A Critical Role for a Peritumoral Stromal Reaction in the Induction of T-Cell Migration Responsible for Interleukin-12-induced Tumor Regression

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ABSTRACT

Interleukin (IL-12) has been shown to elicit tumor regression when this cytokine induces the migration of T cells to tumor sites. The present study investigates the role of a peritumoral stromal reaction in IL-12-induced T-cell migration. In the CSA1M and OV-HM tumor models, IL-12 treatment induced tumor regression that is associated with T-cell migration. Neither T-cell migration nor tumor regression was observed in the Meth A and MCH-1-A1 models. Stromal tissue containing neovascular blood vessels developed at the peritumoral area of the former two IL-12-responsive tumors but not at the peritumoral area of the latter two IL-12-unresponsive tumors. The significance of stroma development was investigated using a pair of tumor models (CSA1M and a subline derived from CSA1M designated the CSA1M variant), both of which exhibit the same tumor immunogenicity. In contrast to the parental CSA1M cell line, the variant cell line was not responsive to IL-12, and neither stroma development nor T-cell migration was observed, even after IL-12 treatment. Histological analyses revealed that the parental cell line had peritumoral stroma with intrastromal vessels but only a few vessels in tumor parenchyma, whereas the variant cell line showed no stroma but had abundant vasculature in the tumor parenchyma. Most importantly, only stromal vessels in the parental tumors expressed detectable and enhanced levels of vascular cell adhesion molecule 1 (VCAM-1)/intercellular adhesion molecule 1 (ICAM-1) before and after IL-12 treatment. In contrast, parenchymal vasculature in the variant cell line failed to express VCAM-1/ICAM-1 even after IL-12 treatment. When transferred into recipient tumor-bearing mice, IL-12-stimulated T cells from the parental CSA1M-bearing or the variant CSA1M-bearing mice migrated into the parental but not into the variant tumor mass. Together with our previous finding that T-cell migration depends on the VCAM-1/ICAM-1 adhesive interactions, the present results indicate a critical role for peritumoral stroma/stromal vasculature in the acceptance of tumor-infiltrating T cells that is a prerequisite for IL-12-induced tumor regression.

INTRODUCTION

IL-12 has pleiotropic effects on T cells and natural killer cells (1, 2) including the stimulation of IFN-γ secretion (3, 4). This cytokine has also been shown to exhibit potent antitumor efficacy in a number of murine tumor models (5–7). Among the various IL-12 bioactivities, the critical requirement of IFN-γ for the IL-12-mediated antitumor effect has been recognized, based on the fact that treatment with anti-IFN-γ mAb before IL-12 administration abrogates the antitumor efficacy of IL-12 (6, 7). However, IFN-γ production alone is not sufficient to induce significant therapeutic effects (8). Instead, we have observed that tumor regression is associated with a massive T-cell infiltration into tumor masses and in situ IFN-γ production by tumor-infiltrating T cells (7, 9, 10). The migration of T cells to tumor masses was induced after IL-12 treatment in IL-12-responsive tumor models but was not elicited in IL-12-unresponsive models. Our preceding study also demonstrated that T-cell migration takes place through interactions between adhesion molecules on the endothelium (VCAM-1/ICAM-1) and their T-cell adhesion receptors (VLA-4/ LFA-1; Ref. 11).

There is considerable experimental evidence showing that tumor growth depends on angiogenesis (12). Tumor vasculature is generated in stromal tissue that develops between the tumor and normal host tissue (13). After a newly initiated tumor has attained a size of a few millimeters in diameter, further expansion of the tumor cell population requires the induction of new blood vessels. The induction of angiogenesis is mediated by specific angiogenic factors produced by tumor cells and coexisting macrophages (14). New vasculature functions as a lifeline for delivering gas and nutrients to the tumor and provides a place where host lymphoid cells can enter the tumor. A critical question may be raised regarding why T cells migrate to tumor sites after IL-12 treatment in some tumor models (IL-12-responsive tumors) but fail to infiltrate in other models (IL-12-unresponsive tumors).

