-/-}$ mice rapidly developed tumors (data not shown). These findings demonstrated that endogenous IFN-γ provided by hemopoietic cells was necessary and sufficient for the inhibition of tumor growth, whereas IFN-γ derived from nonhemopoietic cells or extracellular matrix contributes little or not at all to tumor rejection.

### *IFN-γ produced by T and B lymphocytes is not necessary for tumor immunity*

To further clarify which BM-derived cells, especially whether T lymphocytes, are necessary IFN-γ secretors during tumor rejection, we established −R>+ mice by reconstituting lethally irradiated IFN-$\gamma^{+/-}$ mice with a 1:1 mixture of BM cells from IFN-γ knockout and T/B lymphocyte-deficient Rag-1 knockout mice. In these mice, T/B lymphocytes originate from IFN-$\gamma^{-/-}$ BM and cannot produce IFN-γ, whereas the IFN-γ-producing potential of all other types of cells is unimpaired. Ten weeks after the successful reconstitution, mice were left untreated or immunized and challenged as before with MCA205 cells. As shown in Fig. 2, without immunization, tumors grew progressively in all mice and there was no significant difference between the −>+ and −R>+ groups. After immunization, whereas 100% of mice received IFN-γ knockout BM cells got tumors in ~55 days, only 10% of mice did so if they received a mixture of BM cells from IFN-γ-deficient and Rag-1-deficient mice. Tumor growth in immunized −R>+ mice was similar to that in immunized +>+ or immunized IFN-γ-competent control mice (see also Fig. 1). During the observation period of >90 days, the rates of tumor-free mice in these three groups are around 90, 60, and 80%, respectively (Figs. 1 and 2). Together, the results clearly demonstrate that it is not necessary for T cells to produce IFN-γ for the generation of effective tumor immunity.

### *Adoptive transfer of T cells from IFN-γ-deficient mice inhibits tumor growth*

To analyze whether the above finding was limited to MCA205 cells in C57BL/6 mice, or was limited to the experimental model used, i.e., immunization was followed by challenge in BM chimeric mice. We extended our investigation to BALB/c mice and the adoptive T cell transfer model. In view of the more important role in cytokine production by $CD4^+$ T cells than $CD8^+$ T cells, we used T cells isolated from DO11.10 TCR-transgenic mice here. The transgenic $CD4^+$ T cells specifically recognize OVA peptide

