Therapeutic Vaccination against Murine Lymphoma by Intratumoral Injection of Naive Dendritic Cells

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Abstract

Dendritic cells are potent antigen-presenting cells that can induce both immune responses and tolerance depending on their state of activation. Immunologic tolerance to established tumors is a major impediment for the development of effective cancer immunotherapy. Dendritic cells may be deficient in number or in function at the tumor site. To address this problem, we evaluated the ability of immature naïve dendritic cells to induce an antitumor immune response when injected directly into a murine B-cell lymphoma. Mice with advanced transplanted syngeneic tumor were given intratumoral injections of bone marrow–derived dendritic cells. Intratumoral dendritic cell injection alone had no antitumor effect. Systemic chemotherapy alone resulted in only transient tumor regression. However, the intratumoral injection of dendritic cells after chemotherapy led to complete, long-term tumor regression in the majority of treated mice. This dendritic cell–mediated antitumor effect was systemic, resulting in simultaneous elimination of the tumor at second uninjected sites. In addition, it resulted in long-term memory with resistance to tumor rechallenge. Both CD4+ and CD8+ T cells are necessary for the antitumor effect. Furthermore, tumors that occasionally recurred in mice with initial complete tumor regression could be retreated by the same combined chemoinmunotherapy approach. These results show that immunotherapy can succeed in the setting of advanced lymphoma if dendritic cells are restored and loaded with tumor antigens in situ at a single tumor site. (Cancer Res 2005; 65(13): 5958-64)

Introduction

Although the current treatments for human lymphoma can induce a high rate of complete remission, most of these tumors ultimately relapse and cannot be cured. This fact, coupled with the substantial toxicities of current treatments, has motivated the search for novel and more tumor-selective therapies. The potential specificity of the immune system to recognize and eliminate tumor cells is especially relevant in lymphoma (1–3). Passive immunotherapy with monoclonal antibodies (mAb) has changed the management of lymphoma (2, 3), inducing remission and improving survival of patients without adding significant toxicities (4, 5). Active vaccination against lymphoma should be even more specific and more long lasting than passive immunotherapy. One such approach uses bone marrow–derived dendritic cells, the most potent antigen-presenting cells, to present tumor-associated antigens and thereby generate tumor-specific immunity (6–9). Normally, dendritic cells reside as immature cells in peripheral tissues where they sample their environment by taking up and processing proteins and inducing tolerance to self-antigens. These immature dendritic cells become activated, mature, and induce immune responses only when they encounter danger signals, such as local infection or inflammation (8–10). The presence in a wide range of tumors of immature dendritic cells that are unable to stimulate T cells suggests a possible active role for these cells in the failure of tumor-bearing hosts to mount an effective antitumor response (11–16). Indeed, for some model antigens, injection of immature Ag-pulsed dendritic cells can induce a specific tolerogenic response, whereas similarly pulsed dendritic cells, when matured, induced a typical Th1 immune response (17).

Based on the central role of dendritic cells in initiating immune responses, a variety of cancer vaccine strategies using dendritic cells have been investigated (6, 7, 9). Animal studies have shown that dendritic cells, when loaded ex vivo with tumor antigens (peptides or proteins, tumor cell lysates or mRNAs, vectors encoding tumor antigens, or dendritic cell-tumor cell fusions), and administered to tumor-bearing hosts, can elicit T-cell–mediated tumor destruction (6, 7, 18–24). These observations have led to clinical trials designed to investigate the immunologic and clinical effects of antigen-loaded dendritic cells in patients with cancer (25, 26). One of the first dendritic cell vaccination studies was done in patients with lymphoma in which autologous dendritic cells were loaded with tumor-derived idiotype protein (27). Antitumor immune response and tumor regression were documented (28). Other reports have shown promising results in patients with melanoma (25, 26). However, these strategies require the production, loading, and maturation of the dendritic cells ex vivo.

