Intranal Immunization with Tumor Lysate-pulsed Dendritic Cells Enhances Protective Antitumor Immunity

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ABSTRACT

We developed a technique for direct inguinal lymph node injection in mice to compare various routes of immunization with tumor lysate-pulsed dendritic cell (DC) vaccines. Syngeneic, bone marrow-derived, tumor lysate-pulsed DCs administered intranodally generated more potent protective antitumor immunity than s.c. or i.v. DC immunizations. Intranodal immunization with ovalbumin peptide-pulsed DCs induced significantly greater antigen-specific T-lymphocyte expansion in the spleen than either s.c. or i.v. immunization. Furthermore, a significantly more potent, antigen-specific TH1-type response to the ovalbumin peptide was induced by intranodal, compared with s.c. or i.v., immunization. Intranodal immunization, designed to enhance DC-T cell interaction in a lymphoid environment, optimizes induction of T-lymphocyte-mediated protective antitumor immunity. These results support the use of intranodal immunization as a feasible and effective route of DC vaccine administration.

INTRODUCTION

DCs are antigen-presenting cells that are highly effective at T-lymphocyte activation (1). Immature DCs efficiently capture and process antigen (2). These antigen-loaded DCs then migrate to regional lymph nodes, where they interact with resident T lymphocytes (2, 3). In the environment of the regional lymphoid organ, under the influence of certain cytokines and through stimulation of the DC surface molecule CD40, DCs mature and effectively stimulate a T lymphocyte immune response (4–8).

A number of studies in murine tumor models have shown that DCs pulsed with tumor antigens can generate effective antitumor immune responses in both established tumor and vaccine/challenge protocols (9–16). In these studies, DC vaccines have been administered either s.c., intradermally, or i.v., requiring the DCs to migrate to lymphoid organs for T-cell interaction. However, evidence shows that few transferred DCs actually reach lymphoid organs. In a study by Kupiec-Weglinski et al. (17), only 1% of radiolabeled splenic DCs injected s.c. in the footpad of mice reached the draining popliteal lymph nodes. In a similar murine model, Lappin et al. (18) demonstrated a time- and dose-dependent accumulation of FITC-labeled bone marrow-derived DCs within draining popliteal lymph nodes after s.c. footpad injection. Despite injecting $6 \times 10^5$ DCs, the authors found a peak concentration, 48 h after injection, of only 52 DCs/million lymph node cells (approximately 90 DCs/lymph node; Ref. 18). In a study conducted in humans using radioabeled DCs, <0.4% of the injection site activity was detectable in the regional lymph nodes after an intradermal injection, and no activity was detected after a s.c. injection (19). Studies in murine (17, 18, 20, 21), primate (22), and human (19) models have shown that DCs administered i.v. preferentially migrate to the spleen, with only trace or no migration to regional lymph nodes. However, <15% of i.v. administered DCs ultimately reach the spleen (17). Therefore, conventional methods of DC-based immunization results in the delivery of only a small fraction of transferred DCs to lymphoid organs, where they can interact with T cells.

Another possible route of DC administration, which avoids the need for DC migration, is injection directly into lymph nodes. In a human study by Nestle et al. (23), 16 patients with metastatic melanoma underwent vaccination with melanoma peptide-pulsed DCs via intranodal injection. After immunization, 11 of 16 patients developed a delayed-type hypersensitivity response to peptide-pulsed DCs. Five of 16 patients demonstrated a clinical response to the vaccine, including 2 complete responses and 3 partial responses. It is unclear whether these promising results are attributable to the route of DC injection or the composition of the vaccine. Therefore, we set out to test the hypothesis that direct injection of antigen-loaded DCs into lymph nodes will enhance the generation of antigen-specific T cells and protective antitumor immunity.