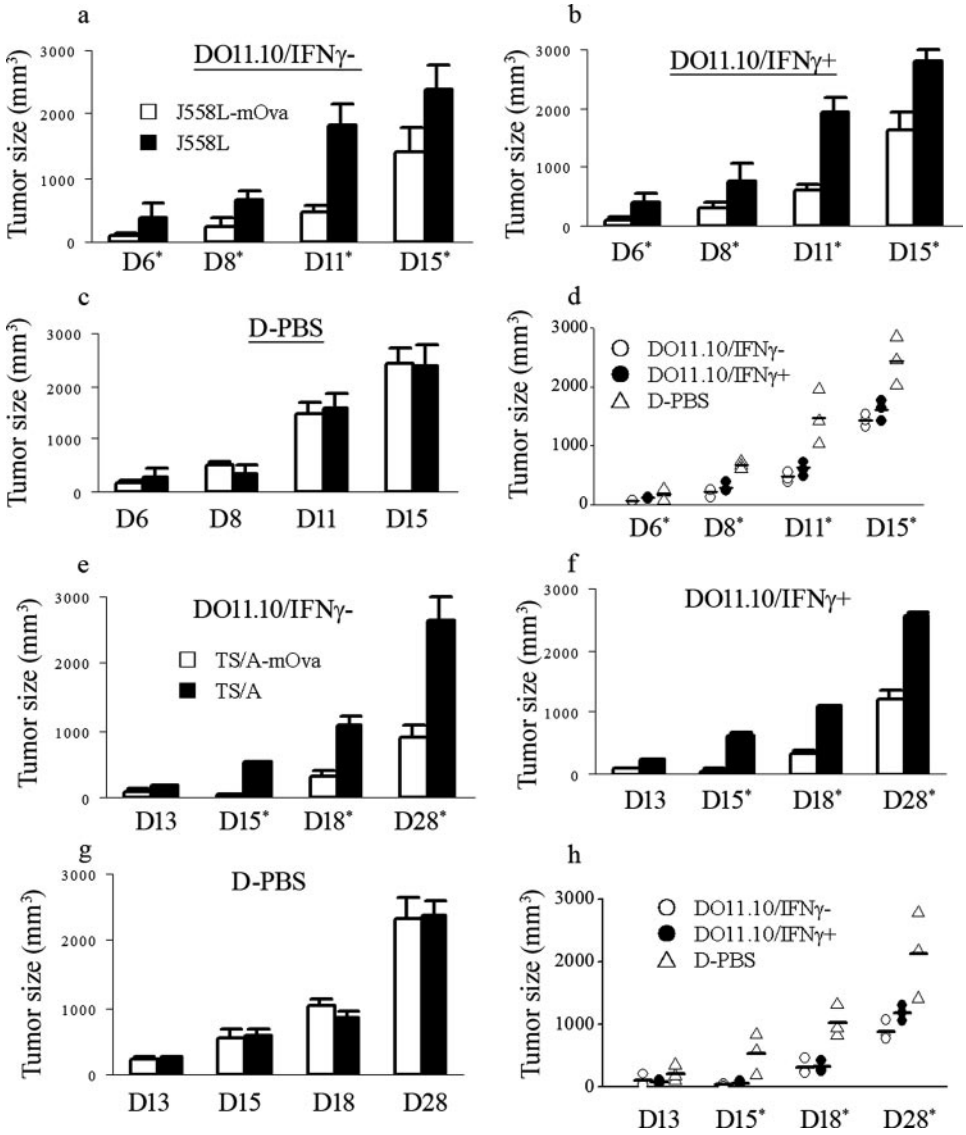


**FIGURE 3.** IFN-γ-deficient T cells can specifically inhibit tumor growth upon adoptive transfer into tumor-bearing mice. *a–c*, Groups of BALB/c mice (3 mice/group) were s.c. inoculated with $2.5 \times 10^6$ J558L cells in the left flank (■) and $2.5 \times 10^6$ J558L-mOva cells in the right flank (□), respectively. Twenty-four hours later, the mice received an adoptive transfer of $1.0 \times 10^7$ splenocytes isolated from (*a*) DO11.10/IFN-$\gamma^-$, (*b*) DO11.10/IFN-$\gamma^+$ mice, or (*c*) just D-PBS as control. Tumor growth was followed. Shown are mean tumor volumes with SDs on the days 6, 8, 11, and 15 after tumor cell inoculation. *d*, Comparison of J558L-mOva tumor volumes in mice transfused with splenocytes from DO11.10/IFN-$\gamma^-$ (○) or DO11.10/IFN-$\gamma^+$ (■), or just transfused with D-PBS (□) on the indicated time points. Each symbol represents the tumor size of a single mouse. *e–g*, A total of $1.0 \times 10^5$ TS/A (■) and $1.0 \times 10^5$ TS/A-mOva cells (□) were s.c. inoculated into the left and right flanks of BALB/c mice (3 mice/group), respectively. Ten days after tumor cell inoculation, mice were i.p. injected with cyclophosphamide (15 mg/kg) and 36 h later, treated by an i.v. adoptive transfer of $1.0 \times 10^7$ splenocytes isolated from DO11.10/IFN-$\gamma^-$ (*e*), DO11.10/IFN-$\gamma^+$ (*f*), or just D-PBS (*g*) as control. Shown are mean tumor volumes with SDs on days 13, 15, 18, and 28. *h*, Comparison of TS/A-mOva tumor volumes in mice treated with splenocytes from DO11.10/IFN-$\gamma^-$ (○) or DO11.10/IFN-$\gamma^+$ (●) or D-PBS (□) on the indicated time points. Each symbol represents the tumor size of a single mouse. *, A significant difference exists between the compared tumor volumes at the indicated time points.

associated with I-$A^d$. The parental J558L and J558L-mOva tumor cells were correspondingly s.c. inoculated at the left and right flanks of BALB/c mice. Twenty-four hours later, T cells from IFN-$\gamma^{+/-}$ or IFN-$\gamma^{-/-}$ DO11.10 mice were i.v. transfused into tumor-bearing mice. As shown in Fig. 3, *a* and *b*, the growth of J558L-mOva tumors was significantly retarded compared with J558L tumors in the same group ($p < 0.05$), no matter whether the DO11.10 T cells used for transfer were IFN-γ competent or deficient. Conversely, in the group receiving no T cell transfer, J558L-mOva or J558L tumors grew similarly ($p > 0.05$) (Fig. 3*c*). Notably, the retardation of J558L-mOva growth caused by IFN-γ-competent DO11.10 T cells was not greater than the retardation caused by IFN-γ-deficient DO11.10 T cells (Fig. 3*d*). Similar results were obtained when a mammary adenocarcinoma tumor cell line TS/A and its transfectant TS/A-mOva were analyzed (Fig. 3, *e* and *h*). These findings suggest that T cell-derived IFN-γ contributes little or not at all to the inhibition of tumor growth. This is in accordance with the results obtained with the BM chimeric mice.

### *IFN-γ from innate immune cells is sufficient for tumor immunity*

Except for T and B lymphocytes, IFN-γ can still be provided by several other kinds of cells, including non-T/B BM cells and the irradiation-resistant recipient BM cells which have survived BM

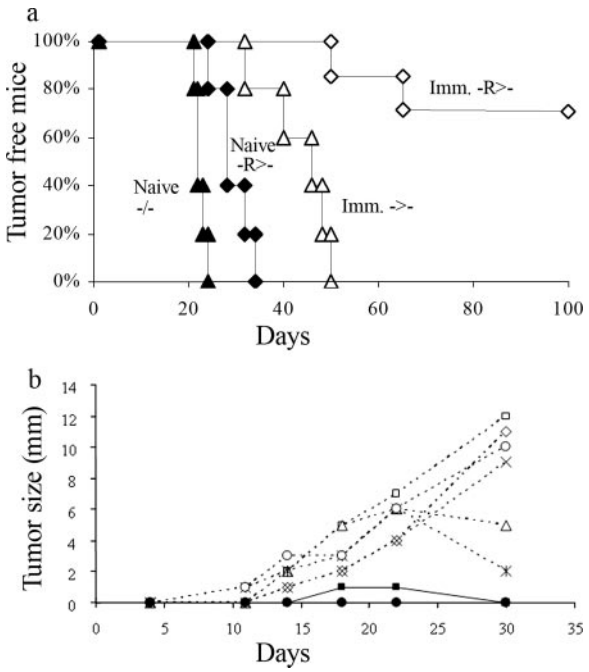


**FIGURE 4.** IFN-γ from BM-derived non-T/B innate immune cells is sufficient for tumor rejection. *a*, Lethally irradiated IFN-$\gamma^{-/-}$ mice (7–10 mice/group) were reconstituted with BM cells from IFN-$\gamma^{-/-}$ mice alone (−>−, triangle) or together with BM cells from Rag-1 knockout mice (−R>−, diamond). BM chimeric mice were left untreated (▲ and ◆) or immunized with $5 \times 10^5$ irradiated MCA205 cells (△ and ◇) and challenged 2 wk later with $1 \times 10^5$ MCA205 cells. Tumor growth was monitored and recorded as in Fig. 1. Similar results have been observed in a further experiment. *b*, Neutralization of IFN-γ activity in −R>− mice eliminated protective antitumor immunity. Groups of immunized −R>− mice were left untreated (two mice, solid lines) or injected with anti-mouse IFN-γ mAb (six mice, broken lines) at 2 days before and 8 days after the challenge of MCA205 tumor cells. Tumor growth was monitored. Each line represents the tumor growth kinetic of a single mouse. One of three experiments with similar results is shown.

reconstitution. To address the question of whether IFN-γ from BM-derived non-T/B innate immune cells was sufficient for tumor immunity, −R>− mice were established by reconstituting lethally irradiated IFN-$\gamma^{-/-}$ mice with a 1:1 BM mixture of IFN-γ knockout and Rag-1 knockout mice. The mice were then immunized and challenged as before with MCA205 cells. As shown in Fig. 4*a*, whereas all the immunized −>− mice got tumors before day 50, 70% of immunized −R>− mice were tumor free for >100 days. The protection was dependent on the secretion of IFN-γ by Rag-1-deficient BM cells, because in the immunized −R>− mice, neutralization of IFN-γ by an IFN-γ-specific mAb, R46A2, led to progressive tumor growth in 66.7% mice (Fig. 4*b*). This result suggests that IFN-γ derived from innate immune cells is sufficient for immune-mediated tumor rejection.

