Induction of Systemic and Therapeutic Antitumor Immunity Using Intratumoral Injection of Dendritic Cells Genetically Modified to Express Interleukin 12

Yasuhiko Nishioka, Motohiro Hirao, Paul D. Robbins, Michael T. Lotze, and Hideaki Tahara

Departments of Surgery (Y.N., M.H., M.T.L., H.T.) and Molecular Genetics and Biochemistry (Y.N., M.H., P.D.R., M.T.L., H.T.), School of Medicine, University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

ABSTRACT

Bone marrow-derived dendritic cells (BM-DCs) retrovirally transduced with genes encoding murine interleukin (IL)-12 stably expressed bioactive IL-12 protein at high levels. Intratumoral injection with IL-12 gene-modified BM-DCs resulted in regression of day 7 established weakly immunogenic tumors (MCA205, B16, and D122). This antitumor effect was substantially better than that of IL-12-transduced syngeneic fibroblasts or nontransduced BM-DCs. Furthermore, intratumoral injection with IL-12-transduced dendritic cells (DCs) induced specific TH1-type responses to the tumor in regional lymph nodes and spleen at levels greater than those of IL-12-transduced fibroblasts or nontransduced BM-DCs. Trafficking studies confirmed that intratumorally injected IL-12-transduced DCs, but not fibroblasts, could migrate to the draining lymph node to the same extent as nontransduced BM-DCs. This strategy designed to deliver genetically modified DCs to tumor sites is associated with systemic and therapeutic antitumor immunity and is an alternative approach to those that use delivery of DCs loaded with tumor antigen. These results support the clinical application of IL-12 gene-modified DCs in patients with cancer.

INTRODUCTION

Critical analysis of early events in the cellular immune response to tumor and viral antigen has identified the DC as the major APC eliciting an effective T-cell response (1). Appropriately activated DCs take up soluble antigen and apoptotic bodies, migrate to the paracortical T-cell-rich area of the lymph nodes, and initiate a series of interactions leading to the selection of antigen-specific T cells and the release of the DC cytokines IFN-α and IL-12. Delivering activated DCs to the site of tumor antigen that are genetically modified to express gene products that enhance their local survival and ability to elicit a response may bypass the requirement for selective recruitment and local activation, which are disordered within the tumor microenvironment. We investigated the antitumor effects of intratumoral injection of murine BM-DCs retrovirally transduced with the gene encoding IL-12. BM-DCs could be efficiently transduced with retroviral vectors expressing hCD80, EGFP, or IL-12.

IL-12 is a heterodimeric cytokine produced by DCs, macrophages, polymophonuclear leukocytes, and keratinocytes (2). IL-12 enhances natural killer cell and CTL activities, plays a key role in the induction of TH1-type immune responses including IFN-γ production (2) and has IFN-γ/IFN-inducible protein 10-dependent antiangiogenic effects (3, 4). We and others have reported potent antitumor effects of IL-12 delivery using IL-12 gene-modified tumor cells or systemic administration of IL-12 protein (5–8). Direct injection of IL-12-transduced fibroblasts also effectively eliminated established tumors with a concomitant induction of effective systemic immunity (9). Based on these results, initial clinical trials of IL-12 gene therapy have been completed using autologous fibroblasts in the context of a Phase I study (10). Partial response was observed in patients with melanoma, breast cancer, and head and neck tumors, persisting for up to 2 years. To further enhance the immune response, we evaluated BM-DCs transduced retrovirally with IL-12 genes in murine tumor models. DCs are capable of producing IL-12 after the ligation of CD40 and MHC class II molecules, presumably only after interaction with T cells (11, 12). IL-12 delivery in conjunction with DCs also enhances CTL response in vitro (13). Considering the antitumor effects mediated by local expression of IL-12 and the capability of DCs to induce an effective systemic immune response, DCs appear to be an excellent candidate to be used as an alternative to fibroblasts. Constitutive production of IL-12 by DCs, coupled with their ability to take up and process tumor antigens, migrate to lymph nodes, and induce an effective immune response (4), would be superior to that observed with nonprofessional APCs such as fibroblasts.