To overcome these limitations, we have developed an approach in which dendritic cells can be loaded and matured with tumor antigens in situ (29). Antigens from apoptotic or necrotic tumor cells can be loaded into immature dendritic cells injected directly into the tumor site. In addition, “danger signals” at the site of dying tumor cells can provide a maturation stimulus for the injected dendritic cells. Exogenously administered immature dendritic cells take up and process tumor antigens released by the dying tumor cells, mature, and become activated in situ and then cross-prime T cells against tumor-derived antigens. This strategy has been tested in a variety of murine solid tumor models using different chemotherapy agents to induce tumor cell death (30–32).

In the current study, we investigated this in situ vaccination strategy for the treatment of a metastatic murine B-cell lymphoma. S.c. injection of this lymphoma leads to local growth as well as systemic spread. We showed that, even for far advanced tumors, local intratumoral injection of dendritic cells after chemotherapy induced long-term, systemic antitumor...
immunity and cure of the animals. This effect was dependent on CD4+ and CD8+ T cells. Tumors that occasionally recurred in mice with initial complete tumor regression still retained their sensitivity to this immunologic maneuver. The combination of chemotherapy to induce tumor death and followed by dendritic cell administration in situ may provide a powerful and convenient mode of inducing systemic antitumor immunity for the treatment of lymphoma, which tends to be responsive to immunologic maneuvers and often require retreatment due to tumor relapse.

Materials and Methods

Mice. Female BALB/c mice (6-8 weeks of age) were purchased from Harlan Sprague-Dawley (San Diego, CA) and were used for all the experiments. Age-matched female C3H and C57BL/6 mice (Harlan Sprague-Dawley) were used as sources of allogeneic dendritic cells. CD8 knockout mice in BALB/c background were a gift from Dr. G. Fathman (Stanford University, Stanford, CA). Animals were housed at the Laboratory Animal Facility at Stanford University Medical Center. All of the experiments were conducted according to the Stanford University Laboratory Animal Facility guidelines.

Cell lines. A20 is a BALB/c-derived B-cell lymphoma expressing MHC class I and II H-2d molecules (33) and was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The idiotype-negative A20 was provided by J. Timmerman (University of California at Los Angeles, Los Angeles, CA). Bat hybridomas GK1.5 (anti-CD4) and 2.43 (anti-CD8) were obtained from ATCC. The isotype control rat hybridoma SFR8-B6 (rat IgG2b antihuman HLA Bw6) was provided by J. Parnes (Stanford University). All cell lines were grown at 37°C in 5% CO2 with complete RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin (both from Life Technologies, Inc., Gaithersburg, MD), and 50 µmol/L 2-mercaptoethanol.

Generation of bone marrow-derived dendritic cells. Primary dendritic cells were obtained from mouse bone marrow precursors as described previously (18, 34). Briefly, murine bone marrow cells were harvested from femurs and tibias and then plated in complete RPMI 1640 containing recombinant murine granulocyte/macrophage-colony stimulating factor (GM-CSF, 10 ng/mL; Sigma Chemical, Co., St. Louis, MO) and recombinant murine interleukin-4 (IL-4, 20 ng/mL; Genzyme, Farmington, MA). On day 3, nonadherent granulocytes were gently removed and fresh medium with GM-CSF and IL-4 was added. On day 6, loosely adherent dendritic cells were obtained from mouse bone marrow precursors as described previously (18, 34). Briefly, murine bone marrow cells were harvested from femurs and tibias and then plated in complete RPMI 1640 containing recombinant murine granulocyte/macrophage-colony stimulating factor (GM-CSF, 10 ng/mL; Sigma Chemical, Co., St. Louis, MO) and recombinant murine interleukin-4 (IL-4, 20 ng/mL; Genzyme, Farmington, MA). On day 3, nonadherent granulocytes were gently removed and fresh medium with GM-CSF and IL-4 was added. On day 6, loosely adherent cells were dislodged and replated. On days 7 to 9 of culture, immature dendritic cells, as nonadherent proliferating aggregates, were collected and washed thrice with PBS before use for in vivo vaccination. For most experiments, freshly prepared dendritic cells were used. However, we found that dendritic cells could be cryopreserved (in 90% fetal bovine serum and 10% DMSO) and thawed with a viability of >80%. The freshly thawed dendritic cells were washed thrice with PBS before use for in vitro vaccination. The cryopreserved dendritic cells were found equally effective as fresh dendritic cells and were used in the treatment experiments in mice with late tumor recurrence.

