Mice Vaccination with Interleukin 12-transduced Colon Cancer Cells Potentiates Rejection of Syngeneic Non-Organ-related Tumor Cells

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ABSTRACT

Cell-based gene therapy after cytokine gene transfer is being investigated for autologous and allogeneic vaccination in cancer therapy. Here we show that mice vaccinated with 3.5 x 10^6 interleukin 12 (IL-12) gene-transduced CT26 colon cancer cells developed a long-lasting antitumor immune memory able to reject not only parental cells but also syngeneic, LM3 mammary, and MCF fibrosarcoma tumorigenic cells. In contrast, mice vaccinated with 0.5-1 x 10^7 CT26 cells transduced with pBabe neo IL-12 retrovirus cells (CT26-IL12) were only able to reject parental cells. An increase in the total circulating levels of IgG2a and a clear shift toward a systemic Th1 response developed, regardless of the amount of injected CT26-IL12 cells. On the contrary, a strong increase in anti-CT26-specific IgG2a levels was observed only when 3.5 x 10^6 CT26-IL12 cells were injected. Immunocompetent mice vaccinated with 3.5 x 10^6 CT26-IL12 cells developed local nodules for a few days, which then ceased growing. These nodules comprised mainly blood vessels, suggesting that an angiogenic process was taking place. CD8+ T cells were responsible for the anti-LM3 tumor cell memory, whereas CD4+ T cells were not involved. Splenocytes and lymphocytes obtained from mice immunized against CT26 cells were able to kill LM3 cells in vitro. Adoptive transfer of lymphocytes obtained from animals immunized against CT26 colon cancer cells suppressed LM3 mammary tumor growth in tumor-bearing mice. The present studies raised the possibility of isolating CTL clones and identifying CTL epitopes shared by different tumor cell types, which can be a target for cancer therapy.

INTRODUCTION

IL-12 is a heterodimeric cytokine that stimulates NK cells (1–3), promotes maturation of CTLs (4, 5), and induces IFN-γ production, stressing its role as an efficient molecule for the initiation of a Th1 response (1, 2). However, in the presence of IL-4, IL-12 may also favor the generation of IL-4-producing T cells from Th0 cells (6, 7) and the exacerbation of an established Th2 response (8, 9).

MATERIALS AND METHODS

Vector Construction, Transfection of Packaging Cells, and Transduction of Tumor Cells. The full-length cDNA corresponding to murine p35 and p40 subunits of IL-12 (kindly provided by Maurice Gately, Hoffmann-La Roche, Inc.) were cloned in the pBabe retroviral vector (16). The p35 subunit was cut with NcoI, blunt-ended with Klenow, and cut with EcoRI. The NcoI/blunt-EcoRI fragment containing p35 was inserted into pBabe Neo linearized with BamHI, blunt-ended with Klenow, and digested with EcoRI. To insert the p40 subunit downstream of the encephalomyocarditis virus internal ribosome entry site, p40 was cut with EcoRI, blunt-ended, and cut with NcoI. This fragment was cloned in pCTIE vector (Novagen, Madison, WI) cut previously with NcoI-EcoRV. This NcoI site within pCTIE contains the ATG corresponding to the translation start site. The IRES-p40 fusion was excised with EcoRI-Sall and cloned into pBabe Neo-p35 cut previously with EcoRI-Sall. The resulting vector was named pBabe neo IL-12. Twenty μg of pBabe neo IL-12 was transfected into GP+env Am12 cells, Geneticin (Life Technologies, Inc., Rockville, MD) was added up to a concentration of 1.5 mg/ml, and resistant cells were used to generate helper-free, virus-containing supernatants. CT26 cells were transduced by exposure to undiluted supernatant. Two days later, cells were split and selected in Geneticin up to a concentration of 1.5 μg/ml. Cells transduced with pBabe neo vector without insert were used as controls.