DCs are specialized APCs, which exist in virtually every tissue, capture antigens in situ, and migrate to lymphoid organs to activate naïve T cells (1, 14). DCs pulsed with synthetic tumor peptides induce an effective antitumor immune response in vitro and after adoptive transfer in mice (15–17). Several human tumor-associated antigens have also now been cloned from melanoma and other tumors. Patients are now being treated with a strategy using DCs pulsed with synthetic tumor peptides. Furthermore, DCs loaded with acid-eluted tumor peptides (18), tumor lysate (19, 20), or RNA (20, 21) are also effective in inducing immunity against tumors for which tumor-specific peptides have yet to be identified. However, these approaches will not be applied to a majority of patients due to technical difficulties and cumbersome nature of the preparation of these materials from human solid tumors.

An alternative approach is to deliver DCs directly to the site of the tumor. We have recently shown that DCs injected intratumorally are capable of capturing tumor antigens in situ at the tumor site, migrating to regional lymph nodes, and inducing a subsequent systemic immune response against the tumor, even without antigen loading. Whereas this immune response was not sufficient to cause eradication of preexisting vigorous tumors, we hypothesized that additional measures, including genetic modification of the DCs, could enhance this systemic antitumor response to a level sufficient for effective treatment. We demonstrate here that intratumoral injection with IL-12 gene-modified but not nontransduced or marker gene-transduced BM-DCs is capable of significantly suppressing the growth of established tumors and inducing a strong antitumor T-cell response. Furthermore, IL-12-transduced DCs were demonstrably more effective in these functions than IL-12-transduced syngeneic fibroblasts.

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2 To whom requests for reprints should be addressed, at Department of Surgery, 300 Kaufmann Building, 3471 Fifth Avenue, Pittsburgh, PA 15213. Phone: (412) 383-9759; FAX: (412) 383-9760; E-mail: taharah@msx.upmc.edu.

3 The abbreviations used are: DC, dendritic cell; IL, interleukin; BM, bone marrow; BM-DCs, BM-derived DC; APC, antigen-presenting cell; EGFP, enhanced green fluorescent protein; CM, complete medium; rm, recombinant mouse; GM-CSF, granulocyte macrophage colony-stimulating factor; hCD80, human CD80; rIL, recombinant human IL; mIL, mouse IL; i.d., intradermally; ME, mercaptoethanol; ConA, concanavalin A; PE, phycoerythrin; TH, T helper cell.


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survived selection with G418 after transduction into NIH3T3 cells. The titer of retroviral supernatants was calculated from the colonies that

Materials and methods

Retroviral Vectors. The construction of retroviruses DFG-mIL-12, TFG-mIL-12, and DFG-hCD80-neo has been described in previous studies (7, 22). MFG-EGFP and MFG-Zeo were created by subcloning the respective fragments obtained from pEGFP-N1 (Clontech, Palo Alto, CA; Ref. 23) and pcDNA3.1/Zeo(-) (Invitrogen, Carlsbad, CA). Retroviral supernatant was generated by transfecting these proviral constructs into BOSC23 or BING packaging cell lines (7). CRE and CRIP cells producing the DFG-hCD80-neo retroviruses used in Table 1 were created by the infection of these packaging cells with BING- or BOSC23-produced retroviruses, respectively, with subsequent selection with G418 (Geneticin; Life Technologies, Inc., Grand Island, NY). The titer of retroviral supernatants was calculated from the colonies that survived selection with G418 after transduction into NIH3T3 cells.

Cell Lines and Mouse Strains. MCA205 methylcholanthrene-induced fibrosarcoma was generously provided by S. A. Rosenberg (National Cancer Institute, Bethesda, MD). The B16-F10 murine melanoma cell line was kindly provided by E. Goretlik (University of Pittsburgh, Pittsburgh, PA). The D122 highly metastatic variant of 3LL tumor cells was kindly provided by L. Eisenbach (Weizmann Institute of Science, Rehovot, Israel). YAC-1 was a generous gift of W. Chambers (University of Pittsburgh). These cell lines were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 μg/ml streptomycin, 100 IU/ml penicillin, and 5 × 10^-5 M 2-ME (all from Life Technologies, Inc.), referred to henceforth as CM.

Female 6–8-week-old C57BL/6 (B6) mice were purchased from Taconic Farms (Germantown, NY) and used for all experiments at the age of 8–10 weeks. Primary culture of syngeneic fibroblasts was obtained from the lungs of B6 mice. Small pieces of lung were minced and stirred in a triple enzyme solution of collagenase IV, hyaluronidase V, and DNase IV (Sigma, St. Louis, MO) for 3 h at room temperature. After rinsing twice with HBSS, cell suspensions were cultured in CM to obtain a primary culture of fibroblasts. IL-12-transduced fibroblasts were generated by infection with the supernatant of CRIP-TFG-mIL-12-neo, followed by selection with G418.