Although the studies described above have used immature DCs for immunization, recent reports suggest that ex vivo maturation of DCs will enhance their potency. Indeed, our lab initially demonstrated that stimulation of CD40 on DCs was required for the generation of protective cell-mediated tumor immunity by DC-based vaccines (24, 25). Labeur et al. (26) have gone on to show that CD40L-activated, tumor lysate-pulsed DCs more potently induced protective immunity than DCs prepared without CD40 activation. Human studies have shown that CD40 stimulation leads to the development of a more mature DC phenotype, with increased ability to stimulate allogeneic T-cell proliferation (27, 28). Because maturation of DCs can change the expression of multiple surface molecules, including chemokine receptors (29), and influence DC migration (30), we also compared the effectiveness of various routes of vaccination with CD40L-stimulated DCs.

MATERIALS AND METHODS

Mice. BALB/c (6–8 weeks of age; female) and C57BL/6 (6–8 weeks of age; male and female) and C57BL/6.Ly5.2 congenic (6–8 weeks of age; male) mice were purchased from the National Cancer Institute (Bethesda, MD). Ovalbumin peptide-specific T-cell receptor transgenic mice [OT-I (H-2b) (31) and OT-II (H-2d)] (32), both a kind gift from Dr. William Heath, Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia] were bred and maintained on a Ly5.2 background in a specific pathogen-free animal facility at Dartmouth Hitchcock Medical Center. All animal studies were preapproved by the Dartmouth Hitchcock Medical Center Institutional Animal Care and Use Committee.

Generation of Bone Marrow-derived DCs. Bone marrow-derived DCs were generated as described previously (13), with minor modifications. Briefly, bone marrow cells flushed from tibias and femurs were depleted of erythrocytes by incubating in 0.9% ammonium chloride for 3 min at 37°C. The cells were washed in HBSS (Sigma Chemical Co., St. Louis, MO) and cultured in CM (33) with 10 ng/ml recombinant mouse GM-CSF (specific activity, 7.42 $\times 10^5$, kindly provided by Immunex, Seattle, WA) and 20 ng/ml recombinant mouse IL-4 (PeproTech, Rocky Hill, NJ) at 1 $\times 10^5$ cells/ml. On the fourth day in culture, the media and floating cells were removed and centrifuged for 5 min at 1500 rpm. The cells were resuspended in CM at 1 $\times 10^5$/ml with 10 ng/ml GM-CSF and 20 ng/ml IL-4 and replaced in the original flasks. On day 7, nonadherent cells were harvested by gentle pipetting. DCs were

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The abbreviations used are: DC, dendritic cell; CM, complete medium; GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; FACS, fluorescence-activated cell sorter; CD40L, CD40 ligand.

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enumerated by FACS (FACScan; Becton Dickinson, Mountain View, CA) analysis through the expression of N418/CD11c-FITC (PharMingen, San Diego, CA). Preparations were typically 40–50% positive for N418/CD11c and 70–80% positive for MHC class II expression. Expression of the T-cell markers CD4 and CD8, and the macrophage marker F4/80 was <5%.

**Antigen Pulsing of DCs.** TS/A mammary adenocarcinoma and MCA-105 sarcoma cell suspensions were prepared by enzymatic digestion in HBSS with 1% collagenase (Sigma) and 0.1% hyaluronidase (Sigma). Tumor lysates were prepared by three cycles of liquid nitrogen and 37°C water-bath freeze-thawing. Day 6 DCs were incubated with tumor lysates at a ratio of 2.5:1 tumor cell equivalents: DCs overnight at 37°C in CM, as described previously (13). DCs were harvested by gentle pipetting and washed twice in HBSS. DCs were resuspended in HBSS at specific vaccine concentrations for use in further studies.

For DCs pulsed with both tumor lysate and CD40L, day 5 DCs were incubated for 4 h at 37°C with tumor lysate as described above. CD40L was generated as described previously (Ref. 34; a kind gift from Dr. Marilyn Kehry, IDEC Pharmaceuticals, La Jolla, CA) then added to the culture at 4% volume for 48 h. Treatment of DCs in this manner up-regulates CD80, CD86, and class II and enhances induction of T-cell proliferation in a mixed lymphocyte reaction (data not shown). The DCs were then harvested and prepared in HBSS as described above.

