Multiple Roles of Interferon-γ in the Mediation of Interleukin 12-induced Tumor Regression

Makoto Ogawa, Wen-Gong Yu, Kazunari Umehara, Masayuki Iwasaki, Rishani Wijesuriya, Takahiro Tsujimura, Takeshi Kubo, Hiromi Fujiwara, and Toshiyuki Hamaoka

ABSTRACT

Administration of recombinant interleukin 12 (IL-12) induces tumor regression that is associated with T-cell infiltration in the OV-HM ovarian carcinoma and CSA1M fibrosarcoma models. After confirming the blocking of regression by injection of anti-IFN-γ monoclonal antibody (mAb), we investigated the mechanisms underlying the requirement of IFN-γ in T-cell migration and tumor regression. T-cell migration was inhibited by injection of anti-IFN-γ mAb to OV-HM tumor-bearing mice prior to IL-12 treatment. We examined, using the lymphoid cell migration assay, whether IFN-γ is required for enhancing the migratory capacity of T cells or the T cell-accepting potential of tumor masses during IL-12 treatment. Spleen cells from IL-12-treated or untreated OV-HM-bearing mice were stained in vitro with a fluorescein chemical and transferred i.v. into OV-HM-bearing mice that were not treated with IL-12. Migration of donor cells was quantitated by counting the number of fluorescent cells on cryostat sections of tumor masses from recipient mice. Compared to spleen cells from OV-HM-bearing mice that were not treated with IL-12, enhanced migration was observed for cells from IL-12-treated OV-HM-bearing mice. Anti-IFN-γ pretreatment of donor mice before IL-12 treatment did not reduce the migratory capacity of T cells, whereas migration was markedly inhibited in recipient mice injected with anti-IFN-γ. Anti-IFN-γ pretreatment decreased vascular cell adhesion molecule-1 (VCAM-1)/intercellular adhesion molecule-1 (ICAM-1)-positive blood vessels at tumor sites. Consistent with this, migration was also inhibited by treatment of recipient mice with either anti-VCAM-1 or anti-ICAM-1 mAb. In contrast to the OV-HM model, T-cell migration was not affected in the CSA1M model following preinjection of anti-IFN-γ mAb. In this model, VCAM-1/ICAM-1-positive blood vessels existed even after anti-IFN-γ treatment, although tumor regression was completely inhibited. These results indicate that IFN-γ plays two distinct roles in expressing the antitumor efficacy of IL-12: one is to support the T-cell acceptability of tumor masses, and the other is to mediate the antitumor effects of migrated T cells.

INTRODUCTION

IL-12 has been shown to induce a number of biological effects (1, 2), including enhancing NK (3) and CTL (4) activities; acting as an NK and T-cell growth factor (5–7); stimulating secretion of various cytokines, particularly IFN-γ, by NK and T cells (3, 8); and promoting maturation of the Th1 helper cell subset (1, 9). In addition, IL-12 induces lymphoid cells to express a number of surface molecules, including adhesion molecules (10, 11). Each of these biological properties has been considered to contribute to controlling tumor growth.

The critical requirement of IFN-γ for the IL-12-mediated tumor effect has been recognized. This is based on the fact that the treatment of anti-IFN-γ mAb prior to IL-12 administration abrogates the anti-tumor efficacy (12, 13), although IFN-γ production alone is not sufficient to induce significant therapeutic effects (14). We have also observed that tumor regression is associated with a massive T-cell infiltration into tumor masses and in situ IFN-γ production (13, 15, 16). Thus, both IFN-γ production and T-cell migration appear to be crucial in the process of IL-12-induced tumor regression. However, it remains to be investigated whether these two requirements are mutually related, i.e., whether IFN-γ is required for (a) enhancing the migratory capacity of T cells; (b) generating the acceptability of tumor masses for migrating T cells; or (c) inducing antitumor responses at tumor sites. In this context, we recently developed a lymphoid cell migration assay (17) in which spleen cells from IL-12-treated donor mice, after in vitro fluorescence labeling, are transferred into recipient mice that are not treated with IL-12 and fluorescence-positive cells are evaluated on cryostat sections of recipient’s tumor masses. This model permitted us to separately examine the roles of IFN-γ on the generation of the migratory capacity in donor T cells and on the preparation of the acceptability in recipient’s tumor masses.