Culture of BM-DCs and Transduction with Retrovirus. BM-DC culture was obtained using methods described previously (15, 18, 24). Briefly, murine BM cells were harvested from the femur and tibia of sacrificed mice. Contaminating erythrocytes were lysed with 0.83 M NH4Cl buffer, and lymphocytes were depleted with a mixture of antibodies (RA3–3A1/6.1, anti-B220; 2.43, anti-Lyt 2; GK1.5, anti-L3T4; all from American Type Culture Collection, Manassas, VA) and rabbit complement (Accurate Chemical and Scientific Corp., Westbury, NY) on day 0. These cells were cultured overnight in CM to remove the adherent macrophages, and then nonadherent cells were placed in fresh CM containing rmGM-CSF (1000 units/ml) and rmIL-4 (1000 units/ml; DC media) on day 1. Cells were generally harvested on day 6. BM-DCs were defined by morphology, phenotype, and strong mixed lymphocyte reaction-stimulating activity. Phenotypic analysis by flow cytometry revealed expression of CD11b, CD11c, CD80, and CD86 as well as MHC class I and class II in the majority of the cultured cells (60–95%). For retroviral transduction, 1 × 10^6 BM cells cultured in DC media for 24 h were aliquoted to 14-ml round-bottomed tubes and suspended in 1 ml of the retroviral supernatant with 8 μg/ml polybrene, 1000 units/ml rmGM-CSF, and 1000 units/ml rmIL-4. These cells were centrifuged at 2500 × g at 30°C–32°C for 2 h (25, 26). After centrifugation, cells were cultured in DC media. The transduction process was repeated on days 3 and 4. Retroviral supernatant from the ecotropic producer cells, BOSC23 and CRE, transduced murine BM-DCs more effectively when compared with amphotropic viruses at comparable titers (data not shown). The retroviral supernatant from BOSC23 cells was used because they produced the highest titered virus (2–8 × 10^6 cfu/ml). To examine the transduction efficiency of murine BM-DCs, we generated retroviral vectors with inserted hCD80 (B7-1) or EGFP genes as transduction markers and determined the efficiency of transduction by flow cytometry. Retrovirally modified DCs at high transduction efficiency (22–75%) could express the transgenes for at least 12 days after the last transduction (on day 4) in culture (data not shown). Transduction efficiency was well correlated with the titer of retroviral supernatants used. Two-color immunofluorescence staining showed that significant numbers of marker (hCD80)-positive cells also expressed high levels of murine CD80 as well as CD86, MHC class II , and DEC-205 (data not shown).

Flow Cytometry. For phenotypic analysis of BM-DCs, PE- or FITC-conjugated monoclonal antibodies against murine cell surface molecules [CD11b, CD11c, CD80, CD86, Gr-1, H-2Kb, I-Ab, and appropriate isotype controls (all from Pharmingen, San Diego, CA)] were used, and cytometric analysis was performed using a FACScan (Becton Dickinson, San Jose, CA). DEC-205 was detected by staining with NLDC-145 antibody (Serotec Ltd., Oxford, England).

| Table 1 Phenotypic characteristics of the IL-12-transduced DCs | Mean fluorescence intensity |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| DCs             | MHC class I     | MHC class II    | CD11c           | CD54            | CD80            | CD86            | DEC205          |
| Nontransduced   | 28              | 84              | 21              | 132             | 114             | 122             | ND              |
| MFG-Zeo         | 30              | 119             | 19              | 178             | 166             | 156             | ND              |
| DFG-IL-12       | 188^           | 144             | 150^           |                  |                 |                 |                 |

a Nontransduced and transduced DCs were harvested 6 days after the initiation of the culture and stained with the antibodies listed here. Background intensities were 4–6.

b ND, not done.

P < 0.05 compared with those of nontransduced or MFG-Zeo transduced DCs.
Oxford, United Kingdom). The transduction marker hCD80 was stained with FITC-conjugated anti-hCD80 antibody (PharMingen), which does not cross-react with mouse CD80.