For DCs pulsed with ovalbumin peptides 323–339 (ISQ) and 357–364 (SNF; Research Genetics, Inc., Huntsville, AL), day 5 DCs were incubated at 37°C in CM with the peptides at 50 μg/ml for 90 min. The cells were harvested and washed three times with HBSS and then resuspended at 1 × 10^6 cells/ml in CM with 10 ng/ml GM-CSF and 20 ng/ml IL-4. CD40L DC pulsing occurred as described above. The cells were incubated in CM with 10 ng/ml GM-CSF and 20 ng/ml IL-4 at 37°C until day 7 and then harvested and prepared as described previously.

**In Vivo Immunization and Tumor Challenge.** BALB/c or C57BL/6 mice were immunized by one of four different routes of vaccine administration: s.c., i.v., intrasplenic, or intranodal. Varying doses of DC vaccine were administered vaccines were injected either alone, with 50 μg/ml ISQ, or with 50 μg/ml SNF. The cells were incubated for 24 h at 37°C. The plates were centrifuged for 2 min at 1000 rpm to pellet the cells, and the supernatants were harvested and assayed for the production of IFN-γ by ELISA using unconjugated rat anti-mouse IFN-γ and a biotinylated anti-IFN-γ antibody (PharMingen). ELISAs were developed using streptavidin-horseradish peroxidase (Amersham, Arlington Heights, IL) and tetramethylbenzidine substrate (Sigma). Comparison of the means was made using a paired t test.

**RESULTS**

Since to our knowledge intranodal vaccine administration had not been investigated previously in murine models, we developed a method to inject mouse inguinal lymph nodes. While wearing 2.5× magnifying loupes and using a 100-μl glass syringe with a 30-gauge needle, we were able to reliably inject murine inguinal lymph nodes. To confirm that the DCs were being injected intranodally, we injected C57 BL/6 mice with 5 × 10^5 congenic Ly5.2 DCs into the inguinal lymph nodes in vivo. We then excised the lymph nodes and by FACS compared the number of Ly5.2 DCs recovered to the yield from injecting a lymph node ex vivo. There was no significant difference in the number of DCs recovered from an ex vivo or in vivo injection (data not shown).

We next assessed the influence of the route of immunization on the ability of a tumor lysate-pulsed DC vaccine to induce protective antitumor immunity. C57BL/6 mice were immunized either intranodally or s.c. with a high (10 × 10^5) or low (2.5 × 10^5) dose of MCA-105 lysate-pulsed DCs. As shown in Fig. 1, all four treatment groups showed significant antitumor immunity compared with non-immunized mice (P ≤ 0.04). Mice immunized intranodally with the high-dose vaccine were the most effective at preventing the growth of MCA-105 pulmonary metastases (1.7 ± 1), whereas those immunized s.c. with the low-dose vaccine were the least effective (85 ± 35). Intranodal injection of the vaccine was more effective than s.c. at both the low dose (P = 0.04) and the high dose (P = 0.04). Intranodal
Fig. 2. Intranodal immunization with TS/A tumor lysate-pulsed DCs is more effective than s.c. or i.v. immunization. Mice were immunized intranodally (LN), s.c. (SC), or i.v. (IV) with either $2.5 \times 10^5$ (A) or $4 \times 10^5$ (B) TS/A tumor lysate-pulsed DCs. On day 7, TS/A tumor cells were injected i.v., and on day 24, pulmonary metastases were enumerated. Data are presented as the mean number of metastases; bars, SE. Statistical significance of differences: A, no vaccine versus LN, $P = 0.01$; no vaccine versus s.c. or i.v., $P = 0.04$ and 0.01, respectively; B, s.c. versus LN, $P = 0.02$; i.v. versus LN, $P = 0.005$. Injection of $2.5 \times 10^5$ DCs generated equivalent protective immunity as $1 \times 10^6$ DCs injected s.c., indicating that the efficacy of intranodal immunization is ~4-fold greater than s.c. This experiment was repeated three times with similar results.