In vitro tumor treatment protocol. A20 tumor cells were thawed from a common frozen stock and passed in vitro in complete medium for 4 days before use. On the day of tumor challenge, cells were washed thrice in HBSS and diluted to the appropriate concentration. 105 cells/mouse in 100 µL of HBSS were implanted s.c. into BALB/c mice in the lower right flank. On day 17, the average tumor sizes were ~ 1 cm in diameter. Mice bearing local tumors within a range 0.8 to 1.5 cm in diameter were chosen. A dose range (25-200 mg/kg body weight) of cyclophosphamide (Sigma) was first tested for antitumor effect. A dose-dependent effect was found and 100 mg/kg body weight given i.p. on 2 consecutive days (day 17-18 posttumor inoculation) was chosen for all experiments. Two injections of dendritic cells (each injection was 2 x 106 cells/mouse in 50 µL of HBSS) were given into the tumor on 2 and 4 days postchemotherapy (days 19 and 21 posttumor inoculation). The local tumor size was measured biweekly using a caliper and expressed as the product of the two maximal perpendicular diameters (mm2). Animals were sacrificed when the tumor diameter exceeded 2 cm, when there was tumor ulceration, or when there were other signs of animal distress.

In vivo T-cell depletion. Animals were depleted of CD4+ or CD8+ cells by i.p. injection of anti-CD4 (GKL5 hybridoma) or anti-CD8 (2.43 hybridoma) ascitic fluid. Ascitic fluid was harvested from pristine-primed mice, diluted in PBS, and serially filtered before use. Antibodies were injected i.p. on days 6, 5, 4, and 0 before intratumoral dendritic cell injection, and then weekly to maintain the depletion status. A group of mice received irrelevant isotype control (SFBR8-B6 hybridoma) as a control. These depletion conditions were validated by flow cytometry analysis of splenocytes using phycoerythrin-conjugated anti-CD4 (Caltag, Burlingame, CA) and anti-CD8 (PharMingen, San Diego, CA) mAbs that were noncompeting with the above antibodies used for in vivo depletion; 95% of the relevant cell subset was depleted, whereas all of the other subsets remained within normal levels (data not shown).

In vitro cytotoxicity assay. Spleens were pooled from two to three representative mice and the splenocytes were restimulated at 5 x 106 cells/ml with irradiated (5,000 eGy) A20 cells (1 x 106/mL) for 6 days. IL-2 (10 units/µL; Chiron, Emeryville, CA) was added to the cultures on day 3. Viable cells were harvested and tested in a 4-hour standard 51Cr release assay as described previously (18). Briefly, [51Cr]-labeled A20 were incubated with effector cells at different effector-to-target (E/T) ratios in triplicate wells, and [51Cr] release was determined by analyzing the supernatants in a γ counter. The percentage of specific release was calculated according to the formula: 100 x (experimental release − spontaneous release) / (maximal release − spontaneous release). Spontaneous release and maximum release were obtained from wells containing target cells incubated in medium alone or in 2% SDS, respectively.