Animal Experiments. Mice (four or five animals/group) were generally injected i.d. in the right flank with 1 × 10⁵ MCA205, B16, and D122 cells on day 0. On day 7, when tumor size reached approximately 10–20 mm², tumors were injected with saline (0.1 ml), 2 × 10⁵ IL-12-transduced syngeneic fibroblasts, or 2 × 10⁶ nontransduced or IL-12-transduced BM-DCs obtained in the manner described above with cytokine stimulation for 6 days. IL-12-transduced fibroblasts and BM-DCs were labeled with red fluorescent cell linker (PKH-26; Sigma) as described previously (27, 28) immediately before injection. In brief, IL-12-transduced fibroblasts and BM-DCs were incubated with PKH-26 at a concentration of 2 × 10⁻⁶ M at room temperature for 5 min, rinsed extensively with HBSS, examined for viability and number using the trypan blue exclusion method, and used for injection. Viabilities of DCs and fibroblasts were better than 95% and 77%, respectively, after the labeling. The mice were sacrificed 24 h after the injection, and the draining lymph nodes were harvested, fixed in Zamboni’s fixiture [2% paraformaldehyde, 10% picric acid, 0.1 M phosphate buffer (pH 7.2)] for 12 h at 4°C, embedded in OCT compound, and frozen. Serial 6-μm sections were made from these samples using a cryostat and examined with a fluorescent microscope (Olympus BH2). Evaluation of the results was performed in a blinded fashion.

Statistical Analysis. Statistical analysis was performed using the unpaired two-tailed Student’s t test. Pearson’s linear regression was applied to examine the correlation. Differences were considered significant when P is less than 0.05.

RESULTS

Murine BM-DCs Can Be Genetically Engineered to Produce High Levels of Biologically Active IL-12. Retroviral delivery of genes into human DCs can be achieved by transduction of human CD34⁺ progenitor cells, with subsequent differentiation into DCs induced by defined cytokines (29, 30). In this study, we examined the genetic modification of murine BM-DCs with retrovirus. Transduction with an EGFP retroviral vector revealed that murine BM-DCs could be retrovirally transduced at high efficiency (Fig. 1). Furthermore, BM-DCs could also be transduced with a retroviral vector expressing both IL-12 genes (DFG-mIL-12). After completion of transduction procedures performed on days 2, 3, and 4, the concentration of mIL-12 p70 heterodimer released into the culture media was measured in an ELISA. As shown in Fig. 2A, accumulation of heterodimeric IL-12 (p70) was observed in cultures of DFG-mIL-12-transduced BM-DCs but not in cultures of nontransduced or marker gene-transduced cells. On day 6, DCs were harvested, washed twice,
IL-12-transduced DENDRITIC CELLS

Fig. 2. IL-12 production from IL-12-transduced DCs ranged from 0.80 to 0.05. We next compared the antitumor effects of IL-12-transduced DCs with those of IL-12-transduced fibroblasts (Fig. 5). As we reported previously (9), IL-12-transduced fibroblasts suppressed the growth of MCA205, whereas a single injection did not lead to rejection. However, the IL-12-transduced DCs could suppress tumor growth more efficiently when compared with IL-12-transduced fibroblasts that expressed IL-12 at a similar but slightly higher level (IL-12 production of gene-modified DCs and fibroblasts was 13 and 22 ng/10^6 cells/48 h, respectively). We also examined the treatment with multiple intratumoral injections of IL-12-transduced DCs on day 7 and day 14 (Fig. 6). Repeated injection of IL-12-transduced DCs resulted in a more significant and prolonged regression (over 60 days) of the tumor when compared with experiments in which a single injection was performed.