To establish whether these findings were generalizable and to evaluate the efficacy of i.v. immunization, similar experiments were conducted in BALB/c mice with the TS/A tumor. Fig. 2 demonstrates the effectiveness of intranodal, s.c., and i.v. immunization at two different vaccine doses. At the low dose (Fig. 2A), only mice immunized intranodally showed significant antitumor immunity compared with nonimmunized mice ($P = 0.01$). In addition, mice immunized intranodally were significantly better at preventing the development of pulmonary metastases than mice immunized s.c. ($P = 0.02$) or i.v. ($P = 0.01$). At the higher dose (Fig. 2B), all three treatment groups showed significant antitumor immunity compared with nonimmunized mice ($P \leq 0.01$). Again, mice immunized intranodally developed significantly fewer pulmonary metastases when compared with mice immunized s.c. or i.v. ($P = 0.05$ and 0.02, respectively). There was no significant difference in the ability of mice immunized s.c. compared with i.v. to prevent the development of TS/A pulmonary metastases ($P = 0.72$).

Intrasplenic DC immunization was also evaluated using both the MCA-105 and TS/A models in protection/challenge experiments. As shown in Fig. 3A, C57BL/6 mice receiving either an intrasplenic or intranodal immunization with MCA-105 tumor lysate-pulsed DCs showed significant antitumor immunity compared with nonimmunized mice ($P = 0.02$ and 0.03, respectively.) No significant difference was discerned between these two routes of immunization ($P = 0.16$). Similarly, BALB/c mice immunized either intrasplenic or intranodal with TS/A tumor lysate-pulsed DCs also showed significant antitumor immunity compared with nonimmunized mice ($P = 0.04$ and 0.01, respectively; Fig. 3B). Again, no significant difference was discerned between these two routes of immunization ($P = 0.31$).

To test whether intranodal DC injection induced more potent protective antitumor immunity because more tumor-reactive T cells were generated, we used a transgenic system. Transgenic, SNF-specific CD8 and ISQ-specific CD4 T lymphocytes were administered i.v. to naïve C57BL/6 mice. The next day, the mice were immunized with ISQ- and SNF-pulsed C57BL/6 DCs either intranodally, s.c. (bilateral, hind-footpads), or i.v. Five days later, transgenic T lymphocytes present in the spleen and popliteal and inguinal lymph nodes were enumerated as described in “Materials and Methods.” As shown in Fig. 4A, less than $1 \times 10^5$ transgenic T lymphocytes were detected in the spleens of nonimmunized mice. A similarly low number of transgenic T lymphocytes were detected in the spleens of mice immunized either intranodally or i.v. with unpulsed DCs (data not shown). Significant transgenic CD8 T-lymphocyte expansion was found in the intranodal, i.v., and s.c. antigen-loaded DC vaccine groups as compared with nonimmunized mice (Fig. 4A; $P < 0.05$). In mice immunized intranodally, the number of transgenic CD8 T lymphocytes recovered from the spleens ($120 \times 10^5 \pm 13 \times 10^5$) was 2.4 times greater than the number recovered from the spleens of mice immunized i.v. ($51 \times 10^5 \pm 4.6 \times 10^5$; $P < 0.05$) and 6.3 times greater than mice immunized s.c. ($19 \times 10^5 \pm 3.2 \times 10^5$; $P < 0.05$). Transgenic CD8 expansion was also significantly greater in mice immunized i.v. as compared with mice immunized s.c. ($P < 0.05$).