The present study investigated whether tumor stroma-associated vasculature has a role in the induction of T-cell migration and, if so, why T-cell migration is not induced in IL-12-unresponsive models. The results show that peritumoral stroma develops in IL-12-responsive tumor models, and T-cell migration occurred in the peritumoral stroma but not in the tumor parenchyma. In unresponsive models, tumors failed to develop peritumoral stroma/stromal vessels; instead, they had well-developed vasculature in the parenchyma. There was a fundamental difference in the expression of VCAM-1/ICAM-1 between peritumoral stroma vessels and parenchymal vessels: the former expressed detectable levels of VCAM-1/ICAM-1 before IL-12 treatment and up-regulated its expression after the treatment. In marked contrast, tumor parenchymal vessels in both IL-12-responsive and -unresponsive models failed to express these adhesion molecules even after IL-12 treatment. These results demonstrate the distinctiveness of peritumoral stroma vasculature in terms of the acceptability of tumor-infiltrating T cells and indicate that IL-12-induced T-cell migration depends on the capacity of the tumor to develop such peritumoral stroma/stromal vasculature.

MATERIALS AND METHODS

Tumor Cell Lines. The following four tumor cell lines were used: (a) CSA1M fibrosarcoma (15); (b) OV-HM ovarian carcinoma (16); (c) Meth A fibrosarcoma; and (d) MCH-1-A1 fibrosarcoma (17). CSA1M and OV-HM tumors were kindly provided by Dr. Takato O. Yoshih (Hamamatsu University School of Medicine, Hamamatsu, Japan) and Dr. Ohtsura Niiwa (Kyoto University, Kyoto, Japan). CSA1M fibrosarcoma was induced in a male BALB/c mouse with the Schmidt-Ruppin strain of Rous sarcoma virus and shown to produce no sarcoma virus (15). During passages of CSA1M tumor cells in vitro cultures, morphological heterogeneity in tumor cells emerged spontaneously. The variant cell line of the CSA1M tumor was isolated through...
limiting dilution, based on morphological distinctiveness. The standard CSA1M and its variant were designated parental CSA1M and the CSA1M variant, respectively. An ovarian tumor, OV2944, was induced in a female (C57BL/6 × C3H/He)F1 mouse by a single whole-body neutron irradiation, and a cloned cell line designated OV-HM was isolated from the parental cell line (18). MCH-1-A1 was a fibrosarcoma cell clone, the parental line of which was induced in a female C3H/He mouse with methylcholanthrene in our laboratory (17). These tumor cell lines were maintained in RPMI 1640 supplemented with 10% FCS at 37°C in a humidified atmosphere with 5% CO2, and Meth A tumor was maintained by i.p. passages in syngeneic BALB/c mice.

Mice. Male BALB/c, female C3H/He, and female (C57BL/6 × C3H/He)F1 mice were obtained from Shizuoka Experimental Animal Center (Hamatsuto, Japan) and used at 6–9 week of age.

Reagents. Murine rIL-12 was obtained from Genetics Institute Inc. (Cambridge, MA). It was purified from the supernatants of Chinese hamster ovary cells transfected with the p35 and p40 cDNA plasmids. A fluorescent dye, PKH-26-GL, was purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of Tumor-bearing Mice. Mice were inoculated s.c. with CSA1M (3 × 103 cells/mouse), OV-HM (5 × 103 cells/mouse), Meth A (5 × 103 cells/mouse), or MCH-1-A1 (5 × 103 cells/mouse) tumor cells.

IL-12 Treatment. rIL-12 (0.5 μg/time) was administered i.p. to tumor-bearing mice once daily for 3 days.

