Effective Combination of Chemotherapy and Dendritic Cell Administration for the Treatment of Advanced-Stage Experimental Breast Cancer

Bin Yu, Sergei Kusmartsev, Fengdong Cheng, Maria Paolini, Yulia Nefedova, Eduardo Sotomayor, and Dmitry Gabrilovich

H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Tampa, Florida 33612

ABSTRACT

Purpose: The goal of this study was to investigate the utility of a new approach to the treatment of advanced stage breast cancer, a combination of chemotherapy and dendritic cell (DC) administration.

Experimental Design: Mice bearing mammary adenocarcinoma expressing a model tumor antigen, influenza virus HA (DA3-HA), and parental tumor (DA3) were treated with different doses of paclitaxel with or without DCs. Paclitaxel was injected three times weekly, DCs were injected either i.v. or into tumor site (t.s.) 36 h after each injection of paclitaxel. Apoptosis was measured using Annexin V binding or terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assays. CD8-mediated response of T cells to HA-derived peptide epitope was measured in an enzyme-linked immunospot (ELISPOT) assay. CD4-mediated response of T cells to HA-derived peptide was measured in proliferation assay. Nonspecific T-cell proliferation was measured in response to ConA and immobilized anti-CD3 and anti-CD28 antibodies.

Results: We have selected the dose of paclitaxel that induced a substantial level of apoptosis and moderate inhibition of T-cell function. Combined treatment resulted in the induction of HA-specific CD8-mediated response in all nine of the tested mice, and CD4-mediated responses in four of six treated mice. These effects were observed only if DCs were injected into tumor site, but not when injected i.v. No specific responses were found in mice treated with either chemotherapy or DCs alone. Injection of dexamethasone together with paclitaxel did not affect the induction of immune responses. Significant antitumor effect of combined treatment was observed in DA3-HA tumor-bearing mice as well as in mice bearing parental DA3 tumor.

Conclusion: The combination of DC administration with repeated cycles of chemotherapy and dexamethasone (conditions similar to real clinical practice) resulted in the induction of antitumor response despite the immunosuppression induced by such treatment.

INTRODUCTION

The demonstration that cells of the immune system are capable of destroying chemotherapy-resistant tumor cells (1, 2) points to cancer immunotherapy as a modality that may potentially broaden the treatment of patients with cancer. These findings, coupled with the advances in identification of TAAs,2 and the better understanding of the cellular and molecular mechanisms regulating host-tumor interactions have led to a renewed impetus to the development of novel immunotherapeutic strategies. Most TAAs exist in the form of MHC class I-restricted peptides or tumor-associated molecules, e.g., products of tumor suppressor genes or oncogenes. However, the use of defined TAAs has serious limitations. These limitations offset the theoretical advantage of having a well-characterized target. Defined TAAs are often not expressed as the same epitope on the majority of targeted tumors. Tumor-associated proteins often undergo a posttranslational modification that allows escape from recognition after immunization with the native form of antigen. The phenomenon of MHC class I restriction requires selection of the patients based on MHC class I alleles. The particular MHC class I molecule may be absent on some tumor cells. These limitations were in part the stimulus for a more comprehensive approach to the effective use of TAAs.

Tumor cells themselves are the richest TAA source. However, the mere presence of DCs in the vicinity of tumor has been insufficient to induce antitumor responses. DCs were recently identified to have unique pathways for phagocytosis, processing, and presentation of antigens from apoptotic cells (3, 4), and are capable of inducing potent immune responses (5, 6). It appears that the optimal presentation of antigens from tumor cells would require not only phagocytosis of apoptotic cells by DCs that have not matured yet, but also activation and maturation of DCs via exposure to the necrotic tumor cells or their products (7, 8). These new findings suggest a therapeutic possibility: exposure of DCs to tumor apoptotic bodies to provide TAAs together with an activation/maturation environment. The combination of apoptosis-inducing treatment with DC adminis-

2 The abbreviations used are: TAA, tumor-associated antigen; DC, dendritic cell; HA, hemagglutinin; FACS, fluorescence-activated cell sorting; IL, interleukin; GM-CSF, granulocyte macrophage colony-stimulating factor; ELISPOT, enzyme-linked immunospot (assay); TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; TCR, T cell receptor.
tration also eliminates the requirement for patient selection based on MHC class I type, for future clinical development. Similarly, it eliminates subject selection criteria based on the expression of defined TAAs and reduces the problem of TAA loss during tumor progression. Recently, several groups, including ours, described induction of antitumor response after DC administration into tumors with high spontaneous rates of apoptosis (9) or after the combination of DC administration with apoptosis-inducing therapy (10, 11). These experiments established proof-of-concept that the combination of the intratumoral placement of \textit{ex vivo} generated autologous DCs in concert with apoptosis-inducing therapy (chemotherapy or radiation) might lead to a generation of systemic antitumor immunity. In cancer patients conventional chemotherapy is given in several cycles over relatively long periods of time. It is known that chemotherapy is associated with systemic immune suppression. The obvious question is whether this environment will be permissible for the induction of effective immune response. This study, for the first time, has tried to address this question. We used a tumor model of mammary adenocarcinoma DA3. DC administration was combined with three cycles of paclitaxel at the dose that induces apoptosis and moderate immune suppression. We have found that such combined treatment induces significant antitumor immune response. This response was associated with marked antitumor effect.