Results

The antitumor effect of intratumoral dendritic cell injection following chemotherapy. BALB/c mice were injected s.c. with 10 million A20 lymphoma cells and were selected for inclusion when their local tumors reached ~1 cm in diameter at day 17 posttumor inoculation. Without treatment, those mice succumbed to local and systemic lymphoma (lymph node, spleen, and liver) within 5 to 7 weeks (Fig. 1A). Therapy with cyclophosphamide at various doses (25, 50, 100, and 200 mg/kg body weight) was tested. Cytoreduction by cyclophosphamide was cyclophosphamide dose-dependent and was evident in the range of 50 to 200 mg/kg (data not shown). Therefore, the dose of cyclophosphamide at 100 mg/kg on 2 consecutive days was chosen for this study. The systemic chemotherapy alone resulted in only transient tumor regression with a slight prolongation of survival, but the majority of treated mice had tumor regrowth and eventually succumbed to systemic lymphoma (Fig. 1B). Intratumoral injection of dendritic cells alone had no antitumor effect (Fig. 1C), indicating that the mere presence of a high number of naive dendritic cells at the tumor site is not sufficient to induce antitumor immunity in advanced-stage tumor-bearing host. However, the addition of local intratumoral injection of dendritic cells after systemic chemotherapy led to complete, long-term regression in the majority of treated mice (Fig. 1D). A dose-dependent effect of intratumoral dendritic cell injection (0.3, 1, and 3 million per injection) following chemotherapy (cyclophosphamide 100 mg/kg) was observed (data not shown), and the dose of 2 million dendritic cell per injection was chosen. Different dendritic cell vaccination schedules (once on day 2 or day 4 postchemotherapy; twice on day 2 and day 8 or day 4 and day 8 postchemotherapy; or thrice on day 2, 4, and 8 postchemotherapy) were tested following chemotherapy (cyclophosphamide 100 mg/kg), and it was found that intratumoral
Intratumoral dendritic cell injections on days 2 and 4 postchemotherapy were chosen for use in all subsequent experiments. This therapeutic effect of intratumoral dendritic cell injection was dependent on injection of the dendritic cells directly into the tumor site as opposed to a regional or distant s.c. site (Fig. 1E-F) or via i.v. route (data not shown). Taken together, the data indicates that both the number of dendritic cells and the functional milieu at the tumor sites are instrumental in generating antitumor immunity. The antitumor effect induced by intratumoral dendritic cell after chemotherapy was restricted to syngeneic dendritic cells because allogeneic dendritic cells (from C3H or C57BL/6 mice) were ineffective as shown in Fig. 2.

Local intratumoral dendritic cell injection after systemic chemotherapy induces systemic and persistent antitumor immunity. The fact that untreated tumor-bearing mice succumb to local and systemic lymphoma (lymph nodes, spleen, and liver) in 5 to 7 weeks and that combination treatment of chemotherapy with intratumoral dendritic cell injection lead to long-term surviving mice (>4 months) indirectly indicate the local intratumoral dendritic cell injection has a systemic antitumor effect. To further support this point, tumors were inoculated s.c. into both lower flanks. The tumor in the right flank was considered as primary tumor and was treated by dendritic cell injection after systemic chemotherapy (cyclophosphamide), whereas the secondary tumor on the left side was simply observed. As shown in Fig. 3, tumors in both flanks regressed simultaneously in the majority of the tumor-bearing mice, indicating that the antitumor effect induced by intratumoral dendritic cell injection was systemic rather than local. By contrast, when the mice were treated with chemotherapy alone, the tumors on both sides regressed temporarily and then recurred and grew progressively.

The systemic antitumor effect induced by intratumoral dendritic cell injection was immune-mediated and with long-term memory because the long-term surviving mice (>4 months) with complete tumor regression were able to resist a rechallenge with the same A20 lymphoma (data not shown). Furthermore, specific cytotoxic T lymphocyte responses against A20 lymphoma cells were detected in the lymph nodes and spleen of the long-term surviving but not from the naïve mice or from chemotheraputic tumor-bearing mice (Fig. 4). These observations suggest that treatment of a single tumor with the intratumoral dendritic cell injection after systemic chemotherapy can generate therapeutic immunity against metastatic tumor.