A Lymphoid Cell Migration Assay. The assay system was essentially the same as described previously (11). The staining of spleen cells with a fluorescent dye (PKH-26-GL) was performed according to the manufacturer’s recommended procedure. Briefly, spleen cells suspended to a concentration of 5 × 107 cells/ml in 1 ml of diluent were allowed to react with 5 × 10−6 m PKH-26-GL dissolved in 1 ml of diluent for 5 min at 37°C. Labeling was stopped by adding 2 ml of FCS, and cells were washed five times with RPMI 1640 containing 10% FCS. Mice with similar tumor sizes (approximately 7 mm in diameter) were used as the recipients for this assay. PKH-26-GL-labeled spleen cells (2 × 107 cells in 1 ml of RPMI 1640) were injected i.v. into the recipient (IL-12-untreated, homologous tumor-bearing) mice. Twenty-four h after the injection, tumor masses were removed, and cryostat sections were prepared. The entry of fluorescence-labeled donor cells was quantified using fluorescence microscopy and expressed as the mean cell number ± SE/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 items were purchased to perform the immunohistochemical examination: (a) biotinylated rat antirat IgG-CD4 and antirat IgG-CD8 (Jackson ImmunoResearch Laboratories, San Francisco, CA); (b) biotinylated antirat CD4, CD8, and CD31 mAbs (PharMingen, San Diego, CA); (c) biotinylated rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA); and (d) the 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 the addition of a biotinylated antibody. After preincubation with 4% BSA solution, the tissues were overlaid with various biotinylated antibodies and incubated in a humidified chamber at room temperature for 1 h. After washing three times, the sections were incubated with peroxidase-conjugated streptavidin solution for 5 min. After three more washes, labeling was visualized with 0.03% 3,3′-diaminobenzidine tetrachloride solution containing 0.1% hydrogen peroxide for several minutes.

RESULTS

A Critical Difference in the Development of Peritumoral Stroma between IL-12-responsive and -unresponsive Tumor Models. Our previous studies showed that CSA1M and OV-HM are IL-12-responsive tumor models, whereas MCH-1-A1 and Meth A are IL-12-unresponsive tumor models (7, 9, 11). Administration of rIL-12 to CSA1M or OV-HM tumor-bearing mice results in complete regression of s.c. growing tumors, but the same protocol of IL-12 treatment fails to induce regression of MCH-1-A1 and Meth A tumors. The infiltration of CD4+ and CD8+ T cells was also shown to correlate with the induction of tumor regression (7, 9, 11). To determine why T-cell migration is induced after IL-12 treatment in some tumor models (CSA1M and OV-HM) but not in other models (MCH-1-A1 and Meth A), a comparison was made of the histological difference in tumor masses from four types of tumor-bearing mice that had not been treated with IL-12. Light microscopic examination revealed that the two IL-12-responsive tumors develop peritumoral stroma between s.c. tissue and tumor parenchyma, whereas the two IL-12-unresponsive tumors do not (Fig. 1). Thus, a difference was found in the development of peritumoral stroma between IL-12-responsive and -unresponsive models.

Correlation between the Development of Peritumoral Stroma and the Induction of Tumor Regression in Two Cell Lines Derived from the CSA1M Tumor. The correlation between the development of peritumoral stroma and the induction of tumor regression was
observed using four tumor models in which tumor antigenicities and mouse strains are different. In this context, we recently developed a convenient pair of tumor models, parental CSA1M and the CSA1M variant; the latter is derived from the former. Table 1 shows the immunogenicity of these two cell lines. Three i.p. immunizations of BALB/c mice with mitomycin C-treated tumor cells produced complete protection against the challenge with homologous viable tumor cells. When these mice were rechallenged with the other tumor cell line (parental or variant), rechallenged heterologous tumor cells were also rejected. Thus, both tumors are immunogenic, and their antigenicity is cross-reactive.