These results show that, in the OV-HM and CSA1M tumor models, in which tumor regression is induced by IL-12 treatment, IFN-γ is not necessarily required for generating the migratory capacity in T cells. In the OV-HM model, the migration of donor T cells was almost completely inhibited when OV-HM-bearing recipient mice were pre-treated with anti-IFN-γ. T-cell migration was dependent on the interactions between VLA-4/ICAM-1 (T cells) and VCAM-1/ICAM-1 (endothelial cells). Anti-IFN-γ pretreatment was found to largely delete VCAM-1/ICAM-1-positive blood vessels, which were otherwise observed at the peritumoral sites, indicating the role of IFN-γ in preparing the acceptability of tumor masses for migrating T cells. In contrast, a similar protocol of anti-IFN-γ pretreatment did not decrease VCAM-1/ICAM-1-positive blood vessels at the peritumoral sites in the CSA1M model. Consistent with this, T-cell migration was not inhibited in the IFN-γ-pretreated CSA1M recipient mice. However, anti-IFN-γ blocked regression of growing CSA1M tumors by IL-12 treatment, suggesting the role of IFN-γ in mediating antitumor events at tumor sites. Together, the results indicate that IFN-γ acts as an antitumor cytokine at various steps, from T-cell migration to intratumoral implementation of antitumor responses.

MATERIALS AND METHODS

Mice. Male BALB/c and female (C57BL/6 × C3H/HeF1) (B6C3F1) mice were obtained from Shizuoka Experimental Animal Center (Hamamatsu, Japan) and used at 6–9 weeks of age.

Tumor Cell Lines. The following two tumor cell lines were used: CSA1M fibrosarcoma (18) and OV-HM ovarian carcinoma (19) were kindly provided by Dr. Takato O. Yoshida (Hamamatsu University School of Medicine, Hamamatsu, Japan) and Dr. Ohtsura Niwa (Kyoto University, Kyoto, Japan). These cloned tumor cell lines were maintained in RPMI 1640 supplemented with 10% FCS at 37°C in a humidified atmosphere with 5% CO2.

Preparation of Tumor-bearing Mice. Mice were inoculated s.c. with CSA1M (5×103/mouse) or OV-HM (5×103/mouse) tumor cells.
**Reagents.** Murine rIL-12 was kindly provided by Genetics Institute Inc. (Cambridge, MA). It was purified from the supernatants of CHO cells transfected with the pSsi and p40 cDNA plasmids. A fluorescent dye, PKH-26-GL, was purchased from Sigma Chemical Co. (St. Louis, MO).

**IL-12 Treatment.** Mice were injected i.p. with rIL-12 in a dose of 0.5 μg/mouse three times every day.

**mAbs.** Antimouse IFN-γ (R4-6A2) mAb, antimouse LFA-1 (M17/5.27), and antimouse ICAM-1 (YN1/7.4) hybridomas were obtained from American Type Culture Collection (Manassas, VA). Antimouse VCA-M (MK1; Ref. 20) and antimouse VLA-4 (PS2; Ref. 21) hybridomas were kindly provided by Dr. K. Miyake (Saga Medical College, Saga, Japan). mAbs were prepared from ascitic fluids of hybridoma cells. The purification was performed by precipitation with ammonium sulfate followed by YFPC gel filtration (Yamazen Corporation, Osaka, Japan). Control rat IgG was obtained from BioMeda (Foster City, CA).

**Depletion of CD4/CD8 T Cells from a Spleen Cell Suspension.** Spleen cells were depleted of CD4/CD8 T cells by immunomagnetic negative selection, as described (22). Briefly, CD4* and CD8* T cells were eliminated by incubation with anti-CD4 and anti-CD8 mAbs, followed by magnetic particles conjugated to goat antirat IgG (Advanced Magnetic, Cambridge, MA).

**Labeling of Cells with a Fluorescent Dye.** Staining was performed according to the manufacturer’s recommended procedure. Briefly, spleen cells suspended to a concentration of 5 × 10^7/ml in 1 ml of diluent were allowed to react with 5 × 10^-6 m PKH-26-GL, solved in 1 ml of diluent for 5 min at 37°C. Labeling was stopped by the addition of 2 ml of FCS, and cells were washed five times with RPMI 1640 containing 10% FCS.