**MATERIALS AND METHODS**

**Mice.** Six 8-week-old female BALB/c mice were purchased from the National Cancer Institute and housed in pathogen-free units in the H. Lee Moffitt Cancer Center, Tampa, FL. TCR-transgenic mice expressing an α/β T-cell receptor specific for MHC class II-restricted SFRFELLFPK peptide, derived from influenza HA, were originally obtained from Harold von Boehmer (Basel Institute for Immunology, Basel, Switzerland) and then were crossed to a BALB/c background for more than 10 generations.

**Description of the Experimental Model.** DA3 cells (provided by Dr. Diana M. Lopez, University of Miami School of Medicine, Miami, FL) is a well-characterized tumor cell line that was derived from the D1-DMBA-3 mammary tumor syngeneic to BALB/c mice (12). The DA3 cell line produces tumors in BALB/c mice with the same growth kinetics as the parent tumor. s.c. injection of 1 × 10⁶ DA3 tumor cells results in a solid tumor in the area of injection after about 5–7 days. Approximately 10 days after s.c. implantation, micrometastases are evident in the draining lymph nodes of tumor-bearing animals, and, as tumor progresses, additional metastases are found in the lungs but not in the liver or spleen. DA3-HA cells were generated by the transfection of DA3 cells with the construct pH1A, which encodes the HA molecule of the influenza virus A/PR/8/34 (H1N1). A stable transfectant was selected and expanded, and expression of the HA gene was confirmed by the presence of HA protein on the cell surface, as determined by FACS analysis using the anti-HA antibody H-18 as described elsewhere (13). HA contains two defined antigenic epitopes. One is MHC class I (K\textsuperscript{b}) restricted (IYSTVASSL). A CD8\textsuperscript{+} T-cell clone specific for a K\textsuperscript{b}-restricted peptide epitope of HA (14) proliferated vigorously and produced significant levels of IL-2 when incubated with DA3-HA cells \textit{in vitro}, whereas no IL-2 was detected in the supernatants of the same T cells cultured with DA3 wild type (data not shown). The other one is MHC class II (I-E\textsuperscript{b}) restricted (SFRFELLFPK). It induces a strong CD4\textsuperscript{+} T-cell response (15, 16). It should be pointed out that the expression of HA did not alter the immunogenicity of DA3 cells, because s.c. injection into BALB/c mice with either DA3-WT or DA3-HA resulted in tumor growth with similar kinetics (data not shown).

**Reagents.** The following antibody-producing hybridomas were obtained from American Type Culture Collection (Manassas, VA) and used as culture supernatants: anti-CD4 (L3T4, TIB-207), anti-CD8 (Lyt-2.2, TIB-210), anti-MHC class II (I-A\textsuperscript{4}, TIB-120). Mouse GM-CSF, and IL-4 were obtained from RDI (Flanders, NJ), ConA, purified anti-CD3 and anti-CD28 antibodies, FITC- or phycoerythrin-conjugated anti-CD11c, CD11b, CD86 (B7-2), and IA\textsuperscript{a} antibodies were purchased from PharMingen (San Diego, CA). Anti-clonotypic TCR (clone 6.5) were obtained from Caltag (Burlingame, CA). Isotype matched FITC- and phycoerythrin-conjugated IgG was used as a control of nonspecific binding. Complete culture medium included RPMI 1640, supplemented with 10% FCS, antibiotics, and 5 × 10⁻⁵ 2-mercaptoethanol.

**Cell Preparation and Analysis of Cell Surface Receptors.** DCs were generated from bone marrow of naïve syngeneic mice in complete culture medium supplemented with 20 ng/ml murine GM-CSF, 10 ng/ml IL-4 (RDI), and 50 μm 2-mercaptoethanol. The cells were maintained at 37°C in a 5% CO₂ humidified atmosphere. One-half of the medium was replaced on day 3. After 5–6 days in culture, cells were collected and enriched by centrifugation over a 13.5% metrizamide gradient (Accurate Chemical, Westbury, NY). The purity of the DC fraction was higher than 80% as determined by FACS analysis of surface molecules expression.

For evaluation of the specific and nonspecific T-cell responses a single-cell suspension was prepared from spleens, and red cells were removed by hypotonic shock using ACK lysis buffer. An enriched population of T cells were obtained from spleens using T-cell enrichment columns (R&D Systems).