### *CD11b$^+$ cells and NK1.1$^+$ cells are potent IFN-γ secretors*

Both CD11b$^+$ cells and NK1.1$^+$ cells belong to innate immune cells and have been shown to have IFN-γ-producing potential. To further clarify the contribution of these cells in provision of local IFN-γ during the effector phase of an antitumor response. The −R>− BM chimeric mice were immunized and challenged with MCA205 cells. Histological analysis of the tumor cell injection site shows that T cells infiltrated in low numbers and dispersed evenly within the tumor, with slightly more CD4$^+$ T cells than CD8$^+$ T cells (Fig. 5, *a* and *b*). In contrast, a large number of CD11b$^+$ cells were found in the center of the tumor, where obvious tumor necrosis was observed (Fig. 5, *c* and *d*). Intracellular IFN-γ staining of tumor infiltrating cells in −R>− mice shows that 3.8% of the CD11b$^+$ and 3% of the NK1.1$^+$ cells were IFN-γ positive (Fig. 5, *e* and *f*). Depletion of NK1.1$^+$ cells led to increased tumor growth in these mice. As shown in Fig. 5*g*, at day 40 after tumor cell challenge, in contrast to six of seven mice (86%) in the immunized group, four of seven mice (57%) in the NK-depleted group kept tumor free. Whether T cells produce IFN-γ or not may not influence this process significantly, because the similar effect of NK depletion on tumor growth has been observed in both wild-type C57BL/6 and −R>− mice (Fig. 5, *g* and *h*). Together, both CD11b$^+$ cells and NK1.1$^+$ cells infiltrated into the tumor mass produced IFN-γ and took part in the tumor rejection. However, other types of innate immune cells have also been shown to be able to produce IFN-γ (24, 30). The possibility that these innate immune cells might participate in IFN-γ production in tumor immunity has to be excluded.

### *Tumor immunity in BM chimeric mice depends on IFN-γ-deficient T cells*

Without IFN-γ-producing capability, are T cells still required for tumor immunity? To address this question, we immunized −R>+ mice as described above and depleted their T cell subsets with either anti-CD4 or anti-CD8 mAb. As shown in Fig. 6*a*, though 90% of the immunized mice rejected the tumor, depletion of CD4$^+$ T cells led to a complete elimination of the antitumor immunity and all mice got a tumor within 40 days. Depletion of CD8$^+$ T cells also significantly increased the tumor growth. Similar results were obtained when −R>− mice were analyzed (Fig. 6*b*). So, IFN-γ-deficient T cells are still necessary for tumor immunity and CD4$^+$ T cells seem to be more important than CD8$^+$ T cells in this model.

### *T cells are required for the induction of IFN-γ both in vivo and in vitro*

To test whether IFN-γ-deficient T cells could participate in tumor rejection by affecting the IFN-γ production of innate immune cells, −R>+ mice were immunized and depleted of CD4$^+$ and CD8$^+$ T cells with specific mAbs. Five days after the challenge of mice with MCA205 cells, the serum IFN-γ level was measured by IFN-γ capture assay, a method used to detect in vivo cytokine levels described first by Finkelman and Morris (36). As shown in Fig. 6*c*, depletion of CD4$^+$ T cells caused a drastic decrease of serum IFN-γ in comparison to the control. In contrast, depletion of CD8$^+$ T cells did not have a significant influence on the serum IFN-γ level. Similar results were obtained when −R>− mice were analyzed (Fig. 6*c*). To further confirm the above finding that the IFN-γ-deficient T cells are necessary for IFN-γ induction, draining lymph node cells were prepared 5 days after tumor challenge of the immunized mice and cultured for 3 days in vitro. Upon stimulation with poly I:C, −R>+ draining lymph node cells secreted 610 ± 102 μg/ml IFN-γ. However, depletion of T cells, especially CD4$^+$ T cells, led to almost complete deprivation of IFN-γ in the supernatant (Fig. 6*d*). Furthermore, as shown in Fig. 6, *e* and *f*, depletion of T cells led to a decreased proliferation of NK1.1$^+$ cells in the culture of lymph node cells isolated from −R>− mice. Together, our results indicate that IFN-γ-deficient T cells, especially CD4$^+$ T cells, play a critical role for the induction of IFN-γ production by BM-derived innate immune cells.