Cell Lines. The CT26 mouse colon carcinoma cell line (17) was obtained from the M. D. Anderson Cancer Research Center (Houston, TX). The LM3 mammary tumor cell line is derived from a mammary tumor which appeared spontaneously in BALB/c mice (18). Both cell types were routinely maintained in DMEM containing 10% (vol/vol) FCS, 2 mM glutamine, 2.5 units/ml penicillin, and 2.5 μg/ml streptomycin. The amphotropic GP+env Am12 packaging cell line was maintained in DMEM containing 10% (vol/vol) FCS, 2 mM glutamine, 2.5 units/ml penicillin, and 2.5 μg/ml streptomycin. The amphotropic GP+env Am12 packaging cell line was maintained in DMEM containing 10% (vol/vol) FCS, 2 mM glutamine, 2.5 units/ml penicillin, and 2.5 μg/ml streptomycin. The amphotropic GP+env Am12 packaging cell line was maintained in DMEM containing 10% (vol/vol) FCS, 2 mM glutamine, 2.5 units/ml penicillin, and 2.5 μg/ml streptomycin.
Assessment of IL-12 Production and IgGs Levels. A capture ELISA using antimonie IL-12 (gA5 capture/5C3 detect mAbs, gifts from Dr. M. Gately, Hoffman-La Roche, Nutley, NJ) was used for quantification of IL-12 production. After overnight incubation at 4°C, plates were blocked at room temperature with BSA and washed with 0.05% Tween-PBS. Cell-conditioned media and IL-12 control samples were added on the plates and incubated overnight at 4°C. After washes with Tween-PBS, plates were incubated over night at 4°C with biotinylated anti-IL-12 antibody. IL-12 levels were calculated using standard curves of murine IL-12.

Total levels of circulating and anti-CT26-specific IgG2a and IgG1 were performed as described previously (20).

Reverse Transcription-PCR Analysis and T Cell Proliferation Assay. For the detection of AhI mRNA, total RNA from the different cell lines was obtained with TRRzol (Life Technologies, Inc.) and reverse transcribed. The 5’ and 3’ primers for detecting AhI transcript were synthesized as described (21). As a control, peptides corresponding to two different genes were used: one corresponding to the CPD1 cdNA (GenBank accession no. V89345) and the other corresponding to the Fru-1,6-biphosphatase cdNA (EC 3.1.3.11). To establish the specific response against AhI peptide, BALB/c mice were injected with 3 × 10^6 CT26-neo or CT26-IL12 cells and challenged with parental cells 2 weeks later. After 1 week, spleen cells were obtained from both types of mice and naive mice were grown in RPMI containing 5% heat-inactivated FBS, L-glutamine, 2-ME, and antibiotics. Cells were incubated for 4 days with the different peptides pulsed with 1 μCi of [3H] thymidine for 7 h and counted (22).

Immunohistochemical Studies. The sites of injection of tumor cells were removed, fixed, and embedded in paraffin. Immunohistochemical studies were performed on 5-μm sections. Sections were preheated in a microwave oven for 12 min in the presence of citrate buffer. Sections were washed, passed through graded alcohols, and incubated with H2O2 in methanol to eliminate endogenous peroxidase. Then, they were incubated with rat antimouse antimacrophages antibody (Ly-6G, PharMingen, San Diego, CA) overnight at 4°C (final concentration, 2.5 μg/ml), and then incubated with biotin-labeled goat antirat antisera (Jackson Immunoresearch Lab, West Grove, PA). After washing, sections were incubated with ABC Vectastain ABC Elite reagent (Vector Laboratories, Burlingame, CA). Staining was developed with diaminobenzidine and sections were counterstained with hematoxylin. For the frozen sections, the site of tumor cell injection was included in OCT (Miles, Elkhart, IN). Seven-μm sections were incubated overnight with either rat antimouse antiamacrophages antibody (F4/80; Serotec, Oxford, United Kingdom; 1/50 final dilution) or rat antimouse anti-CD34 antibody (MEC 14.7; Serotec; 1/20 final dilution). Sections were developed as described previously for paraffin embedded tissues.

Statistical Analysis. The significance of differences was determined by using the Student’s t test, one-way ANOVA, and Tukey-Kramer Multiple Comparison Tests.