**Lymphoid Cell Migration Assay.** The assay system was essentially the same as that described previously (17). Mice with similar tumor sizes (~7 mm in diameter) were used as recipients for this assay. PKH-26-GL-labeled lymphoid cells (2 × 10^7 cells) were injected into homologous tumor-bearing mice that were not treated with IL-12. Twenty-four h after injection, tumor masses were removed, and cryostat sections were prepared. The entry of fluorescence-labeled donor cells was quantitated under a fluorescence microscope and expressed as the mean cell number ± SE per section.

**Histological Examination.** Tumor masses were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with H&E for histological examination.

**Staining Procedure of Immunohistochemical Examination.** The following reagents were purchased to perform immunohistochemical examination: biotinylated rat antimouse VCAM-1 and antimouse ICAM-1 (CALTAG Laboratories, San Francisco, CA); biotinylated antimouse CD4, CD8, and CD21 mAbs (PharMingen, San Diego, CA); biotinylated rat IgG (Jackson Immunoresearch, West Grove, PA); Histofine SA-PO kit and Histofine DAB kit (Nichirei Co. Ltd., Tokyo, Japan). Cryostat sections (5 μm) were cut, air-dried, fixed in 1% paraformaldehyde for 30 min at room temperature, and then washed three times with PBS. The sections were incubated in PBS containing 0.6% hydrogen peroxide at room temperature for 15 min to block endogenous peroxidase activity before a biotinylated mAb was added. After preincubation with 4% BSA solution, the tissues were overlaid with various biotinylated antibodies and incubated in a humidified chamber at room temperature for an h. After washing 3 times, the sections were incubated with peroxidase-conjugated streptavidin solution for 5 min. After additional three washes, the labeling was visualized with 0.03% DAB solution containing 0.1% hydrogen peroxide for several minutes.

**RESULTS**

**Effect of Anti-IFN-γ Pretreatment on IL-12-induced T-Cell Migration and Tumor Regression.** Our earlier studies (13, 15, 23) showed that systemic administration of rIL-12 to tumor-bearing mice induces complete regression of growing tumors that is associated with a massive T-cell infiltration to tumor masses in the CSA1M fibrosarcoma and OV-HM ovarian carcinoma models. We have further found that injections of anti-IFN-γ mAb starting just before (1 day before) IL-12 treatment result in almost complete prevention of tumor regression in both tumor models (13, 15). In this study, we examined the effect of anti-IFN-γ pretreatment on IL-12-induced T-cell migration and tumor regression.
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Fig. 2. Pretreatment with anti-IFN-γ mAb inhibits IL-12-induced infiltration of lymphoid cells to tumor sites. Tumor cell inoculation, anti-IFN-γ treatment, and IL-12 injections were performed in the same protocol as described in Fig. 1. Tumor masses were removed from OV-HM tumor-bearing mice 2 days after the final (third) IL-12 injection. H&E staining. Original magnification, ×500.

inoculum abolished enhanced migration (17). The results of Fig. 5 confirm this and, furthermore, demonstrate that most of migrating cells were also CD4/CD8 T cells when donor cells were prepared from mice treated with IL-12 after anti-IFN-γ injection.

We next examined the effect of anti-IFN-γ injection in recipient mice. Recipient tumor-bearing mice were untreated or were treated with anti-IFN-γ mAb or control rat IgG. Three days later, donor cells prepared from IL-12-treated tumor-bearing mice were transferred into anti-IFN-γ-untreated or -treated recipient mice (Fig. 6). Migration was not inhibited by treatment of recipients with anti-IFN-γ mAb in the CSA1M model, whereas anti-IFN-γ treatment of recipients strikingly reduced the enhanced migration in the OV-HM model. Thus, these differential effects of anti-IFN-γ treatment of recipient mice in the migration assay correlated with the differences in the effects of anti-IFN-γ treatment on the migration induced after IL-12 treatment between OV-HM (Fig. 2) and CSA1M (24) models.

Effect of Anti-IFN-γ Treatment on the Induction of VCAM-1-/ICAM-1-positive Blood Vessels. We previously demonstrated that IL-12 can confer T cells with a capacity to migrate to tumor sites through VLA-4/LFA-1 adhesion pathways (17). Fig. 7 confirms this by demonstrating that treatment of recipient mice with mAbs against ligands for VLA-4 and LFA-1 inhibits the migration of donor cells from IL-12-treated tumor-bearing mice. These observations, showing the crucial roles of VCAM-1/ICAM-1, probably on intratumoral blood vessels in T-cell migration in both OV-HM and CSA1M models, raise the question of why the effect of anti-IFN-γ treatment on T-cell migration differs in these two tumor models.