Tumor-specific Immune Response Can Be Identified after Treatment with Intratumoral Injection of IL-12-transduced BM-DCs. As mentioned above, the local antitumor effects of IL-12-transduced DCs were substantially greater than those observed with IL-12-transduced fibroblasts. We next asked whether intratumoral injection of IL-12-transduced DCs could induce significant systemic immune responses specific for the tumor at a greater extent than that of nontransduced DCs or IL-12-transduced fibroblasts. To address this question, s.c. lymph nodes in the ipsilateral abdominal wall of the inoculated tumor (draining lymph nodes) as well as the spleen were harvested from tumor-bearing mice 7 days after injection with DCs (14 days after tumor inoculation). These lymphoid cells were cocultured with irradiated tumor cells (MCA205) in vitro, and IFN-γ and IL-4 production in the culture supernatant was examined. As shown in our previous study,^4 injections with nontransduced DCs enhanced tumor-specific IFN-γ production by lymphoid cells harvested from draining lymph nodes and spleen when compared with IL-12-transduced fibroblasts (Table 2). Interestingly, intratumoral injection of IL-12-transduced DCs resulted in greater enhancement of IFN-γ production in response to tumor restimulation by these lymphoid cells. Interestingly, DC injection also enhanced IL-4 production to a lesser extent. IFN-γ was specifically released after MCA205 stimulation, but not with B16 or MCA207 tumors (data not shown). These results suggest that intratumorally injected IL-12-transduced DCs could traffic to the draining lymph node and efficiently stimulate lymphocytes in situ to produce IFN-γ as nontransduced DCs, but at a greater extent. To confirm this notion, IL-12-transduced DCs and fibroblasts were labeled with fluorescence dye (PKH-26) and injected intratumorally, and the draining lymph node was examined 24 h after injection (Fig. 7). Although no cells were found to be positive for fluorescence in the lymph node from animals injected with saline (data not shown) and

and transferred to a new plate. At that time, IL-12-transduced DCs produced approximately 80 ng heterodimeric IL-12/10^6 cells/48 h (Fig. 2B). IL-12 production from IL-12-transduced DCs ranged from 8–80 ng/10^6 cells/48 h and was related to the titer of the retroviral supernatant used in each experiment (data not shown). IL-12 protein produced by the gene-modified DCs was confirmed to be biologically active and capable of stimulating IFN-γ production from ConA-treated splenocytes (data not shown). To examine the effect of IL-12 transduction on the DC phenotype, various cell surface molecules were examined by flow cytometry. IL-12-transduced DCs did not differ from nontransduced or Zeo-transduced DCs, except that they expressed increased levels of MHC class I and II molecules (Table 1).

Intratumoral Injection with IL-12 Gene-modified BM-DCs Mediates Significant Growth Suppression of Various Established Tumors. To examine the antitumor effect of intratumoral injection with DCs, 10^6 nontransduced or IL-12-transduced BM-DCs were injected into day 7 established tumors (MCA205, B16, and D122; tumor diameter, 3–5 mm). As shown in Fig. 3, IL-12-transduced DCs significantly suppressed the growth of these tumors, resulting in eventual rejection in two of five tumors. Nonmodified or Zeo-transduced DCs had no statistically significant antitumor effects. In this experiment, the IL-12 production of genetically modified DCs was 29 ng/10^6 cells/48 h. In total, treatment with IL-12-transduced DCs resulted in the rejection of established MCA205 tumors in 5 of 14 mice (36%). These tumor-free mice rejected a subsequent rechallenge with twice as many MCA205 cells, suggesting acquisition of immunological memory for the injected tumor. These antitumor effects of IL-12-transduced DCs were correlated with IL-12 production (Fig. 4; r = −0.80; P < 0.05). We next compared the antitumor effects of IL-12-transduced DCs with those of IL-12-transduced fibroblasts (Fig. 5). As we reported previously (9), IL-12-transduced fibroblasts suppressed the growth of MCA205, whereas a single injection did not lead to rejection. However, the IL-12-transduced DCs could suppress tumor growth more efficiently when compared with IL-12-transduced fibroblasts that expressed IL-12 at a similar but slightly higher level (IL-12 production of gene-modified DCs and fibroblasts was 13 and 22 ng/10^6 cells/48 h, respectively). We also examined the treatment with multiple intratumoral injections of IL-12-transduced DCs on day 7 and day 14 (Fig. 6). Repeated injection of IL-12-transduced DCs resulted in a more significant and prolonged regression (over 60 days) of the tumor when compared with experiments in which a single injection was performed.

Fig. 4. Correlation between IL-12 production by gene-modified DCs and the antitumor effect of intratumorally injected DCs. Mice were injected i.d. with 1 x 10^5 MCA205 cells. IL-12-transduced BM-DCs were injected into established tumors on day 7, and tumor area (in mm^2) was measured on day 28. The correlation (r = −0.80; P < 0.05) was detected between IL-12 production of DCs and tumor area (in mm^2) on day 28 using Pearson’s linear regression.