Fig. 3. Intranodal immunization with tumor lysate-pulsed DCs is equally effective as intrasplenic immunization. Mice received either intranodal (LN) or intrasplenic (SP) immunization with either MCA-105 (A) or TS/A (B) tumor lysate-pulsed DCs. On day 7, MCA-105 (A) or TS/A (B) tumor cells were injected i.v., and on day 24, pulmonary metastases were enumerated. Data are presented as the mean number of pulmonary metastases; bars, SE. Statistical significance of differences: A, no vaccine versus LN or SP, $P \leq 0.03$; B, no vaccine versus LN or SP, $P \leq 0.04$; LN versus SP, $P = 0.04$. No vaccine versus LN, $P = 0.03$.
We then evaluated the splenocytes of immunized mice for the presence of an antigen-specific TH-1 cytokine response to peptide. Transgenic SNF-specific CD8 and ISQ-specific CD4 T lymphocytes were adoptively transferred. One day later, mice were immunized intranodally, i.v., or s.c. with either unpulsed or peptide-pulsed DCs, and splenocytes were harvested on day 5. The splenocytes were then either left unpulsed or pulsed with ISQ or SNF peptides overnight, and the supernatants were assayed for IFN-γ (Fig. 5). Although we observed significant proliferation of CD4 (OT-II) T cells in the spleen when peptide-pulsed DC vaccine was administered intranodally, i.v., or s.c. (Fig. 4A), we did not detect IFN-γ secretion from ISQ-pulsed splenocytes. In contrast, SNF-pulsed splenocytes from mice immunized intranodally with antigen-pulsed DCs produced significant amounts of IFN-γ compared with splenocytes from nonimmunized mice (P = 0.001; Fig. 5). This was not attributable to nonspecific antigen presentation, because splenocytes from mice immunized with nonpulsed DCs did not secrete IFN-γ (Fig. 5). Splenocytes from mice immunized intranodally with antigen-pulsed DCs produced 25 times as much IFN-γ as mice immunized i.v. (P = 0.02) and 31 times more IFN-γ than mice immunized s.c. (P = 0.01). When normalized for the number of OT-1 T cells/well, splenocytes from mice immunized intranodally secreted 6.25 times more IFN-γ than mice immunized i.v. These findings indicate that intranodal injection of peptide-pulsed DCs stimulated significantly greater expansion of antigen-specific CD8 T cells that were 6-fold more effective at generating a TH-1 cytokine response than i.v. immunization.

To assess whether the route of administration also affected the potency of DCs matured ex vivo with CD40L, BALB/c mice were immunized s.c. or intranodally with TS/A tumor lysate and CD40L-pulsed DCs as described in “Materials and Methods.” As shown in Fig. 6, compared with nonimmunized mice, both treatment groups showed significant antitumor immunity against a lethal i.v. challenge of live TS/A tumor cells (P = 0.01). Mice immunized intranodally were significantly better at suppressing the development of pulmonary metastases than those immunized s.c. (P = 0.02).

We also assessed whether the route of administration of CD40L-matured DC vaccine affected the generation of antigen-specific T lymphocytes. Using the transgenic model described previously, after transfer of SNF-specific CD8 and ISQ-specific CD4 T lymphocytes, C57BL/6 mice were immunized either intranodally or s.c. with

Fig. 4. Intranodal immunization with ovalbumin peptide-pulsed DCs induces greater ovalbumin peptide-specific, T-lymphocyte expansion in the spleen (A) and inguinal and popliteal lymph nodes (B) than i.v. or s.c. immunization. One day after adoptive transfer of ovalbumin peptide-specific CD8 and CD4 T lymphocytes, mice were immunized intranodally (LN), s.c. (SC), or i.v. (IV) with ovalbumin peptide-pulsed DCs. On day 5, spleens and inguinal and popliteal lymph nodes were removed, and the antigen-specific T lymphocytes were enumerated by flow cytometry. Data are presented as the mean number of antigen-specific T lymphocytes; bars, SE. Similar results were obtained in two separate experiments. Statistical significance of differences: A, CD8: LN versus i.v., P < 0.05; LN versus s.c., P < 0.05; CD4: LN versus i.v., P = not significant; LN versus s.c., P < 0.05; B, CD8: LN versus i.v. or s.c., P < 0.05; CD4: LN versus i.v. or s.c., P < 0.05.

With regard to CD4 transgenic T lymphocytes, although the absolute number recovered in the spleens was much smaller than that of CD8 T cells, intranodal, i.v., and s.c. immunization again led to significant CD4 T-cell proliferation relative to nonimmunized mice (Fig. 4A; P < 0.05). CD4 transgenic T lymphocyte expansion was significantly greater after intranodal i.v. immunization compared with s.c. (P < 0.05); however, there was no significant difference between intranodal and i.v. immunization (P = 0.28). These experiments were repeated twice with similar results.