We, therefore, investigated whether anti-IFN-γ treatment influences the expression of VCAM-1/ICAM-1 on blood vessels or the induction of VCAM-1-/ICAM-1-positive vessels at tumor sites. This was done by immunohistochemical examination. Cryostat sections of tumor masses from OV-HM or CSA1M tumor-bearing mice 3 days
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Anti-IFN-γ Treatment Inhibits the Induction of VCAM-1-/ICAM-1-positive Blood Vessels in Tumor Masses, even from IL-12-treated OV-HM-bearing Mice. T-cell migration was inhibited not only in recipient mice treated with anti-IFN-γ but not treated with IL-12 in the migration assay (Fig. 6, top) but also in mice receiving IL-12 therapy following anti-IFN-γ pretreatment (Figs. 2 and 3). We finally examined whether anti-IFN-γ treatment also down-regulates the induction of VCAM-1-/ICAM-1-positive blood vessels in tumor masses of mice during IL-12 treatment. Tumor masses were harvested from IL-12-treated tumor-bearing mice with or without anti-IFN-γ pretreatment and stained for CD31, VCAM-1, and ICAM-1 (Fig. 10). The results show that CD31+ blood vessels from IL-12-treated mice not receiving anti-IFN-γ pretreatment expressed high levels of VCAM-1 and ICAM-1 and that ICAM-1 positivity was additionally detected on cells other than CD31+ endothelial cells. In contrast, pretreatment with anti-IFN-γ before IL-12 treatment resulted in a striking down-regression of VCAM-1-/ICAM-1 expression. Slight levels of ICAM-1 expression were constitutively detected but VCAM-1 expression was hardly observed in normal s.c. tissue before IL-12 treatment. After IL-12 treatment, up-regulation of VCAM-1-/ICAM-1 was observed at tumor sites especially in the peritumoral regions (stroma) but was not seen in s.c. tissue not associated with tumors (data not shown). Thus, anti-IFN-γ treatment inhibits the expression of intratumoral vascular adhesion molecules required for T-cell migration.

DISCUSSION

The results obtained here demonstrate that IL-12 treatment induces a massive infiltration of CD4+ and CD8+ T cells into tumor masses after anti-IFN-γ treatment were stained for CD31 (as a marker for endothelial cells), VCAM-1, or ICAM-1 (Figs. 8 and 9). Fig. 8 illustrates that the control OV-HM tumor masses have many CD31+ vessels (especially at the outer area of the mass) expressing high levels of VCAM-1 and ICAM-1, but the mass from anti-IFN-γ-treated mice have fewer CD31+ vessels, expressing strikingly and appreciably reduced levels of VCAM-1 and ICAM-1, respectively.

In contrast to the OV-HM model, the incidence of CD31+ vessels did not differ between untreated and anti-IFN-γ-treated groups in the CSA1M model (Fig. 9). Moreover, the vessels expressed VCAM-1 and ICAM-1, irrespective of whether anti-IFN-γ treatment was performed (Fig. 9). Taken together (Figs. 6–9), the results indicate that anti-IFN-γ treatment inhibits VLA-4/LFA-1-dependent T-cell migration by down-regulating the induction VCAM-1-/ICAM-1-positive blood vessels at tumor sites only in the OV-HM tumor model.
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in the OV-HM model and that this T cell migration is dependent on IFN-γ activity because the migration is largely inhibited by anti-IFN-γ pretreatment. The lymphoid cell migration assay revealed that IFN-γ is required not for the acquisition of the tumor-infiltrating capacity by T cells but for the induction of VCAM-1/ICAM-1-positive intratumoral vasculature in the above tumor model. In contrast, neither the expression of VCAM-1/ICAM-1 on intratumoral vessels nor T-cell migration is inhibited by anti-IFN-γ pretreatment in the CSA1M tumor model. Our previous study showed that anti-IFN-γ treatment prevents the regression of CSA1M tumors by blocking the activity of IFN-γ produced by tumor-infiltrating T cells (24). Taken together, the results indicate multiple roles of IFN-γ produced by IL-12-stimulated T cells in the scenario of IL-12-induced tumor regression.