Fig. 5. DC delivery of IL-12 at the site of tumor is more effective than delivery by syngeneic fibroblasts. Mice were injected i.d. with 1 x 10^5 MCA205 cells. On day 7, HBSS (○), 10^5 Zeo-transduced (△), or IL-12-transduced BM-DCs (□) or IL-12-transduced syngeneic fibroblasts (●) were injected into established tumors. Similar results were obtained in three separate experiments. Data are presented as the mean ± SE.

Fig. 6. Repeated injection of IL-12 gene-transduced DCs reveals profound antitumor effects and prolonged regression. Mice were injected i.d. with 1 x 10^5 MCA205 cells. On days 7 and 14, HBSS (○), 10^5 IL-12-transduced BM-DCs (□), or IL-12-transduced syngeneic fibroblasts (△) were injected into established tumors. Similar results were obtained in two separate experiments. Data are presented as the mean ± SE.
IL-12–transduced syngeneic fibroblasts. Cells were cocultured with irradiated MCA205 (5000 rads) in a 24-well plate for 36 h. The supernatants were assessed with ELISA.

The number of fluorescence-positive cells in the lymph nodes from animals injected with labeled nontransduced (B; ×100) or IL-12–transduced BM-DCs (C; ×100). The number of the fluorescence-positive cells in the lymph nodes did not appear to differ significantly between the animals injected with nontransduced BM-DCs and the animals injected with IL-12–transduced DCs. Next, the CTL activity of splenocytes from treated mice was also evaluated, as shown in Fig. 8. Intratumoral injection with IL-12–transduced fibroblasts induced significant CTL activity, as compared with HBSS-injected animals (Fig. 8A). Zeo-transduced DCs were more effective in inducing CTL activity than IL-12–transduced fibroblasts, consistent with the results of our previous study using nontransduced DCs.4 IL-12–transduced DCs induced significantly higher CTL activity than that observed using other strategies. This activity appeared to be specific for the MCA205 tumor (Fig. 8B) and could be partially blocked with an anti-CD8 antibody (40–50%; data not shown). To confirm the induction of systemic and therapeutic immunity, we examined the growth of contralateral nontreated tumor, which is distant from the injected tumor with IL-12–transduced DCs.

Mice were injected i.d. with 1 × 10^5 MCA205 and B16 tumor cells in both flanks, and IL-12–transduced DCs were injected in the tumor in the right flank on day 7. Intratumoral injection with IL-12–transduced DCs significantly suppressed the growth of the uninjected tumor as well as that of the injected tumor (Fig. 9). In these experiments, no IL-12 was detected in murine sera 2 days after injection with IL-12–transduced DCs.

DISCUSSION

We have demonstrated successful genetic modification of murine BM-DCs with retroviral vectors. Previous reports have demonstrated the feasibility of retroviral gene modification of human DCs by transduction of human CD34⁺ progenitor cells with the subsequent addition of cytokine to promote differentiation and maturation (29, 30). To date, transduction of murine DCs has been far less successful. We have successfully transduced murine BM-DCs using a centrifugal method with retroviral supernatant. The use of retroviral supernatant, instead of cocultivation with retroviral producer cells (31), has advantages over other strategies. Specifically, (a) there is no direct toxicity to the DCs; (b) stable gene expression is attained; (c) minimal virus-specific CTL response is noted, unlike adenoviral vectors (32); and (d) extensive clinical experience has been attained with the use of retroviral supernatants.

BM-DCs retrovirally transduced with IL-12 expressed increased amounts of both MHC class I and class II surface molecules without other apparent changes in DC phenotype including the expression of costimulatory molecules. These phenotypic changes could be due in part to the direct effect of IL-12 or, more likely, indirectly through IFN-γ production by contaminating T cells or natural killer cells.

**Table 2. BM-DCs injected directly into tumors induce IFN-γ production by lymphoid cells harvested from the draining lymph node and spleen**

<table>
<thead>
<tr>
<th>Group</th>
<th>Lymph nodes</th>
<th>Spleen</th>
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<tbody>
<tr>
<td></td>
<td>IL-4a</td>
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<tr>
<td>BM-DC-Zeo</td>
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</tr>
<tr>
<td>BM-DC-IL-12</td>
<td>80 ± 10³</td>
<td>16,130 ± 83³</td>
</tr>
<tr>
<td>Fibroblast-IL-12</td>
<td>&lt;32</td>
<td>189 ± 12²</td>
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*Draining lymph nodes and spleen were harvested 7 days after intratumoral (MCA205) injection with HBSS, nontransduced, or Zeo- or IL-12–transduced BM-DCs or IL-12–transduced syngeneic fibroblasts. Cells were cocultured with irradiated MCA205 (5000 rads) in a 24-well plate for 36 h. The supernatants were assessed with ELISA.