We next compared the antitumor efficacy of IL-12 in the parental CSA1M and variant models (Fig. 2). Tumor regression was induced in the parental CSA1M model, as has been shown in our previous studies (7, 9, 11). In contrast, almost no efficacy of IL-12 was observed in the CSA1M variant model. Regression of variant tumors was not induced, even when the number of IL-12 injections was increased 3-fold (nine times; data not shown). Thus, the parental CSA1M and variant tumors with the same immunogenicity represent IL-12-responsive and -unresponsive models, respectively. Histological examination of the parental CSA1M and variant tumor masses before and after IL-12 treatment shows that peritumoral stroma is induced in the parental CSA1M but not in the variant tumor (Fig. 3, upper panels) and that lymphoid cell infiltration after IL-12 treatment occurs only in the parental IL-12-responsive model (Fig. 3, lower panels). Thus, the correlation between the induction of peritumoral stroma, lymphoid cell migration,

### Table 1

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<th>Mice immunized with CSA1M cellsa</th>
<th>Incidence of rejection after challenge</th>
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a BALB/c mice were immunized with mitomycin C-treated parental or variant tumor cells (10^7 cells/time) four times at 5-day intervals.
b One week after the last immunization, immunized mice and control mice were challenged with 3 × 10^5 viable homologous parental or variant tumor cells.
c The mice that rejected the homologous tumor cell challenge were rechallenged with heterologous tumor cells, as were new control mice.
and tumor regression is also seen using two tumor sublines derived from the same tumor.

The Site of T-Cell Migration within a Tumor Mass in IL-12-responsive Models. Fig. 3 (lower left panel) indicates that migrating lymphoid cells are located mainly in the peritumoral stromal area. We examined the site of lymphoid cell migration more accurately by using a lymphoid cell migration assay. Tumor (CSA1M or OV-HM)-bearing mice were treated with IL-12. Spleen cells were obtained 2 days after the third IL-12 injection and labeled with PKH-26-GL. The labeled spleen cells were transferred i.v. into homologous tumor-bearing mice not treated with IL-12. Twenty-four h later, tumor masses were removed, and cryostat sections were prepared. Trans-illuminal and fluoromicroscopic micrographs of the same section are shown.

We also examined the distribution of tumor-associated blood vessels throughout the tumor mass. Blood vessels were stained with anti-CD31 mAb. Fig. 5 shows that the parental CSA1M tumor develops peritumoral stroma that is rich in blood vessels, whereas the parenchyma of the tumor has only few small vessels. In the variant tumor, peritumoral stroma was not generated, and peritumoral stroma vessels were not observed. However, this tumor had well-developed vasculature throughout the tumor parenchyma. This is consistent with the fact that the growth rate of the variant tumor was much higher than that of the parental tumor (Fig. 2). Thus, the lack of lymphoid cell migration in the variant tumor after IL-12 treatment is not ascribed to the failure of this tumor to develop intratumoral vasculature.

A Fundamental Difference in the Capacity to Express VCAM-1 and ICAM-1 between Tumor Vasculature at the Peritumoral Stroma and Tumor Parenchyma. Considering that the interaction between adhesive receptors on T cells (VLA-4/LFA-1) and their ligands (VCAM-1/ICAM-1) on endothelial cells is a prerequisite for T-cell migration (11), we examined the expression of VCAM-1/ICAM-1 in blood vessels in the original and variant tumors from...
IL-12-untreated mice. Fig. 6 shows that CD31<sup>+</sup> vessels at the peritumoral stroma of the original tumor express detectable levels of VCAM-1/ICAM-1. In contrast, well-developed vasculature in the parenchyma of the variant tumor did not express these adhesion molecules. It should also be noted that parenchymal vessels in the original tumor failed to express VCAM-1/ICAM-1.