Here, we show a major role for IFN-γ in the induction of tumor regression in the OV-HM tumor model. The results indicate that this role is associated with the regulation of vascular adhesion molecules on intratumoral vasculature responsible for T-cell migration. The interaction of T cells with endothelial cells is principally mediated by two integrin molecular pathways: the binding of LFA-1 to ICAM-1 and the binding of VLA-4 to VCAM-1 (25). The involvement of these two interactive units in the recruitment of T cells to inflamed sites has been well established by the blocking experiments using mAbs against these adhesion molecules (26–31). It is well known that the expression of VCAM-1/ICAM-1 is up-regulated at the inflamed sites by inflammatory cytokines such as IFN-γ (31). Our results illustrated that detectable levels of cellular infiltration and VCAM-1/ICAM-1 expression are already observed within tumor masses before IL-12 treatment in the OV-HM tumor model. In the migration assay, donor T cells prepared from IL-12-treated tumor-bearing mice migrated to tumor masses of recipient mice not exposed to IL-12 in a VCAM-1-/ICAM-1-dependent manner. Treatment of recipients with anti-IFN-γ mAb prior to donor cell transfer inhibited T-cell migration along with down-regulation of intratumoral VCAM-1/ICAM-1 expression. Thus, it is reasonable to assume that IFN-γ produced by a few tumor-infiltrating cells contributed to inducing the expression of VCAM-1/ICAM-1 before IL-12 treatment.

The expression of VCAM-1/ICAM-1 is strikingly up-regulated in tumor masses from IL-12-treated OV-HM-bearing mice. This was

Fig. 8. Down-regulation of VCAM-1/ICAM-1 expression on blood vessels in tumor masses from anti-IFN-γ-treated OV-HM-bearing mice. OV-HM tumor masses were harvested from mice 3 days after treatment with anti-IFN-γ mAb or control rat IgG. Sections of tumor masses were stained for CD31, VCAM-1, and ICAM-1.

Fig. 7. Inhibition of T-cell migration by treatment of recipient mice with anti-VCAM-1 or anti-ICAM-1 mAb before donor cell transfer. Donor spleen cells from IL-12-treated tumor-bearing mice were transferred into recipient mice that had been injected with 1 mg/mouse anti-VCAM-1 or anti-ICAM-1 mAb 12 h before. The results are representative of two similar experiments.

In the OV-HM model and that this T cell migration is dependent on IFN-γ activity because the migration is largely inhibited by anti-IFN-γ pretreatment. The lymphoid cell migration assay revealed that IFN-γ is required not for the acquisition of the tumor-infiltrating capacity by T cells but for the induction of VCAM-1/ICAM-1-positive intratumoral vasculature in the above tumor model. In contrast, neither the expression of VCAM-1/ICAM-1 on intratumoral vessels nor T-cell migration is inhibited by anti-IFN-γ pretreatment in the CSA1M tumor model. Our previous study showed that anti-IFN-γ treatment prevents the regression of CSA1M tumors by blocking the activity of IFN-γ produced by tumor-infiltrating T cells (24). Taken together, the results indicate multiple roles of IFN-γ produced by IL-12-stimulated T cells in the scenario of IL-12-induced tumor regression.

Here, we show a major role for IFN-γ in the induction of tumor regression in the OV-HM tumor model. The results indicate that this role is associated with the regulation of vascular adhesion molecules on intratumoral vasculature responsible for T-cell migration. The interaction of T cells with endothelial cells is principally mediated by two integrin molecular pathways: the binding of LFA-1 to ICAM-1 and the binding of VLA-4 to VCAM-1 (25). The involvement of these two interactive units in the recruitment of T cells to inflamed sites has been well established by the blocking experiments using mAbs against these adhesion molecules (26–31). It is well known that the expression of VCAM-1/ICAM-1 is up-regulated at the inflamed sites by inflammatory cytokines such as IFN-γ (31). Our results illustrated that detectable levels of cellular infiltration and VCAM-1/ICAM-1 expression are already observed within tumor masses before IL-12 treatment in the OV-HM tumor model. In the migration assay, donor T cells prepared from IL-12-treated tumor-bearing mice migrated to tumor masses of recipient mice not exposed to IL-12 in a VCAM-1-/ICAM-1-dependent manner. Treatment of recipients with anti-IFN-γ mAb prior to donor cell transfer inhibited T-cell migration along with down-regulation of intratumoral VCAM-1/ICAM-1 expression. Thus, it is reasonable to assume that IFN-γ produced by a few tumor-infiltrating cells contributed to inducing the expression of VCAM-1/ICAM-1 before IL-12 treatment.