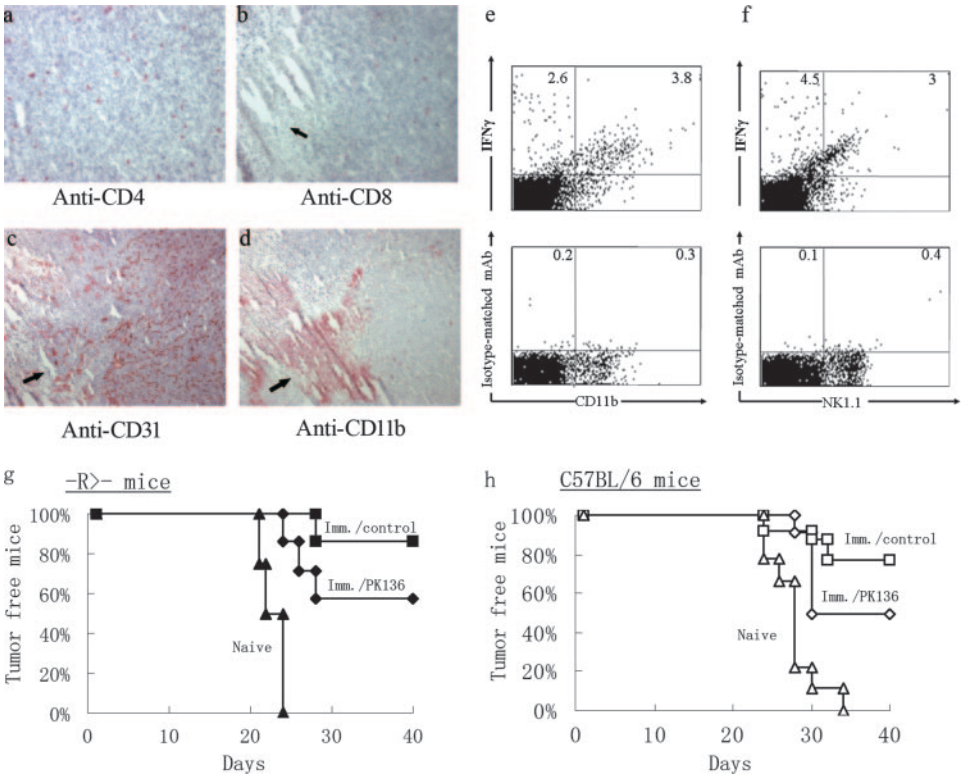


**FIGURE 5.** CD11b$^+$ and NK1.1$^+$ cells are potent IFN-γ producers. *a* and *d*, Heavy infiltration of CD11b$^+$ cells into the tumor. The −R>− BM chimeric mice were immunized and 7 days after the challenge with $1 \times 10^6$ MCA205 tumor cells, tissue sections were stained with mAbs specific for (*a*) CD4$^+$, (*b*) CD8$^+$, (*c*) CD31$^+$, and (*d*) CD11b$^+$ cells. The necrotic area is indicated with a black arrow. Magnification: *a* and *b*, ×100; *c* and *d*, ×40. *e* and *f*, Intracellular IFN-γ staining of tumor-infiltrating cells. Single-cell suspensions prepared from tissue sections as described above were stained for intracellular IFN-γ and cell surface markers specific for (*e*) CD11b and (*f*) NK1.1. Isotype-matched mAb was used as control. Shown are the percentages of IFN-γ-positive cells in size-gated leukocytes. *g* and *h*, Effect of NK1.1$^+$ cell depletion on tumor growth. The −R>− BM chimeric mice (*g*) and as control, wild-type C57BL/6 mice (*h*) were left untreated (▲, $n = 4$ and △, $n = 9$) or immunized (■, $n = 7$ and □, $n = 13$) and challenged with MCA205 cells. Two days before and 8 days after the tumor challenge, one group of immunized mice was treated with mAb PK136 to deplete NK cells (◆, $n = 7$ and ◇, $n = 11$). Shown are the percentages of tumor-free mice at different time points after tumor cell challenge.

### *IL-2 mediates the cooperation between T cells and innate immune cells*

The next question to address was how T cells interact with innate immune cells. IL-2 is a cytokine which has been shown to be able to promote IFN-γ and TNF-α release by NK cells and macrophages (37–39) and to increase the tumoricidal activities of these cells (40, 41). As shown in Fig. 7, *a* and *b*, the draining lymph node cells from −R>− BM chimeric mice secreted a high amount of IL-2, especially when the cells were stimulated with Con A. Depletion of T cells, either CD4$^+$ or CD8$^+$, decreased IL-2 levels in the culture supernatant (Fig. 7, *a* and *b*). In comparison to the draining lymph node cells from −R>− mice, cells from the immunized −>− mice secreted significantly less IL-2 (Fig. 7, *a* and *b*). This may suggest that the IFN-γ-producing capability of innate immune cells promotes the IL-2 production by T lymphocytes. Subsequently, we analyzed the IFN-γ production by the draining lymph node cells and the effect of IL-2 neutralization on IFN-γ production. Fig. 7*c* shows that draining lymph node cells isolated from immunized −R>− mice secreted high amounts of IFN-γ when stimulated with MCA205 tumor cells. However, if IL-2 was neutralized, IFN-γ levels in the supernatant sharply decreased (Fig. 7*c*). If NK cells were depleted in vivo by PK136 as described in Fig. 5, *g* and *h*, IFN-γ in the supernatant of draining lymph node was almost completely eliminated (Fig. 7*c*), indicating that the major source of IFN-γ in the draining lymph node is NK cells. These findings suggest that IL-2 could be responsible for mediating the interaction between T cells and innate immune cells.

Collectively, close cooperation between T cells and innate immune cells exists, which is most likely mediated by IL-2, and it is the cooperation that leads to IFN-γ production and tumor regression. During this process, one of the major tasks of T cells is to induce IFN-γ production by innate immune cells, but not to produce IFN-γ themselves.

## Discussion

In the present study, we show that IFN-γ essential for tumor immunity is solely produced by BM-derived cells. Non-BM-derived cells contribute little or not at all in provision of IFN-γ in the effector phase of antitumor responses, although IFN-γ mRNA has been detected in non-BM-derived cells, such as in fibroblasts (25, 26). This conclusion is based on the observation that in the BM chimeras, whether recipient mice possess IFN-γ-producing ability could not affect tumor growth, but the IFN-γ-producing ability of donor mice was crucially important. As shown in Fig. 1, there was no significant difference in tumor growth between immunized +>− mice and +>+ mice. In both cases, 60% of the mice were tumor free during the observation period of 90 days. Similarly, there was no significant difference in the tumor growth between immunized −>+ and −>− mice. In both cases, all mice got tumors around day 60.

Surprisingly, T cells are not necessary IFN-γ producers for tumor immunity. In immunized −R>+ mice, in which T cells are derived from IFN-$\gamma^{-/-}$ BM and cannot produce IFN-γ, the growth of MCA205 tumor was still suppressed, just like in immunized