Fig. 4B shows transgenic CD8 and CD4 T lymphocytes recovered from the inguinal and popliteal lymph nodes of transgenic T-lymphocyte reconstituted mice immunized with ISQ- and SNF-pulsed DCs. Both CD8 and CD4 transgenic T-cell proliferation was significantly greater after intranodal, i.v., or s.c. immunization compared with nonimmunized mice (P < 0.05). CD8 and CD4 transgenic T-cell expansion was significantly greater in mice immunized intranodally compared with mice immunized i.v. or s.c. (P < 0.05). There was no significant difference in antigen-specific T-cell proliferation between mice immunized i.v. compared with s.c. (P ≥ 0.13).

Fig. 5. Intranodal immunization with ovalbumin peptide-pulsed DCs induces a more potent, specific TH-1-type cytokine response than i.v. or s.c. immunization. One day after adoptive transfer of ovalbumin peptide-specific CD8 and CD4 T lymphocytes, mice were immunized intranodally (LN), s.c. (SC), or i.v. (IV) with ovalbumin peptide-pulsed or nonpulsed DCs. On day 5, spleens were removed, and the splenocytes were tested for IFN-γ secretion in response to ovalbumin peptide by ELISA. Data are presented as the means; bars, SE. Similar results were obtained in two separate experiments. Statistical significance of differences: LN versus i.v., P = 0.02; LN versus s.c., P = 0.01.
CD40L-conditioned ISQ- and SNF-pulsed DCs. Five days later, the spleens and lymph nodes were removed, and the numbers of transgenic CD8 and CD4 lymphocytes were enumerated. As shown in Fig. 7, relative to nonimmunized mice, significant CD8 and CD4 transgenic T-cell proliferation was observed in the spleens of mice immunized either intranodally or s.c. with the CD40L-stimulated DC vaccine (P < 0.05). CD8 and CD4 antigen-specific T-cell expansion in the spleens of mice immunized intranodally was 8-fold higher than mice given the same vaccine s.c. (P < 0.05). Expansion of transgenic CD8 and CD4 T cells in the lymph node was also significantly increased after intranodal immunization compared with either nonimmunized mice or mice immunized s.c. (P < 0.05).

DISCUSSION

Evidence of a significant role for the route of immunization in the induction of systemic immunity is mounting. In a murine tumor model, a recent study by Irvine et al. (36) showed that i.v. and i.m. immunization with recombinant tumor antigen-expressing poxviruses was significantly more effective at inducing antitumor immunity than either s.c. or tail scarification. Studies evaluating DC vaccine administration also indicate a significant role for the route of immunization. In a murine study of protective immunity against adenovirus-peptide-expressing tumors, a statistically significant increase in survival was seen after i.v. immunization with adenoviral peptide-pulsed, spleen-derived DCs, as compared with s.c. immunization. However, when bone marrow-derived DCs were used, no statistically significant difference in survival based upon the route of immunization was seen (37). In another murine study by Takahashi et al. (38), i.v. administration of HIV-1 viral peptide-pulsed, spleen-derived, irradiated DCs was found to be more efficient at CD8+ CTL generation than either s.c. or i.p. administration. On the contrary, Lappin et al. (18) demonstrated a significant increase in contact hypersensitivity responses in a murine model after s.c. administration of trinitrobenzenesulfate-pulsed, bone marrow-derived DCs as compared with i.v. Thus, there appears to be some controversy in the literature regarding the optimal route of immunization with a DC vaccine. Although these discrepancies in DC efficacy may be based upon the site of DC origination, they raise important questions relevant to the design of future clinical trials of DC vaccines. In our tumor model, both s.c. and i.v. immunization with tumor lysate-pulsed, bone marrow-derived DCs can induce significant antitumor immunity. As reported by Toes et al. (37), we did not appreciate a significant difference between s.c. and i.v. routes of bone marrow-derived DC vaccine immunization (Fig. 2).