The expression of VCAM-1/ICAM-1 is strikingly up-regulated in tumor masses from IL-12-treated OV-HM-bearing mice. This was
Fig. 9. The expression of VCAM-1/ICAM-1 on intratumoral vessels is not inhibited by anti-IFN-γ mAb pretreatment in the CSA1M model. CSA1M tumor masses were harvested from mice 3 days after treatment with anti-IFN-γ mAb or control rat IgG.

also observed in another IL-12-curable model, CSA1M, but not in IL-12-noncurable tumor models, in which only marginal levels of cellular infiltration were observed, even after IL-12 treatment. The enhanced VCAM-1/ICAM-1 expression in tumor masses from IL-12-treated OV-HM-bearing mice was almost completely inhibited by anti-IFN-γ pretreatment. Consequently, T-cell migration and tumor regression were prevented. Thus, IFN-γ is required for inducing the T-cell acceptability of tumor masses, as exemplified by the expression of VCAM-1/ICAM-1 in the OV-HM model.

In contrast to the OV-HM model, the expression of VCAM-1/ICAM-1 was not largely affected by anti-IFN-γ treatment in the CSA1M model. This was, indeed, the case for CSA1M tumor masses from mice not treated with IL-12 (Fig. 9) and even from IL-12-treated mice. In this tumor model, the up-regulation of VCAM-1/ICAM-1 may be assumed to be efficiently achieved by inflammatory cytokines (31) other than IFN-γ when IFN-γ is neutralized. Because the expression of these adhesion molecules was not largely inhibited in CSA1M tumor masses, T-cell migration was observed in anti-IFN-γ-pretreated recipient mice in the present migration assay and in mice receiving IL-12 therapy following anti-IFN-γ injections (24). Despite positive T-cell migration, regression of CSA1M tumors was inhibited. This was shown to be associated with the neutralization of intratumoral IFN-γ activity responsible for the manifestation of IFN-γ-mediated anti-tumor effects (24). Namely, anti-IFN-γ mAb injection prior to IL-12 treatment almost completely abrogated the in situ expression of inducible nitric oxide synthase and IFN-γ-inducible protein-10, as examples of IFN-γ-inducible genes (24). This also shows the efficacy of IFN-γ neutralization in the CSA1M tumor model. Thus, differential effects of anti-IFN-γ treatment on the expression of VCAM-1/ICAM-1 were observed in IL-12-treated mice, as exemplified by OV-HM tumors, in which IFN-γ was required for the manifestation of IFN-γ-mediated anti-tumor effects, whereas such a requirement was not observed in CSA1M tumors, in which the IFN-γ-inducible expression of adhesion molecules was efficiently achieved by other cytokines.

Fig. 10. Anti-IFN-γ mAb treatment inhibits the expression of VCAM-1/ICAM-1 on intratumoral vessels in IL-12-treated mice. OV-HM tumor-bearing mice were pretreated with anti-IFN-γ mAb or control IgG and, 3 days later, received IL-12 therapy (3 injections). Tumor masses were harvested 2 days after the final IL-12 injection.

VCAM-1/ICAM-1 permitted the evaluation of two distinct roles of IFN-γ in tumor regression in two IL-12-curable tumor models. It would also be of importance to consider the effect of IL-12 on the acquisition of tumor-infiltrating capacity by T cells. In the migration assay, T cells from tumor-bearing mice not treated with IL-12 failed to migrate to tumor sites, and T cells exhibited the tumor-infiltrating capacity only after IL-12 treatment (Ref. 24 and this study). Thus, it is obvious that IL-12 confers T cells with the tumor-infiltrating capacity only after IL-12 treatment (Ref. 24 and 31).

The present results indicate that IFN-γ is required for up-regulating the expression of adhesion molecules responsible for enhanced T-cell migration. IFN-γ also functions as a key cytokine in the mediation of the activation of various antitumor mechanisms at tumor sites (reviewed in Ref. 35). In addition to the IL-12 function that is necessary for up-regulating the tumor-infiltrating capacity of T cells, there are multiple mechanisms for the manifestation of antitumor effects of IL-12. It is also true that IL-12 is unable to exhibit antitumor effects in some tumor models. Recognizing each of the above mechanisms and examining the operation of these mechanisms could contribute to the designing of ways to correct the defects of antitumor pathways in tumor models in which tumor regression is not induced by IL-12 treatment.

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