Both CD4+ and CD8+ T cells were involved in tumor regression. To determine the role CD4+ and CD8+ T cells in the tumor regression in mice treated with combination of chemotherapy and intratumoral dendritic cell injection, mice were depleted of different subsets of T cells with mAbs. Antibodies were given 12 days posttumor inoculation but 1 week before the intratumoral dendritic cell injection to assure the proper T-cell depletion at the time of dendritic cell injection.
(>95% depletion; data not shown), and then were given weekly to maintain the T-cell depletion status. As shown in Fig. 5B-C, both CD4+ and CD8+ T cells are involved in the tumor regression induced by intratumoral dendritic cell injection. Similarly, mice with CD8+ T cells depleted before tumor inoculation (data not shown) or CD8 knockout mice (Fig. 4D) also show the essential role of CD8+ T cells in tumor regression induced by intratumoral dendritic cell injection. It is likely that the CD4+ helper T cells are involved in the priming and activation of CD8+ effector T cells.

**Effective retreatment of the late recurring tumor in long-term surviving mice.** Mice with complete tumor regression after the combined systemic chemotherapy and the intratumoral dendritic cell immunotherapy were observed long-term because of the theoretical concern of the induction of autoimmune diseases via the presentation of self-antigens by the intratumoral injected dendritic cells. Those long-term surviving mice seem to maintain their complete tumor regression status.

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**Figure 2.** Tumor regression induced by intratumoral injection of dendritic cells from syngeneic but not from allogeneic mice. Treatment was similar as Fig. 1 except that dendritic cells from either syngeneic BALB/c (A) or allogeneic C57BL/6 (B) or C3H (C) were injected intratumorally following chemotherapy. The numbers of long-term surviving mice with complete tumor regression in each group were indicated. A. cyclophosphamide chemotherapy alone. B. cyclophosphamide chemotherapy + syngeneic dendritic cells. C. cyclophosphamide chemotherapy + allogeneic dendritic cells from C57BL/6. D. cyclophosphamide chemotherapy + allogeneic dendritic cells from C3H.

**Figure 3.** Unilateral intratumoral dendritic cell injection resulted in tumor regression on the contralateral side. On day 0, syngeneic BALB/c mice were inoculated with A20 lymphoma cells s.c. in bilateral lower flanks (10⁷ cells to each side). On days 17 and 18, tumor-bearing mice were treated either with systemic chemotherapy alone (filled symbols) or systemic chemotherapy followed by intratumoral injection of dendritic cells (open symbols) into the tumor only on the right flank. Tumor growth from both side of flanks were followed and the mean tumor size from each group were shown. The numbers of long-term surviving mice with complete tumor regression on left or right side were indicated.

**Figure 4.** CTL activity in splenocytes of long-term surviving mice. Four months after the sequential treatment with chemotherapy and intratumoral dendritic cell injection, the resulting long-term surviving mice with complete tumor regression were examined for the presence of tumor-specific CTL activities. Splenocytes isolated from the long-term surviving mice or from control mice (naive or chemo-treated tumor-bearing mice) were stimulated for 5 days in vitro with irradiated (5,000 cGy) A20 tumor cells. Cytotoxic activities against A20 lymphoma target cells were measured in a standard ⁵¹Cr release assay. Data are expressed as mean specific lysis of triplicate value (%) at different E/T cell ratios.
healthy up to 1 year after the initial treatment except that it was consistently observed that some of those mice (one to two in a group of 10 mice) had tumor recurrence at the initial tumor injection site or at other metastatic sites after a prolonged period (1-4 months) of tumor-free time. Then, large-scale experimental groups were set up to obtain a reasonable number of mice with late tumor recurrence to address whether those mice are still retreatable by the same treatment strategy. Cryopreserved dendritic cells were found to be as effective as the freshly prepared dendritic cells. Therefore, the cryopreserved dendritic cells were used in treatment for the individually recurring tumor when the tumor reached ~1 cm in diameter. In one such experiment, the initial combined treatment of chemotherapy and intratumoral dendritic cell injection led to complete tumor regression in 23 of 28 tumor-bearing mice (data not shown), but 5 of those 23 mice had late tumor recurrence as shown in Fig. 6A. These five mice with late tumor recurrence were again induced into a complete tumor regression by intratumoral dendritic cell injection after chemotherapy. In another separate experiment, the initial combined treatment of chemotherapy and intratumoral dendritic cell injection led to complete tumor regression in 51 of 64 tumor-bearing mice (data not shown), but 8 of those 51 mice had late tumor recurrence as shown in Fig. 6B. As before, chemotherapy alone could not induce sustained tumor regression in the majority of the mice (Fig. 6B).