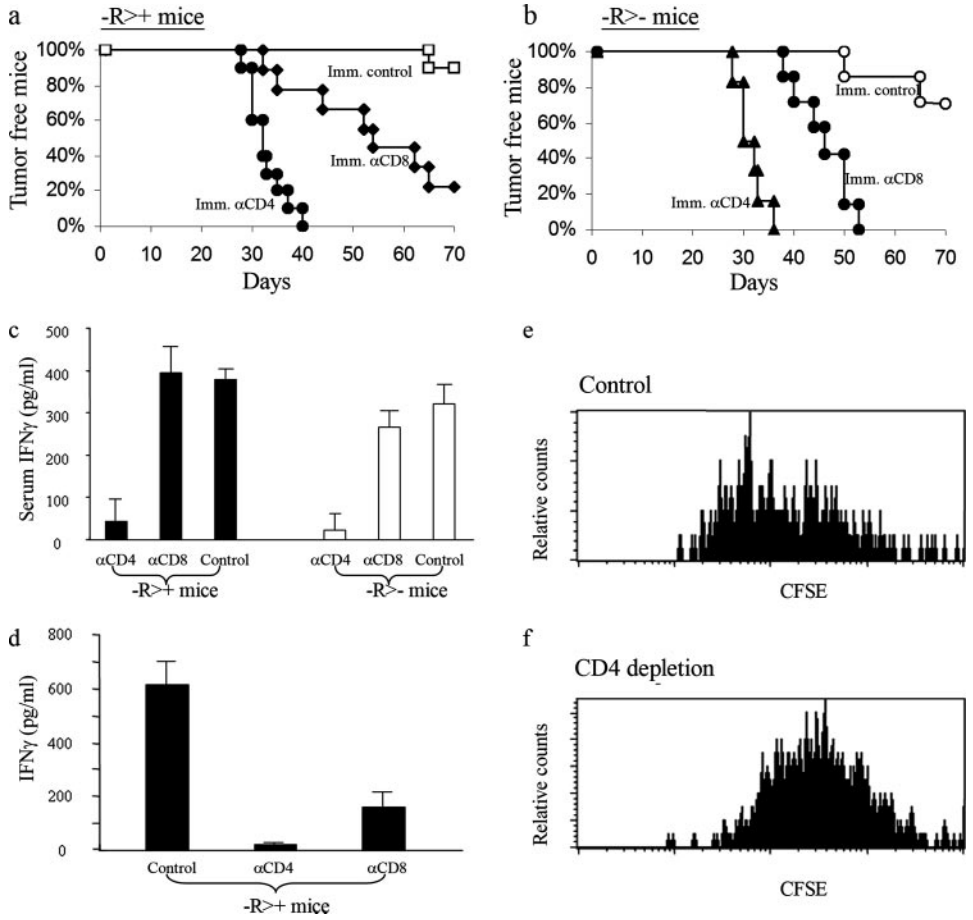


**FIGURE 6.** The IFN-γ-deficient T cells are necessary for tumor regression and the induction of IFN-γ from innate immune cells. *a*, Groups of −R>+ mice (10 mice/group) were immunized and 2 wk later, challenged with MCA205 cells. Two days before tumor challenge, mice were treated with D-PBS as control (□) or depleted of $CD4^+$ (●) or $CD8^+$ T cells (□) by injection of mAbs. Tumor growth was followed. *b*, A similar experiment was done with −R>− mice (6–7 mice/group) treated with D-PBS (○) anti-mouse $CD4^+$ (▲) or $CD8^+$ mAbs (●). Shown are percentages of tumor-free mice at different time points after tumor cell challenge. *c*, Depletion of IFN-γ-deficient $CD4^+$ T cells reduces the serum level of IFN-γ. Five days after tumor challenge, serum IFN-γ levels of the mice as described in (*a*) and (*b*) were determined. A summary of the results derived from two to three mice in each group is shown. Representative results from one of three experiments are shown. *d*, Depletion of IFN-γ-deficient T cells decreases IFN-γ production by innate immune cells in vitro. Draining lymph node cells from −R>+ mice as described above were prepared 5 days after tumor challenge and cultured with 50 μg/ml poly I:C, a well-known NK cell stimulator, for 3 days. IFN-γ level in the culture supernatant was determined using an ELISA kit. Shown are the results derived from two to three mice in each group. Two experiments were done with similar results. *e* and *f*, IFN-γ-deficient T cells stimulate NK cell proliferation. Draining lymph node cells from the immunized −R>− mice were isolated 5 days after tumor challenge. The cells were labeled then with CFDA-SE and cultured with mitomycin-C-treated MCA205 cells (40:1) for 3 days. After staining of the cells with PE-labeled PK136, the $NK1.1^+$ cells were gated and analyzed for the CFDA-SE intensity. Representative results are shown for cells isolated from the control mice (*e*) and cells isolated from the $CD4^+$ T cell-depleted mice (*f*).

wild-type mice (Fig. 2). This finding is not relevant only to the MCA205 tumor, because we found that IFN-γ-deficient tumor-specific T cells also inhibited the growth of J558L and TS/A tumors upon adoptive transfer. The independence of tumor growth suppression on T cell-derived IFN-γ is in accordance with recent clinical observations that T cells exhibiting higher IFN-γ-producing ability in vitro could not eradicate melanoma metastasis more efficiently than T cells with lower IFN-γ-producing ability when transfused into cancer patients (19, 42–44). However, we cannot exclude the possibility that the critical source of IFN-γ necessary for tumor rejection would vary in different tumor models and multiple mechanisms can sometimes operate in the same model (45).

The role of IFN-γ produced by innate immune cells, such as NK cells and macrophages, in tumor immunity, has been investigated in several studies including ours (13, 27, 29, 30). However, no direct evidence has been obtained to show whether sufficient IFN-γ can be provided by innate immune cells. This is partially due to the difficulties of establishing such an experimental model in which only innate immune cells have IFN-γ-producing potential. In this study, we show that IFN-γ produced by innate immune cells is indeed sufficient for tumor immunity. In immunized −R>− mice, in which no cells except innate immune cells possess IFN-γ-producing ability, tumor growth was still inhibited, as efficiently as in immunized wild-type mice (Fig. 4*a*). However, tumors grew progressively in immunized −R>− mice if the endogenous IFN-γ had been neutralized (Fig. 4*b*). Furthermore, we observed a heavy infiltration of tumor mass by $CD11b^+$ cells (Fig. 5*d*). Tumor regression was partially abolished by NK cell depletion (Fig. 5, *g* and *h*). $NK1.1^+$ cells and $CD11b^+$ cells isolated from tumor mass expressed IFN-γ (Fig. 5, *e* and *f*). The evidence above obviously suggests that during tumor regression, innate immune cells are recruited, activated, and can produce IFN-γ. However, whether NK cells, macrophages, or other types of innate immune cells are the most important IFN-γ producers is still unknown and requires further investigation.