RESULTS

In Vivo Studies. None of the BALB/c mice that received injections of 2 × 10^5 to 1 × 10^6 CT26-IL12 cells developed tumors (Table 1). Mice that received injections of 3 or 5 × 10^6 CT26-IL12 cells showed palpable nodules between days 7 and 15 after injection, after which the nodules ceased growing and regressed (Fig. 1, A and B). Surprisingly, these nodules containing a central necrotic area were composed of blood vessels, active fibroblasts, and an immune infiltrate composed mainly of neutrophils (Fig. 2, B–D). Macrophages and mast cells were scarcely seen (not shown). Blood vessels and the presence of macrophages was confirmed by using specific antibodies (not shown). By contrast, all of the mice injected with CT26-neo cells showed large tumor masses (Fig. 2). Only a few neutrophils and vessels were seen (Fig. 2A). By using the technique of everted skin (23), we confirmed that injection of CT26-IL12 cells induced neovascularization compared with CT26-neo cells (2.41 ± 0.6 versus 1.74 ± 0.43 vessels/mm² skin; P < 0.001; Fig. 2, E and F). In addition, all of the athymic nude mice injected with 3 × 10^6 CT26-neo cells developed tumors. But only 50% of the nude mice injected with CT26-IL12 cells developed tumors, with great delay, suggesting the involvement of both T cells and non-T cells in the initial rejection of CT26-IL12 cells (Fig. 1C). Histological analysis of the site of injection of CT26-IL12 cells and of the tumors formed after injection with CT26-IL12 cells showed no evidence of neovessel formation (not shown).

Antitumor Immune Memory. All of the mice that received injections of CT26-IL12 cells were able to reject a contralateral challenge with parental cells even when challenged with 3 × 10^6 cells (Table 1). Mice were also able to reject a second challenge with parental cells performed 8 weeks after the first injection (Table 1).

To evaluate whether the immune memory might induce cross-protection against non-organ-related tumor cells, mice that rejected CT26-IL12 cells were challenged with three different nonimmunogenic, syngeneic, non-organ-related tumor cell types. None of the mice that rejected 2 or 5 × 10^6 CT26-IL12 cells was able to reject a challenge with 3 × 10^5 LM3 mammary tumor cells (Table 1).

None of the BALB/c mice that received injections of 2 × 10^5 CT26-IL12 cells were able to reject a first injection of 3 × 10^5 CT26-IL12 cells and a double challenge with parental CT26 cells rejected a challenge with LM3 cells, but only 50% rejected a second injection of 3 × 10^5 CT26-IL12 cells. However, 60–90% of mice that rejected 3 or 5 × 10^6 CT26-IL12 cell growth rejected 3 × 10^5 LM3 cells injection when challenged 3–9 weeks later (Table 1). In addition, 75% of mice that rejected a first injection of 3 × 10^5 CT26-IL12 cells and a double challenge with parental CT26 cells rejected a challenge with LM3 cells.
cells performed 4 months later (Table 1). Moreover, 50% (12 of 24) of mice vaccinated with 3 $\times$ 10^6 CT26-IL12 cells that rejected a challenge with parental cells 2 weeks later were able to reject a challenge with tumorigenic inocula of MCE fibrosarcoma cells (Table 1). Moreover, 10 of 12 of these mice rejected a second challenge with MCE cells performed 4 weeks later. Under the same conditions, these mice were unable to reject a challenge with LB T lymphoma cells. All of the control mice injected with LM3, MCE, and LB cells developed tumors and were not protected against a challenge with parental cells (not shown).

Levels of Circulating and CT26-specific IgG1 and IgG2a Subclasses. Vaccination of mice with CT26-IL12 cells induced in all cases a strong increase in IgG2a circulating levels, leading to a Th1 systemic status regardless of the amount of injected cells (Table 2). Vaccination with 0.5 and 1.0 $\times$ 10^6 CT26-IL12 cells led to moderate increases both in IgG1 and IgG2a anti-CT26-specific levels. On the contrary, vaccination with 3 and 5 $\times$ 10^6 CT26-IL12 cells induced a 5- to 10-fold increase in anti-CT26-specific IgG2a levels and decreased levels or no change in anti-CT26-specific IgG1, showing a clear shift toward a Th1-dominated response (Table 2). None of the serum samples obtained from these mice recognized LM3 cells regardless of whether the samples corresponded to mice that did or did not reject the LM3 challenge (not shown).