Figure 5. Both CD4 and CD8 T cells are involved in the tumor regression. Wild-type (A-C) or CD8 knockout (D) tumor-bearing mice were treated with systemic chemotherapy and intratumoral dendritic cell injection in the same way as in Fig. 1. CD4 T cells (B) or CD8 T cells (C) were depleted with mAbs to murine CD4 or CD8, respectively, in tumor-bearing mice before the intratumoral dendritic cell injection as described in Materials and Methods. The numbers of long-term surviving mice with complete tumor regression in each group were indicated.


Figure 6. Retreatment of late-recurring tumor with cryopreserved dendritic cells and chemotherapy. Cryopreserved dendritic cells were found equally effective as fresh dendritic cells and, therefore, were used for the treatment of late-recurring tumor in mice with initial complete tumor regression. When the recurring tumor reached a diameter of 1 cm, retreatment with combination of chemotherapy (cyclophosphamide, 100 mg/kg body weight, i.p., daily for 2 consecutive days) and subsequent intratumoral injection of dendritic cells (2 million per injection, 2 and 4 days postchemotherapy) or chemotherapy alone. The timing of initial treatment (chemotherapy + dendritic cells) and retreatment (chemotherapy + dendritic cells or chemotherapy alone) are indicated by the arrows. A, retreatment with chemotherapy + intratumoral dendritic cell injection. B, retreatment with chemotherapy alone.
Discussion

A number of studies have shown that antitumor immunity can be achieved by generating dendritic cells in vitro, loading the dendritic cells ex vivo with tumor-specific antigens and reinfusing the dendritic cells back to the recipient host (6, 7, 9). In contrast, the present study shows the effectiveness of an antitumor strategy that eliminates the steps of loading and maturing the dendritic cells ex vivo. We achieved this by combining intratumor administration of dendritic cells with systemic chemotherapy. Using a metastatic murine lymphoma model, the data show that the combination of systemic chemotherapy plus direct intratumoral administration of naive dendritic cells results in complete tumor regression that requires both CD4 and CD8 T cells. Importantly, the chemotherapy + dendritic cell–treated animals had acquired long-term antitumor immunity even in advanced tumor-bearing stage. Late tumor recurrence, which occasionally occurred in mice with initial complete tumor regression, was still treatable with this approach.

Since the report of our initial study using the combined chemotherapies (cyclophosphamide and Adriamycin) and intratumoral dendritic cell vaccination for treatment of murine tumors (CT26 colon adenocarcinoma and B16 melanoma; ref. 29), several groups have reported results of this combined strategy in treating different murine tumor models using different chemotherapeutic agents (30–32). Tanaka et al. (30) reported the use of 5-fluorouracil and cisplatin in combination with intratumoral dendritic cell vaccination in treating M38 colon adenocarcinoma. Yu et al. (31) reported the use of paclitaxel in combination with intratumoral dendritic cell vaccination in treating DA3 experimental breast cancer. Shin et al. (32) reported the use of vincristine in combination with intratumoral dendritic cell vaccination in treating MCA102 fibrosarcoma. Our current study extends this strategy for the first time to a metastatic murine lymphoma model. It not only confirmed the power of this approach in inducing T-cell–dependent therapeutic antitumor immunity leading to regression of preestablished tumors but also revealed additional interesting observations: the potency even in the setting of advanced tumor-bearing stage and the effective retreatment of late recurring tumors with cryopreserved dendritic cells after chemotherapy. In addition, the following practical issues regarding intratumoral dendritic cell vaccination were first systemically addressed in this lymphoma tumor model: dependence of the dendritic cell injection on tumor site rather than peritumor or given distantly or systemically and the equal efficacy of cryopreserved dendritic cells compared with fresh dendritic cells.