A similar immunohistochemical examination was performed for tumor masses taken from IL-12-treated parental and variant tumor-bearing mice. Fig. 7 shows that vessels at the peritumoral stroma of the parental tumor express enhanced levels of VCAM-1/ICAM-1, whereas the up-regulation of VCAM-1/ICAM-1 expression was not observed in parenchymal vessels of the parental and variant tumors. Taken together, the results indicate a fundamental difference in the expression of VCAM-1/ICAM-1 between peritumoral and parenchymal vessels.

**T-Cell Migration Depends on the T-Cell Acceptability of Tumor Masses in the Parental CSA1M and Variant Tumor System.** We finally investigated whether T cells in the CSA1M variant tumor-bearing mice develop the capacity to migrate to tumor sites when they are treated in vivo with IL-12. For this purpose, the criss-cross transfer of donor cells from IL-12-treated parental and variant tumor-bearing mice to IL-12-untreated tumor (parental and variant)-bearing recipient mice was conducted in a lymphoid cell migration assay. As shown in Fig. 8, donor cells from IL-12-treated parental tumor-bearing mice exhibited enhanced migration to the parental tumor mass compared to the migration seen by donor cells from IL-12-untreated mice, which is consistent with previous results (11). No significant increase in the number of migrating cells was seen for donor cells from IL-12-treated normal mice compared to those from untreated normal mice (data not shown), as reported previously (11). Comparable levels of enhanced migration to the parental tumor were observed with donor cells from IL-12-treated variant tumor-bearing mice. Our previous study (11) demonstrated that migrating cells in donor spleen cells from the original CSA1M-bearing mice were predominantly T cells. This was also the case for donor cells from the variant CSA1M-bearing mice, because the elimination of CD4<sup>+</sup> and CD8<sup>+</sup> cells in the donor cell inoculum almost completely inhibited migration (data not shown). In contrast to the migration to the parental tumor, portions of the same donor cells from either parental or variant-bearing mice failed to migrate to the variant tumor mass. These results indicate that IL-12 treatment can enhance the migratory capacity of T cells in variant tumor-bearing mice. Thus, the results strongly suggest that the failure of these T cells to migrate to the variant tumor mass is ascribed to the lack of the T-cell acceptability in the variant tumor mass.

**DISCUSSION**

The results obtained in this study show that the efficacy of IL-12 in inducing T-cell migration to tumor sites depends on the development
of peritumoral stromal tissue containing stromal vasculature. The results also demonstrate that there is a fundamental difference between vessels developed at the peritumoral stroma and the tumor parenchymal area in terms of their functional ability to facilitate T-cell migration. The former type of blood vessels expressed VCAM-1/ICAM-1 before IL-12 treatment and up-regulated its expression after IL-12 treatment. In contrast, the latter type of vessels failed to express these adhesion molecules before and even after IL-12 treatment. The migration of T cells is induced through their adhesive interaction with endothelial cells expressing VCAM-1/ICAM-1 (11). Therefore, the development of peritumoral stroma capable of providing VCAM-1/ICAM-1-positive vessels correlated with the induction of intratumoral T-cell migration. Thus, this study demonstrates the critical role of peritumoral stroma/stromal vessels in inducing intratumoral T-cell migration responsible for tumor regression.

Tumor stroma is connective tissue developing between normal host tissues and tumor cells (13, 19). All solid tumors require stroma when they grow beyond minimal size (20). Stroma provides the vascular supply that tumors require for the uptake of nutrients and gas exchange. How stroma is developed after tumor initiation and how angiogenesis is induced in association with the development of stroma (13) have been studied. Stroma development and angiogenesis are a pair of complex events consisting of multiple processes. These include the production of vascular permeability factor/vascular endothelial growth factor, the extravasation of plasma proteins from leaky blood vessels, the deposition of a provisional ECM, and the migration of endothelial cells and fibroblasts (13, 21). Whereas the exact scenario of stroma development and angiogenesis remains to be further elucidated, all solid growing tumors simultaneously develop two events, stroma and angiogenesis, regardless of the extents.