Characterization of the Anti-LM3 Immune Response. Depletion of CD8+ T cells abrogated the anti-LM3 immune memory, whereas depletion of CD4+ T cells had no effect (Table 3). Interestingly, arrested CT26-IL12 tumors resumed growth 1 week after the depletion of CD8+ cells, suggesting the existence of remaining viable tumor cells (Table 3). A significant increase in anti-CT26 CTL activity was observed with spleen cells obtained from mice vaccinated with 3 $\times$ 10^6 CT26-IL12 cells. This cytolitic activity was able to lyse both parental and LM3 cells (Table 4). The CTL activity against LM3 cells was even more evident when lymphocytes obtained from the draining lymph nodes were used (Table 4). No difference was observed when sera from the immunized animals was used as an adjuvant added in the assay (not shown).

The previously described immunodominant MHC class I-restricted AH1 peptide (21) does not seem to be the target of the CTLs derived from mice vaccinated with CT26-IL12 cells. Reverse transcription-PCR studies demonstrated that CT26 and LM3 cells as well as B16 murine melanoma cells expressed the MuLV env antigen mRNA from which AH1 is derived (not shown). A 6- to 7-fold increase in the stimulation index of spleen cells obtained from mice vaccinated with CT26-neo cells was observed when stimulated to proliferate in response to AH1 peptides (Fig. 3). Under similar conditions, no increase in the stimulation index was observed with spleen cells obtained from mice vaccinated with CT26-IL12 cells (Fig. 3).

In Vivo Adoptive Transfer Experiments. To assess the in vivo effect of immune cells, we adoptively transferred spleen cells and lymphocytes, draining the site of tumor cell injection into mice bearing 1-day-old LM3 tumors. In an initial experiment, spleen cells obtained from mice vaccinated with CT26-IL12 cells (Sp-IL12) were able to delay LM3 tumor growth, compared with spleen cells obtained from control mice vaccinated with CT26-neo cells (Sp-neo; not shown). In a second experiment, Sp-IL12 cells re-stimulated in vitro with mitomycin C-treated CT26-cells signifi-
cantly delayed LM3 growth in 5 of 12 mice, whereas Sp-neo cells had no effect (Fig. 4, A and B). Moreover, LM3 primary tumors ceased growing, and lung metastases did not develop in two of the mice after adoptive transfer of Sp-IL12 cells (Fig. 4, A and B, and Table 5). Adoptive transfer of Ly-neo cells had no effect on LM3 primary tumor growth but partially inhibited the development of large metastatic nodules. On the contrary, a complete suppression of LM3 primary tumor growth and the absence of lung metastases

Table 2. Levels of total serum and CT26-specific IgG2a and IgG1 subclasses, after mice received injections of tumor cells expressing or not expressing IL-12.