Besides chemotherapy, other apoptosis-inducing agents and local radiation therapy have also been investigated in combination with intratumoral dendritic cell vaccination (35–37). Candido et al. (35) were the first to use the apoptosis-inducing agent, tumor necrosis factor-α, to enhance intratumoral dendritic cell vaccination in a murine breast cancer model. The combination of irradiated tumor cells with intratumoral dendritic cell vaccination have also been explored recently by a number of groups (36, 37). In our own experiments, local radiation therapy greatly enhanced the intratumoral dendritic cell vaccination in the A20 lymphoma model but was less powerful compared with chemotherapy with intratumoral dendritic cell vaccination in the advanced tumor-bearing stage (data not known). All these tumor-killing strategies followed by intratumoral dendritic cell vaccination are based on the observation that immature dendritic cells can efficiently engulf apoptotic or necrotic cells and present antigenic peptides to MHC class I– and class II–restricted T cells (38–41). Uptake of apoptotic bodies from normal cell turnover by immature dendritic cells, without appropriate secondary danger signals, may maintain peripheral T-cell tolerance (10, 42). Simultaneous release of necrotic cells or their products, as in chemotherapy, may provide appropriate danger signals for the maturation and activation of dendritic cells (40, 43). In addition, many of the chemotherapy agents, including cyclophosphamide, have been shown to exhibit both immunosuppressive and immunomodulating effects depending on the dosage used and the temporal relationship between drug administration and antigen challenge (44–46). Early studies showed that cyclophosphamide caused a selective reduction in the suppressor T-cell population (47), and recent studies suggested that cyclophosphamide exerts its immunomodulating effects in tumor-bearing animals through the induction of type I IFN (48, 49). The contribution of an immunomodulating effect as opposed to a tumor killing effect of cyclophosphamide remains to be determined in the our system.

An interesting observation in this study is the late tumor recurrence in the long-term surviving mice with an initial complete tumor regression after the combined treatment, a phenomenon observed frequently in clinical situations. Many potential mechanisms have been described that enable tumor evasion of host immunity (50–55). Because the tumor regression in our model was shown to be mediated by T cells, which are generally long lived, the recurrent tumors must have arisen in the presence of preexisting antitumor immunity and have evaded host immunity. Deletion of the high-affinity tumor antigen-specific T cells from the host or the emergence of tumor antigen-loss variants or chemoresistant tumor variants are among the many possibilities. In the current study, besides the known A20 idiotype protein, clearly other potential antigens were presented because the long-term surviving mice were also completely protected from the tumor rechallenge with an idiotype-negative A20 lymphoma cell line (data not shown). The identification of potential antigens presented by dendritic cells and the mechanisms of tumor recurrence merit further investigation. It is important to note that, whatever the tumor evasion mechanisms, the recurrent tumor was still susceptible to the effects of immunotherapy newly induced after tumor recurrence.

In this study, additional in vitro maturation of dendritic cells was intentionally avoided before the intratumoral injection with the hope that chemo-induced danger signals might be strong enough to induce dendritic cell maturation in situ during or after the antigen uptake process. Our preliminary experiment revealed that the direct intratumoral injection of naive dendritic cells matured in vitro by CpG oligonucleotide was equally effective (data not shown). Additional strategies to promote dendritic cell maturation in situ are currently being explored to make this approach more effective as abundant literature indicating that tumor or regulatory T cells secrete a number of suppressive factors (vascular endothelial growth factor, IL-10, transforming growth factor β, etc.) to inhibit dendritic cell maturation (15, 56–58).

The results in the present study have clinical implications. Because complete tumor regression and long-lasting tumor immunity was observed in this advanced murine lymphoma...
model, we suggest that the same strategy could be applied to treat patients with lymphomas. Lymphomas are among the most sensitive malignancies to chemotherapy, radiotherapy, and mAbs, but usually require retreatment due to tumor relapse. These treatments may be rendered even more effective by simultaneously and repetitively inducing an active immune response against the tumor with minimal additional toxicity by the maneuver described here.

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References

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