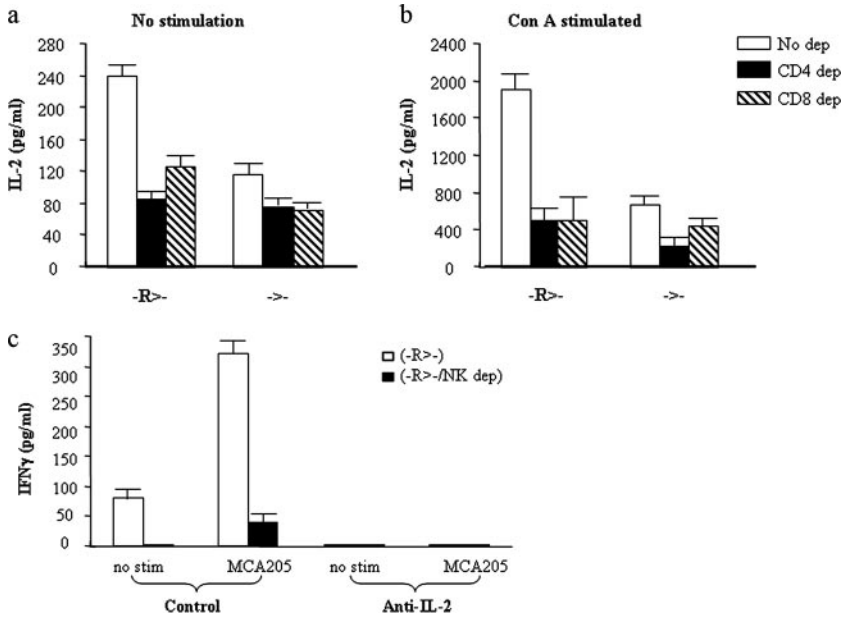


**FIGURE 7.** IL-2 may mediate the interaction between T cells and innate immune cells. *a* and *b*, T cells derived from the draining lymph nodes produce IL-2. Draining lymph node cells were prepared from the immunized −R>− and as control from −>− mice 5 days after MCA205 challenge and cultured for 3 days (*a*) without or (*b*) with the addition of Con A to stimulate T cells. The mouse IL-2 in culture supernatants was detected by ELISA. *c*, Neutralization of the IL-2 activity eliminates IFN-γ production by innate immune cells in vitro. Groups of −R>− mice were immunized and challenged with MCA205 cells as described above. Two days before challenge, mice were left untreated (□) or depleted of NK1.1$^+$ cells (■). Draining lymph node cells were isolated 5 days after tumor challenge and cultured without or with the stimulation of mitomycin C-treated MCA205 cells (40:1). The IL-2 activity was neutralized by the addition of anti-mouse IL-2 mAb in the cell culture. Shown are IFN-γ amounts detected by ELISA in culture supernatants. Three mice in each group were analyzed. Three experiments were done with similar results.

IFN-γ-deficient T cells are still absolutely required for the inhibition of tumor growth, with CD4$^+$ T cells being more important than CD8$^+$ T cells. In immunized −R>+ mice, depletion of CD4$^+$ T cells completely abrogated the protection against tumor incidence, while depletion of CD8$^+$ T cells led to tumor outgrowth only in 80% of mice (Fig. 6*a*). The result obtained here is different from our previous finding that CD8$^+$ T cells play more important roles in inhibiting MCA205 growth in immunized wild-type C57BL/6 mice (12). This discrepancy may be caused by the different animal models, namely, BM chimeric vs wild-type mice. Furthermore, in −R>+ and −R>− mice, IFN-γ is solely produced by innate immune cells, while IFN-γ-deficient T cells, either CD4$^+$ or CD8$^+$, can only provide help, such as by producing IL-2. In contrast, IFN-γ can be directly produced by T cells in wild-type mice.

Close interaction exists between T cells and innate immune cells and this interaction is crucial for IFN-γ production. In CD4$^+$ T cell-depleted BM chimeric mice, the level of IFN-γ produced by innate immune cells in serum was drastically decreased as detected by capture assay (Fig. 6*c*), though the effect on IFN-γ production by tumors in these mice cannot be excluded (Fig. 6, *a* and *b*). The influence of T cells on IFN-γ production by innate immune cells can also be observed in vitro. In the culture of draining lymph node cells, depletion of IFN-γ-deficient T cells led to the decrease of IFN-γ level in the supernatant (Fig. 6*d*) and the delayed proliferation of NK cells (Fig. 6, *e* and *f*). Furthermore, innate immune cells may also influence the activities of T cells. As shown in Fig. 7, *a* and *b*, the IL-2 level in the single-cell suspension of draining lymph nodes isolated from −>− mice is significantly lower than the IL-2 level in the cells isolated from −R>− mice. IL-2 may play important roles during the interaction between T cells and innate immune cells. In −R>− mice, IFN-γ-deficient T cells can secrete a high amount of IL-2 (Fig. 7, *a* and *b*). Neutralization of IL-2, just like T cell depletion, reduced IFN-γ production by innate immune cells (Fig. 7*c*).

Gao et al. (28) have recently reported that γδ T cells are a necessary early source of IFN-γ for protection against 3-methylcolanthrene-induced tumorigenesis and transplanted B16 tumors. However, which cells are necessary later source of IFN-γ, e.g., the production of IFN-γ in the effector phase of immune responses, has not been addressed. In the present study, we show that T lymphocytes play important roles in the induction of IFN-γ essential for tumor immunity, not by producing IFN-γ directly, but by promoting IFN-γ production by innate immune cells.

Analyzing the role of T cells in the provision of IFN-γ during the effector phase has important physiological relevance. Recently, great progresses have been made in both adoptive T cell transfer-based and cancer vaccine-based immunotherapy (46–48). During these therapies, effector T cells, which are either in vitro activated and transfused into patients or in vivo activated by cancer vaccine, are of great importance. Dissecting the traits of effector T cells necessary for tumor regression will help to improve the efficacy of the therapeutic strategies. In this study, making use of various BM chimeric mice, we clearly demonstrate that in the effector phase of immune-mediated tumor regression, the major task of T cells is to induce IFN-γ production by innate immune cells, not to produce IFN-γ themselves. This finding will help us to understand the complex interactions between various cells during tumor regression and to develop new strategies for the immunotherapy of cancers.

## Acknowledgments

We thank Tanja Specowius, Nahid Hakyi, and Xueqiang Zhao for the technical assistance. We thank Thomas Blankenstein for support.

## Disclosures

The authors have no financial conflict of interest.