We are not aware of any other published murine studies evaluating intranodal immunization. Our study shows that immunization of mice with intranodal injections of tumor lysate-pulsed DCs could induce antitumor immunity and resulted in significantly better antitumor protection against pulmonary metastases compared with mice immunized s.c. or i.v. (Figs. 1 and 2). In addition, our data indicate that the potency of protective immunity induced by intranodal immunization is 4-fold higher than s.c. immunization. These in vivo antitumor effects were observed in two different strains of mice (C57BL/6 and BALB/c) against two histologically distinct, syngeneic tumors (MCA-105 sarcoma and TS/A adenocarcinoma), indicating that it is a generalizable observation.

To understand the mechanism for the enhanced efficacy of intranodal vaccination, we compared the effect of various routes of vaccine administration on their ability to generate antigen-specific T lymphocytes with a TH-1 cytokine profile. Previous experiments in our laboratory have shown that IFN-γ secretion by CD8 lymphocytes is required for therapeutic effectiveness against established micrometastases (33). Our results using the transgenic OT-I/OT-II system show that intranodal immunization dramatically increases the generation of antigen-specific CD8 and CD4 lymphocytes compared with i.v. or s.c. DC immunization. Furthermore, antigen-specific secretion of IFN-γ by the expanded T cells after intranodal immunization was >6-fold greater than i.v. immunization on a cell-to-cell basis. Intranodal immunization may be more effective than other routes simply because it is a reliable method for getting more DCs to contact T lymphocytes in a lymphoid environment than other methods of immunization. We are also currently evaluating the effect of intranodal immunization on DC trafficking and survival.

We have demonstrated that the route of DC vaccine administration has a quantitative effect on the development of a TH-1 T-cell response. Others have shown that the route of DC vaccine administration may have a qualitative influence on the TH-1/TH-2 cytokine response profile. In a study by Morikawa et al. (39), using reverse transcription-PCR to measure the presence of mRNA of various cytokines in mouse spleen and lymph node cells, keyhole limpet hemocyanin-pulsed, bone marrow-derived DCs were shown to stim-
roduce a TH-1 cytokine response in the draining lymph nodes when administered s.c. and a TH-2 cytokine response when administered i.v. We are currently evaluating the effect of the route of vaccine administration on the pattern of cytokine production by lymphocytes in the transgenic autologous system.

Evidence is accumulating from both murine and human studies that maturation of DCs through CD40 stimulation leads to more potent T-lymphocyte activation. We initially demonstrated that CD40 triggering was necessary for the generation of protective cell-mediated tumor immunity by both DC-based and adjuvant-based vaccines (24, 25). A study by Labeur et al. (26) has also shown that CD40L-activated, tumor-lysat-pulsed DCs led to more potent induction of protective immunity than DCs prepared without CD40 activation. Human studies using DCs generated from peripheral bone marrow cells cultured in GM-CSF and IL-4 have shown that CD40 stimulation leads to the development of a more mature DC phenotype and increased ability to cause allogeneic T-cell proliferation in a mixed lymphocyte reaction (28). Others have shown that CD40L stimulation of DCs will enhance allogeneic T-cell proliferation and lead to the generation of DCs that are able to induce peripheral bone marrow cells to lyse autologous leukemia cells (27). As with non-CD40L-stimulated DCs, our data show that intranodal immunization with tumor lysate-pulsed, CD40L-stimulated DCs induces significantly greater antitumor immunity in a murine protection/challenge model compared with s.c. immunization. Furthermore, CD40L-stimulated, antigen-loaded DCs administered intranodally were also capable of inducing significantly more antigen-specific CD4 and CD8 T-cell proliferation in both the spleen and lymph nodes compared with nonimmunized mice and mice immunized s.c.

In summary, we have demonstrated that intranodal injection of both mature and immature DC tumor vaccines generates a more potent antitumor immune response than conventional s.c. or i.v. routes of vaccination. These results are directly applicable to the design of DC-based tumor vaccine studies in patients.
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