Data represent the mean ± SD pg/ml/10⁶ cells/36 h of duplicated samples.

Significant (P < 0.05) versus HBSS.

Significant (P < 0.05) versus HBSS and IL-12–transduced fibroblasts.

Significant (P < 0.05) versus all other groups.
Recent report have shown that IL-12 acts directly on DCs to promote nuclear localization of nuclear factor κB and primes DCs for IL-12 production (33). These changes could explain, in part, why IL-12-transduced DCs are more effective in in vivo tumor treatment applications.

We have previously demonstrated that administration of DCs pulsed with synthetic tumor-associated peptides serves as an effective therapeutic antitumor vaccine (15, 18). However, T-cell-defined epitopes have been identified only for a limited number of human tumor types. Several approaches to overcome this problem, including pulsing DCs with acid-eluted bulk tumor peptides (18), tumor extracts and RNA (20, 21), or fusion of tumor with DC (34), have been used for DC-based vaccination strategies against tumors. Although these approaches will allow the treatment of tumors for which tumor-associated antigen is not well characterized, there are still significant problems, particularly in the preparation of clinical samples from human solid cancers. In this study, intratumoral injection with BM-DCs was used as an alternative approach that does not require the pulsing of DCs with tumor-derived materials.

We have recently demonstrated that a specific antitumor immune response can be induced with intratumoral injection of nontransduced DCs. This study suggests that intratumorally injected nontransduced immature DCs can acquire and process tumor antigen(s) in situ, migrate to lymphoid organs via blood or lymphoid vessels, and then initiate a significant tumor-specific immune response. When mature DCs were used, a smaller number of DCs were observed in the draining lymph node, and subsequent induction of tumor-specific systemic immunity was significantly impaired. This observation is consistent with the previous reports showing that DCs have antigen-capturing and processing as well as trafficking abilities only during their immature phase (35–37). Thus, using flow cytometry and/or mixed lymphocyte reaction assay, we confirmed at each experiment in the current study that the prepared DCs have an immature phenotype (data not shown).

Significant antitumor effects were observed in animals bearing various established tumors (MCA205, B16, and D122) when IL-12-transduced BM-DCs were injected, but not when nontransduced DCs were injected. Complete tumor eradication was observed in 30–50% of mice treated with a single injection of IL-12-transduced DCs, even when the weakly immunogenic MCA205 tumor was used. These results suggest that overexpression of IL-12 by DCs at the tumor site or, alternatively, in the secondary lymphoid sites could be important for the antitumor response in addition to the phenotypic changes of IL-12-transduced DCs mentioned above. Chemokine production induced by IL-12/IFN-γ elaboration at the tumor site would be responsible for these effects, at least in part, throughput enhanced recruitment of cytolytic effector cells into tumors as well as possibly antiangiogenic effects (2, 3, 38). Furthermore, the local antitumor effects of IL-12-transduced DCs were substantially better than those of fibroblasts expressing equivalent amounts of IL-12. These results may partially reflect the absence of tumor growth-promoting factors that can also be produced by fibroblasts (39, 40).

Systemic immune responses, as demonstrated by CTL activity and IFN-γ production described above, were also significantly higher and tumor specific when IL-12-transduced DCs were used. High cytolytic activity in association with a TH1-type response could indeed contribute to the profound in vivo antitumor effects that we observed. Experiments involving a subsequent rechallenge with additional tumors further confirmed systemic antitumor immunity after treatment with IL-12-transduced DCs into tumors. In contrast, responses induced by IL-12-transduced fibroblasts were far less potent. Trafficking studies of intratumorally injected IL-12 transduced DCs confirmed that the DC function of preferential recruitment to regional...
lymph nodes is not impaired by constitutive IL-12 production. In summary, we present evidence that intratumoral injection with IL-12 gene-modified DCs is feasible, mediates an effective antitumor response that is superior to that observed with IL-12-transduced fibroblasts or nontransduced DCs, and is capable of inducing substantial systemic antitumor immunity. These results strongly suggest that an evaluation of intratumoral injection of IL-12 gene-modified BM-DCs in humans should be performed. A clinical trial evaluating this approach is in preparation.

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