## References

1. Ikeda, H., L. J. Old, and R. D. Schreiber. 2002. The roles of IFN γ in protection against tumor development and cancer immunoediting. *Cytokine Growth Factor Rev.* 13: 95–109.
2. Blankenstein, T., and Z. Qin. 2003. The role of IFN-γ in tumor transplantation immunity and inhibition of chemical carcinogenesis. *Curr. Opin. Immunol.* 15: 148–154.
3. Dunn, G. P., L. J. Old, and R. D. Schreiber. 2004. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 21: 137–148.
4. Hung, K., R. Hayashi, A. Lafond-Walker, C. Lowenstein, D. Pardoll, and H. Levitsky. 1998. The central role of CD4$^+$ T cells in the antitumor immune response. *J. Exp. Med.* 188: 2357–2368.
5. Qin, Z., and T. Blankenstein. 2000. CD4$^+$ T cell-mediated tumor rejection involves inhibition of angiogenesis that is dependent on IFN γ receptor expression by nonhematopoietic cells. *Immunity* 12: 677–686.
6. Gansbacher, B., R. Bannerji, B. Daniels, K. Zier, K. Cronin, and E. Gilboa. 1990. Retroviral vector-mediated γ-interferon gene transfer into tumor cells generates potent and long lasting antitumor immunity. *Cancer Res.* 50: 7820–7825.
7. Hock, H., M. Dorsch, U. Kunzendorf, Z. Qin, T. Diamantstein, and T. Blankenstein. 1993. Mechanisms of rejection induced by tumor cell-targeted