<table>
<thead>
<tr>
<th>No. of cells</th>
<th>Serum</th>
<th>Total IgG2a</th>
<th>Total IgG1</th>
<th>IgG2a/IgG1</th>
<th>Anti-CT26 IgG2a</th>
<th>Anti-CT26 IgG1</th>
<th>IgG2a: IgG1</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 × 10⁵</td>
<td>Normal</td>
<td>102 ± 1.1</td>
<td>6.7 ± 1.8</td>
<td>15.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CT26-Neo</td>
<td>100 ± 1.3</td>
<td>7.1 ± 1.3</td>
<td>14.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CT26-IL12</td>
<td>306 ± 8.9</td>
<td>4.5 ± 1.0</td>
<td>68.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5 × 10⁵</td>
<td>Normal</td>
<td>34 ± 1.0</td>
<td>1.8 ± 1.0</td>
<td>18.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CT26-Neo</td>
<td>46 ± 2.0</td>
<td>4.8 ± 2.1</td>
<td>9.6</td>
<td>21 ± 3.8</td>
<td>34 ± 3.1</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>CT26-IL12</td>
<td>166 ± 10</td>
<td>2.1 ± 2.1</td>
<td>75.5</td>
<td>63 ± 1.9</td>
<td>64 ± 5.6</td>
<td>0.98</td>
</tr>
<tr>
<td>1 × 10⁶</td>
<td>Normal</td>
<td>39 ± 1.4</td>
<td>6.5 ± 0.1</td>
<td>6.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CT26-Neo</td>
<td>131 ± 11</td>
<td>17.2 ± 1.1</td>
<td>7.6</td>
<td>20 ± 4.1</td>
<td>21 ± 8.1</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>CT26-IL12</td>
<td>409 ± 11</td>
<td>11.2 ± 2.8</td>
<td>36.5</td>
<td>83 ± 1.8</td>
<td>83 ± 1.8</td>
<td>2.59</td>
</tr>
<tr>
<td>3 × 10⁶</td>
<td>Normal</td>
<td>34.1 ± 1.1</td>
<td>1.8 ± 0.9</td>
<td>18.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CT26-Neo</td>
<td>46 ± 1.8</td>
<td>3.0 ± 1.5</td>
<td>15.4</td>
<td>18 ± 2.9</td>
<td>41 ± 2.3</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>CT26-IL12</td>
<td>142 ± 18</td>
<td>3.6 ± 2.1</td>
<td>39.5</td>
<td>200 ± 5.1</td>
<td>3 ± 3.7</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>39 ± 1.4</td>
<td>6.5 ± 0.1</td>
<td>6.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 × 10⁶</td>
<td>CT26-Neo</td>
<td>146 ± 45</td>
<td>11.9 ± 4.3</td>
<td>12.2</td>
<td>25 ± 3.1</td>
<td>5 ± 6.9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>CT26-IL12</td>
<td>475 ± 1.4</td>
<td>10.4 ± 1.7</td>
<td>45.6</td>
<td>212 ± 3.2</td>
<td>3 ± 5.2</td>
<td>7.07</td>
</tr>
</tbody>
</table>

* Data are expressed as μg/ml (mean ± SD).
* Ratio in arbitrary units. Each value was obtained as an average of 8–10 mice.
* Data are expressed as ng of bound Ig/10⁶ cells (mean ± SD).
Balb/c mice received injections of $3 \times 10^6$ CT26-IL12 cells. Twelve days later, depletion of T lymphocyte subsets was started by injecting 0.5 mg in 0.3 ml of PBS of mAb YTS 191.1 for CD4$^+$ cells and YTS 169.4 for CD8$^+$ cells (50). Two days later, mice were challenged with $3 \times 10^5$ LM3 cells. The same amount of antibody was injected each 7 days for 2 months. Antibodies were prepared as described (19).

was observed in mice adoptively transferred with Ly-IL12 cells (Fig. 4, C and D, and Table 5).

### DISCUSSION

The present study provides for the first time evidence that mice vaccinated with CT26 colon cancer cells producing IL-12 developed an immune-mediated, antitumor cross-protection that enabled them to reject not only a challenge with parental cells but also with syngeneic mammary tumor cells and MCE fibrosarcoma cells. No antitumor cross-tolerance developed against T lymphoma cells. Adoptive transfer of spleen cells and lymphocytes obtained from animals vaccinated with CT26-IL12 cells suppressed mammary tumor growth and metastases development in tumor-bearing mice. The antitumor activity of IL-12 after either the injection of the recombinant protein or the gene transfer has been reported in different murine models (24). But, Phase II clinical trials aimed at treating cancer patients with the recombinant protein were halted because of the high toxicity of IL-12 (25). Studies in rodents and squirrel monkeys demonstrated splenomegaly, pulmonary edema, myelosuppression, and leukopenia as major toxic effects (26, 27). The use of a predose for desensitizing the host to the toxic effects of IL-12 failed to improve its antitumor efficacy (28). But local production of IL-12 after gene expression by tumor cells or fibroblasts inhibited tumor growth (24) with no accompanying splenomegaly, although NK cell activity was significantly induced (29). Therefore, the assessment of IL-12 antitumor effect after gene transfer is of potential clinical interest, because local production of IL-12 appears less toxic than IL-12 protein therapy.