Four tumor cell lines (CSA1M, OV-HM, Meth A, and MCH-1-A1) and a subline of the CSA1M tumor differed in the development of peritumoral stroma. Only the CSA1M and OV-HM tumors developed peritumoral stromal tissue around the tumor parenchyma. In contrast to such an obvious stroma, the stromal tissue that would be induced inside the tumor parenchyma was obscure in all of the above-mentioned tumors. However, a large number of blood vessels were induced inside the parenchyma of the peritumoral stroma-negative CSA1M variant (this study) as well as the peritumoral stroma-positive OV-HM tumor masses (22). According to the scenario described above, these intraparenchymal vessels are considered to be induced in association with a small quantity of stromal tissue that supports the tumor cells within the tumor mass.

A series of our previous studies (7, 9–11) has shown that T-cell migration leading to tumor eradication is induced after IL-12 treatment in some but not all tumor models. The present study showed that T-cell migration occurs at the peritumoral stromal area but not at the parenchymal area; therefore, the development of peritumoral stroma

Fig. 7. Expression of VCAM-1/ICAM-1 on CD31 vessels in the peritumoral stroma but not in the parenchyma of tumors from IL-12-treated mice. Sections of parental CSA1M and variant tumors were removed 2 days after the third IL-12 injection. Sections were stained for CD31, ICAM-1, and VCAM-1. Original magnification, ×660.
ROLE OF PERITUMORAL STROMA IN T-CELL MIGRATION

Fig. 8. Spleen cells from parental CSA1M- or variant-bearing mice migrate into the original tumor mass but not into the variant tumor mass. Spleen cells from IL-12-ununtreated or IL-12-treated CSA1M- or variant-bearing mice were labeled with PKH-26-GL. The labeled cells (2 × 10⁷ cells/mouse) were subjected to a lymphoid cell migration assay. The number of fluorescent dye-positive cells was evaluated on cryostat sections of recipient tumor masses and expressed as the mean ± SE of three sections/tumor mass. The results are representative of three similar experiments.

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REFERENCES


underlies the T-cell migration responsible for IL-12-induced tumor regression. In this context, it is important whether stroma is generated, particularly at the peritumoral area.

In relation to the particular importance of peritumoral stroma, two fundamental questions emerge from the present study. First, it remains to be solved why the generation of peritumoral stroma is present in some tumor models but not in others. The development of peritumoral stroma may be regarded as a part of excessive production of stromal proteins in the entire tumor mass or may result from peritumoral site-selective overproduction of stromal proteins. In either case, stromal proteins consisting of various types of ECM proteins [collagens (23, 24), fibronectin (25, 26), and tenascin (27)] are produced by macrophages, fibroblasts, and endothelial cells (13, 25). High levels of stromal protein deposit could be due to enhanced migration of ECM protein-producing cells and/or elevated production of ECM proteins. It is possible that either of these events is mediated by soluble factors produced by tumor cells or elaborated by host cells during their interaction with tumor cells. For example, IFN-α has been shown to stimulate the production of fibronectin (28), an ECM protein. Thus, studies will be required to investigate whether soluble factors capable of regulating the generation of peritumoral stroma exist. In this context, the parental CSA1M and variant sublines could provide an intriguing pair of tumor models because the latter is derived from the former, and both have the same antigen specificity.

Another important question concerns the differential function of peritumoral vasculature at the peritumoral stroma and parenchymal area. T-cell endothelial adherence and transendothelial migration are prerequisites for T-cell infiltration into infiltrated sites (29). Adhesion molecules (30, 31) and chemokines (32, 33) have been proposed as essential elements for determining T-cell traffic to tissues by acting combinatorially (34–36). Our previous studies have shown that systemic administration of IL-12 induces a complete regression of growing tumors that is associated with a massive T-cell migration to tumor masses (7, 9–11). The results further demonstrated that such a T-cell migration occurs in a VLA-4/LFA-1-dependent manner (11), indicat-
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