gene transfer of interleukin 2, interleukin 4, interleukin 7, tumor necrosis factor, or interferon γ. *Proc. Natl. Acad. Sci. USA* 90: 2774–2778.
8. Dighe, A. S., E. Richards, L. J. Old, and R. D. Schreiber. 1994. Enhanced in vivo growth and resistance to rejection of tumor cells expressing dominant negative IFN γ receptors. *Immunity* 1: 447–456.
9. Platzer, C., G. Richter, K. Uberla, H. Hock, T. Diamantstein, and T. Blankenstein. 1992. Interleukin-4-mediated tumor suppression in nude mice involves interferon-γ. *Eur. J. Immunol.* 22: 1729–1733.
10. Fathallah-Shaykh, H. M., L. J. Zhao, A. I. Kafrouni, G. M. Smith, and J. Forman. 2000. Gene transfer of IFN-γ into established brain tumors represses growth by antiangiogenesis. *J. Immunol.* 164: 217–222.
11. Ruegg, C., A. Yilmaz, G. Bieler, J. Bamat, P. Chaubert, and F. J. Lejeune. 1998. Evidence for the involvement of endothelial cell integrin $\alpha_V\beta_3$ in the disruption of the tumor vasculature induced by TNF and IFN-γ. *Nat. Med.* 4: 408–414.
12. Qin, Z., J. Schwartzkopff, F. Pradera, T. Kammertoens, B. Seliger, H. Pircher, and T. Blankenstein. 2003. A critical requirement of interferon γ-mediated angiostasis for tumor rejection by $CD8^+$ T cells. *Cancer Res.* 63: 4095–4100.
13. Ibe, S., Z. Qin, T. Schuler, S. Preiss, and T. Blankenstein. 2001. Tumor rejection by disturbing tumor stroma cell interactions. *J. Exp. Med.* 194: 1549–1559.
14. Barth, R. J., Jr., J. J. Mule, P. J. Spiess, and S. A. Rosenberg. 1991. Interferon γ and tumor necrosis factor have a role in tumor regressions mediated by murine $CD8^+$ tumor-infiltrating lymphocytes. *J. Exp. Med.* 173: 647–658.
15. Becker, C., H. Pohla, B. Frankenberger, T. Schuler, M. Assenmacher, D. J. Schendel, and T. Blankenstein. 2001. Adoptive tumor therapy with T lymphocytes enriched through an IFN-γ capture assay. *Nat. Med.* 7: 1159–1162.
16. Dobrzanski, M. J., J. B. Reome, and R. W. Dutton. 1999. Therapeutic effects of tumor-reactive type 1 and type 2 $CD8^+$ T cell subpopulations in established pulmonary metastases. *J. Immunol.* 162: 6671–6680.
17. Prevost-Blondel, A., M. Neuenhahn, M. Rawiel, and H. Pircher. 2000. Differential requirement of perforin and IFN-γ in CD8 T cell-mediated immune responses against B16.F10 melanoma cells expressing a viral antigen. *Eur. J. Immunol.* 30: 2507–2515.
18. Rodolfo, M., C. Zilocchi, P. Accornero, B. Cappetti, I. Arioli, and M. P. Colombo. 1999. IL-4-transduced tumor cell vaccine induces immunoregulatory type 2 CD8 T lymphocytes that cure lung metastases upon adoptive transfer. *J. Immunol.* 163: 1923–1928.
19. Gattinoni, L., C. A. Klebanoff, D. C. Palmer, C. Wrzesinski, K. Kerstann, Z. Yu, S. E. Finkelstein, M. R. Theoret, S. A. Rosenberg, and N. P. Restifo. 2005. Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred $CD8^+$ T cells. *J. Clin. Invest.* 115: 1616–1626.
20. Gattinoni, L., D. J. Powell, Jr., S. A. Rosenberg, and N. P. Restifo. 2006. Adoptive immunotherapy for cancer: building on success. *Nat. Rev. Immunol.* 6: 383–393.
21. Yin, Z., D. H. Zhang, T. Welte, G. Bahtiyar, S. Jung, L. Liu, X. Y. Fu, A. Ray, and J. Craft. 2000. Dominance of IL-12 over IL-4 in γδ T cell differentiation leads to default production of IFN-γ: failure to down-regulate IL-12 receptor β2-chain expression. *J. Immunol.* 164: 3056–3064.
22. Harris, D. P., L. Haynes, P. C. Sayles, D. K. Duso, S. M. Eaton, N. M. Lepak, L. L. Johnson, S. L. Swain, and F. E. Lund. 2000. Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat. Immunol.* 1: 475–482.
23. Munder, M., M. Mallo, K. Eichmann, and M. Modolell. 1998. Murine macrophages secrete interferon γ upon combined stimulation with interleukin (IL)-12 and IL-18: a novel pathway of autocrine macrophage activation. *J. Exp. Med.* 187: 2103–2108.
24. Rottenberg, M. E., A. Gigliotti-Rothfuchs, and H. Wigzell. 2002. The role of IFN-γ in the outcome of chlamydial infection. *Curr. Opin. Immunol.* 14: 444–451.
25. Saed, G. M., W. Zhang, and M. P. Diamond. 2001. Molecular characterization of fibroblasts isolated from human peritoneum and adhesions. *Fertil. Steril.* 75: 763–768.
26. Mustafa, M., B. Wondimu, M. Bakhiet, and T. Modeer. 2000. Induction of interferon γ in human gingival fibroblasts challenged with phytohaemagglutinin. *Cytokine* 12: 368–373.
27. Shankaran, V., H. Ikeda, A. T. Bruce, J. M. White, P. E. Swanson, L. J. Old, and R. D. Schreiber. 2001. IFNγ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 410: 1107–1111.
28. Gao, Y., W. Yang, M. Pan, E. Scully, M. Girardi, L. H. Augenlicht, J. Craft, and Z. Yin. 2003. γδ T cells provide an early source of interferon γ in tumor immunity. *J. Exp. Med.* 198: 433–442.
29. Hayakawa, Y., K. Takeda, H. Yagita, M. J. Smyth, L. Van Kaer, K. Okumura, and I. Saiki. 2002. IFN-γ-mediated inhibition of tumor angiogenesis by natural killer T-cell ligand, α-galactosylceramide. *Blood* 100: 1728–1733.
30. Taieb, J., N. Chaput, C. Menard, L. Apetoh, E. Ullrich, M. Bonmort, M. Pequignot, N. Casares, M. Terme, C. Flament, et al. 2006. A novel dendritic cell subset involved in tumor immunosurveillance. *Nat. Med.* 12: 214–219.
31. Shu, S. Y., and S. A. Rosenberg. 1985. Adoptive immunotherapy of newly induced murine sarcomas. *Cancer Res.* 45: 1657–1662.
32. Oi, V. T., S. L. Morrison, L. A. Herzenberg, and P. Berg. 1983. Immunoglobulin gene expression in transformed lymphoid cells. *Proc. Natl. Acad. Sci. USA* 80: 825–829.
33. Nanni, P., C. de Giovanni, P. L. Lollini, G. Nicoletti, and G. Prodi. 1983. TS/A: a new metastasizing cell line from a BALB/c spontaneous mammary adenocarcinoma. *Clin. Exp. Metastasis* 1: 373–380.
34. Diebold, S. S., M. Cotten, N. Koch, and M. Zenke. 2001. MHC class II presentation of endogenously expressed antigens by transfected dendritic cells. *Gene Ther.* 8: 487–493.
35. Qin, Z., G. Noffz, M. Mohaupt, and T. Blankenstein. 1997. Interleukin-10 prevents dendritic cell accumulation and vaccination with granulocyte-macrophage colony-stimulating factor gene-modified tumor cells. *J. Immunol.* 159: 770–776.
36. Finkelman, F. D., and S. C. Morris. 1999. Development of an assay to measure in vivo cytokine production in the mouse. *Int. Immunol.* 11: 1811–1818.
37. Economou, J. S., W. H. McBride, R. Essner, K. Rhoades, S. Golub, E. C. Holmes, and D. L. Morton. 1989. Tumour necrosis factor production by IL-2-activated macrophages in vitro and in vivo. *Immunology* 67: 514–519.
38. Puddu, P., M. Carollo, I. Pietraforte, F. Spadaro, M. Tombesi, C. Ramoni, F. Belardelli, and S. Gessani. 2005. IL-2 induces expression and secretion of IFN-γ in murine peritoneal macrophages. *J. Leukocyte Biol.* 78: 686–695.
39. Trinchieri, G., M. Matsumoto-Kobayashi, S. C. Clark, J. Seehra, L. London, and B. Perussia. 1984. Response of resting human peripheral blood natural killer cells to interleukin 2. *J. Exp. Med.* 160: 1147–1169.
40. Phillips, J. H., and L. L. Lanier. 1986. Dissection of the lymphokine-activated killer phenomenon: relative contribution of peripheral blood natural killer cells and T lymphocytes to cytolysis. *J. Exp. Med.* 164: 814–825.
41. Verstovsek, S., D. Maccubbin, M. J. Ehrke, and E. Mihich. 1992. Tumoricidal activation of murine resident peritoneal macrophages by interleukin 2 and tumor necrosis factor α. *Cancer Res.* 52: 3880–3885.
42. Dudley, M. E., J. Wunderlich, M. I. Nishimura, D. Yu, J. C. Yang, S. L. Topalian, D. J. Schwartzentruber, P. Hwu, F. M. Marincola, R. Sherry, et al. 2001. Adoptive transfer of cloned melanoma-reactive T lymphocytes for the treatment of patients with metastatic melanoma. *J. Immunother.* 24: 363–373.
43. Dudley, M. E., J. R. Wunderlich, J. C. Yang, P. Hwu, D. J. Schwartzentruber, S. L. Topalian, R. M. Sherry, F. M. Marincola, S. F. Leitman, C. A. Seipp, et al. 2002. A phase I study of nonmyeloablative chemotherapy and adoptive transfer of autologous tumor antigen-specific T lymphocytes in patients with metastatic melanoma. *J. Immunother.* 25: 243–251.
44. Yee, C., J. A. Thompson, D. Byrd, S. R. Riddell, P. Roche, E. Celis, and P. D. Greenberg. 2002. Adoptive T cell therapy using antigen-specific $CD8^+$ T cell clones for the treatment of patients with metastatic melanoma: in vivo persistence, migration, and antitumor effect of transferred T cells. *Proc. Natl. Acad. Sci. USA* 99: 16168–16173.
45. Schuler, T., and T. Blankenstein. 2003. Cutting edge: $CD8^+$ effector T cells reject tumors by direct antigen recognition but indirect action on host cells. *J. Immunol.* 170: 4427–4431.
46. Dudley, M. E., J. R. Wunderlich, P. F. Robbins, J. C. Yang, P. Hwu, D. J. Schwartzentruber, S. L. Topalian, R. Sherry, N. P. Restifo, A. M. Hubicki, et al. 2002. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* 298: 850–854.
47. Kolb, H. J., C. Schmid, A. J. Barrett, and D. J. Schendel. 2004. Graft-versus-leukemia reactions in allogeneic chimeras. *Blood* 103: 767–776.
48. Mocellin, S., S. Mandruzzato, V. Bronte, M. Lise, and D. Nitti. 2004. Part I: vaccines for solid tumours. *Lancet Oncol.* 5: 681–689.