The present study demonstrated that mice vaccinated with high doses of CT26-IL12 cells developed an immune-mediated, long-lasting antitumor cross-protection able to reject up to three challenges with parental and non-organ-related tumor cells even 4 months after the initial injection. This CT26-IL12-mediated immune memory was strong enough to induce the development of CTL activity able to eliminate mammary tumor cells in vitro and in vivo. Previous studies have shown that CTLs obtained from mice vaccinated with C26 cells producing IL-12 were able to kill other colon cancer cell lines derived from C26 cells (30). However, no previous evidence appeared in the literature regarding the capacity of immunized animals to generate CTL activity able to recognize and eliminate non-organ-related tumor cells. Indeed, mice immune to SCK mammary carcinoma cells expressing the costimulatory molecule B7 and after administration of IL-12 were unable to reject syngeneic S1 sarcoma cells (31). Mice immune to MB49 bladder cancer or to MCA207 sarcoma after expression of IL-12 were unable to reject syngeneic MC38 sarcoma (32) and B16 melanoma cells (15), respectively.

Only mice vaccinated with 3 or $5 \times 10^5$ CT26-IL12 cells showed a strong shift toward a CT26-specific Th1 response and were immunized not only against parental cells but also against LM3 and MCE malignant cells. Previous studies have shown that systemic administration of IL-12 or IL-12 gene transfer can induce a Th1 differentiation pattern, which seems to be largely dependent on the induction of IFN$\gamma$ by NK cells (33). IFN$\gamma$ production was highly dependent on continued administration of IL-12, and serum IFN$\gamma$ levels decreased markedly 48 h after stopping IL-12 (34). In addition, IL-12 was shown to induce the production of the Th2 cytokine IL-10 as a control mechanism to stop an ongoing Th1 response (35, 36) and to exacerbate an
established Th2 response (9, 36), suggesting that IL-12 may stimulate both a Th1 and a Th2 response. The present data suggests that vaccination with a high number of CT26 cells producing sustained amounts of IL-12 might support the establishment of a long-term Th1 response and prevent the appearance of a Th2 response. The fact that CD8+ T cell depletion led to regrowth of CT26-IL12 dormant tumor cells indicates that the continuous production of IL-12 by viable CT26 cells might support the recruitment of immune cells, the strong bias toward a long term-Th1 response, and the induction of antitumor cross protection.

The present data also supports recent findings demonstrating that the target of CTLs obtained from animals carrying C26 tumors and cured after vaccination with IL-12-transduced cells is not the AH1 immunodominant peptide (37). These studies confirmed previous evidence that IL-12 may modulate the immunodominance of T cell epitopes (37, 38). Unlike IL-10, whose expression in engineered CT26 cells up-regulated MHC class I expression (19), IL-12 production by CT26 cells down-regulated MHC Class I (not shown), possibly favoring an initial non-T cell antitumor response.

A major role for NK cells and macrophages in the primary antitumor response after IL-12 expression is supported by previous evidence from the literature (39 – 41) and our own data with nude mice. Tumor antigens shared by the different cell types might be the target of CD8+ T cells that appeared to be responsible for the cross-protection because depletion of CD4+ cells before the challenge had no effect. In a previous study, CD4+ T cells were shown to play a key role in the rejection of parental cells after vaccination of mice with B16 cells producing GM-CSF (42). CTLs might play an important role in the CT26 model because B16 cells constitu-

Table 5 Lung metastases development after adoptive transfer of spleen cells and lymphocytes to LM3 tumor-bearing mice

Mice were sacrificed when LM3 tumors from the different groups reached an average size of 1.5 cm³. The experiment was ended when the remaining Sp-neo+LM3 mice also reached an average size of 1.5 cm³ (day 39). Lung metastases were histologically analyzed at autopsy.

<table>
<thead>
<tr>
<th>Injected cells</th>
<th>27</th>
<th>30</th>
<th>39</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM3</td>
<td>2/2 (50% &gt; 0.1 cm³)</td>
<td>3/3 (60% &gt; 0.1 cm³)</td>
<td></td>
</tr>
<tr>
<td>Sp-neo+LM3</td>
<td>3/4 (70% &gt; 0.1 cm³)</td>
<td>4/4 (50% &gt; 0.1 cm³)</td>
<td></td>
</tr>
<tr>
<td>Sp-IL12+LM3</td>
<td>4/4 (22% &gt; 0.1 cm³)</td>
<td>3/3 (60% &gt; 0.1 cm³)</td>
<td></td>
</tr>
<tr>
<td>Ly-neo+LM3</td>
<td>3/3 (0% &gt; 0.1 cm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ly-IL12+LM3</td>
<td>0/3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mice bearing metastases/total mice. Between parentheses, percentage of metastatic nodules larger than 0.1 cm². The number of metastatic nodules varied from 0 to 10, depending on the treatment and the time of sacrifice. For more details see Fig. 2.
tively express Fasl, which may generate an immunoprivileged zone (42). Alternatively, the main role of GM-CSF is the differentiation of bone marrow progenitors to antigen presenting cells (APC), which may act by cross priming CD8+ T cell through the activation of CD4+ T cells (43); whereas IL-12 can act both by up-regulating MHC expression in APC via IFNγ expression by infiltrating NK or T cells, or act directly by stimulating Th1 differentiation and CTL activity (4, 5).

The anti-angiogenic property of IL-12 was well documented in different in vitro and in vivo studies (14, 44). Only recently, the severe tumor hypoxia-dependent apoptosis that appeared after IL-12 dependent inhibition of tumor cell-induced angiogenesis suggested that inhibition of angiogenesis might affect tumor growth (44). The IL-12-dependent angiogenesis inhibition seems to be mediated by IFN-γ and IP-10, which by itself was also shown to be chemotactic for monocytes and T cells (45). An IFNγ/IP-10 dependent mechanism seems to mediate the IL-12-induced infiltration of activated macrophages to different organs (46). IP-10 gene transfer of two different cell lines led to tumor cell rejection via a T-cell-dependent mechanism (47). The present data shows the development of a transient CT26-IL12-dependent pro-angiogenic process. Whether this pro-angiogenic process is an IL-12-mediated direct effect or mediated by an intermediate compound is still unknown, but it might actively contribute to the support of the initial recruitment of immune cells to the site of tumor cell inoculation. It is conceivable to assume that the overall IL-12/IFNγ/IP-10 effect as angiostatic and chemotactic factors for immune cells might occur in sequential steps. In an initial stage, an IL-12-mediated pro-angiogenic scenario can be envisioned to allow the immune cells to infiltrate the tumor mass. The IFNγ/IP-10-mediated angiostatic effect induced by IL-12 will occur as a secondary event once the immune cells have reached the tumor mass and started to produce angiostatic cytokines. The histology of the site of CT26-IL12 injection demonstrated mainly the presence of neutrophils. T cells were also suggested to be involved in this angiogenic process because of the absence of angiogenesis in studies of nude mice. Although tumor cells were not visualized in histological sections, CT26-IL12 tumor growth after depletion of CD8+ T cells demonstrated the presence of viable CT26-IL12 tumor cells in the angiogenic nodules. Sustained production of angiogenic factors like IL-8, MGSa, and other ELR-CXC chemokines might be produced either by neutrophils, by endothelial cells, or by the tumor cells themselves (48). Moreover, T lymphocytes from tumor-bearing mice with the participation of tumor cells were shown to trigger an angiogenic cascade driven by oxygen derivatives (49, 50). Therefore, the different cell types that constitute a tumor might contribute to the development of a localized, transient angiogenic process that is able to support the initial recruitment of immune cells.

The identification of tumor-associated antigens and CTL epitopes shared by many patients’ tumors led to the initiation of clinical protocols involving treatment with allogeneic tumor cell vaccines (51). Most of the tumor antigens and CTL epitopes were obtained from human melanoma samples, whereas the identification of such kinds of molecules from other malignant tissues was less frequent (51). Recently, Kayaga et al. (52) have shown that whole allogeneic tumor cell vaccines expressing GM-CSF can be used successfully in a melanoma murine model. The present studies raised the possibility of identifying tumor-associated antigens and CTL epitopes shared by different tumor types that also can be a target for the production of vaccines with multiple antigens or for adoptive transfer of CTLs.

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REFERENCES


Mice Vaccination with Interleukin 12-transduced Colon Cancer Cells Potentiates Rejection of Syngeneic Non-Organ-related Tumor Cells

Soraya Adris, Eduardo Chuluyan, Alicia Bravo, et al.

Cancer Res 2000;60:6696-6703.