**Antigen-specific T-Cell Proliferation.** Splenocytes were placed in triplicates into U-bottomed 96-well plates (10⁵ well) in the presence of 10 μg/ml HA-derived peptide (amino acids 110–120, SFRFELLFPK) or control OVA-derived peptide (amino acids 323–339). Cell proliferation was evaluated as described above. In parallel, wells were pulsed with [³²P]thymidine (1 μCi/well; Amersham, Arlington Heights, IL). [³²P]thymidine uptake was counted using a liquid scintillation counter and expressed as cpm.

**Non-specific T-Cell Proliferation.** T cells isolated from spleens were seeded in triplicates at a concentration 10⁵/well into U-bottomed 96-well plates. Cells were cultured for 4 days with various concentrations (1–10 μg/ml) of ConA. Cell proliferation was evaluated as described above. In parallel, wells were coated either with 0.25 μg/ml anti-CD3 antibody or with anti-CD3 antibody and 0.5 μg/ml anti-CD28 antibody. After the wells were washed, T-cells (10⁵/well) were incubated in triplicates for 4 days. Cell proliferation was evaluated as described above.
then incubated overnight at 4°C. Tinylated anti-IFN-antibody (PharMingen) was used. Reactions were developed using avidin-alkaline phosphatase and BCIP/NBT (PharMingen). Splenocytes (2 × 10^6 cells/well) were cultured for 24 h at 37°C in a 5% CO₂ incubator in the complete medium alone or in the presence of the specific or control peptides at a concentration of 10 μM. After that time, wells were washed and then incubated overnight at 4°C with a different clone of biotinylated anti-IFN-γ antibody (PharMingen). Reactions were visualized using avidin-alkaline phosphatase and BCIP/NBT substrate. The number of spots per 10^6 splenocytes, which represented the number of IFN-γ producing cells, was calculated blindly by two investigators (S.K. and D.G.).

ELISPOT Assay. The number of IFN-γ-producing cells was measured using ELISPOT assay. Briefly, Millipore MultiScreen-HA plates were coated with antimouse IFN-γ antibody (PharMingen). Splenocytes (2 × 10^6 cells/well) were cultured for 24 h at 37°C in a 5% CO₂ incubator in the complete medium alone or in the presence of the specific or control peptides at a concentration of 10 μM. After that time, wells were washed and then incubated overnight at 4°C with a different clone of biotinylated anti-IFN-γ antibody (PharMingen). Reactions were visualized using avidin-alkaline phosphatase and BCIP/NBT substrate. The number of spots per 10^6 splenocytes, which represented the number of IFN-γ producing cells, was calculated blindly by two investigators (S.K. and D.G.).

IL-2 and IFN-γ ELISA. ELISA was performed using antibodies and protocol developed by PharMingen. The sensitivity of the assays was 6 pg/ml.

Apoptosis Assay. To evaluate induction of apoptosis of tumor cells by Taxol, tumors were excised and minced on ice. Connective tissue was digested by 30-min incubation with 400 units/ml collagenase type V (Sigma) at 37°C, and single-cell suspension was prepared. Apoptosis of tumor cells was measured by staining with FITC-conjugated Annexin-V and PI (PharMingen) and by TUNEL assay using Apo-Direct kit (PharMingen) according to the manufacturer’s instructions.

RESULTS
Evaluation of Apoptosis Induced by Paclitaxel in Breast Tumor. Paclitaxel (Taxol) was selected because together with its analogue Taxotere (docetaxel) it is the most frequently used drug in breast cancer with proven apoptotic effect on tumor cells. First, we identified the dose of paclitaxel sufficient for induction of apoptosis in our experimental tumor model. As an experimental model of breast cancer, we used BALB/c mice inoculated s.c. with 10^6 DA3-HA cells. Paclitaxel was administered i.p. 7–10 days later when tumors reached 0.5–0.7 cm in diameter. Mice were sacrificed at different time points after injection of the drug, single-cell suspension was prepared from tumor tissues, and apoptosis was measured on FACScalibur flow cytometer using TUNEL and Annexin V binding assays. At least three mice were studied at each time point. Spontaneous apoptosis in untreated tumor-bearing mice was 2.5 ± 0.9%. Maximum tolerated dose of paclitaxel (15 mg/kg) induced apoptosis of tumor cells as early as 24 h after the injection (10.2 ± 2.2%; P < 0.05). The total proportion of propidium iodide-positive dead cells increased from 12.1 ± 3.7% in control to 42.2 ± 6.5% (P < 0.05). Peak of apoptosis was detected on days 2 and 3 after injection (15.6 ± 3.2% and 17.1 ± 2.5%, respectively). By day 7, the level of apoptosis was only 5.5 ± 2.2% (P > 0.05). To identify a minimal dose of the drug able to induce apoptosis, DA3-HA-bearing mice were given injections of different doses of paclitaxel, and the level of apoptosis was evaluated 48 h later. No statistically significant differences between the effects of 15 mg/kg and 10 mg/kg doses of paclitaxel were found. Injection of the drug at dose 7.5 mg/kg resulted in a comparable level of apoptosis (12.5 ± 3.7%), whereas 5 mg/kg of paclitaxel induced a substantially lower level of apoptosis (6.3 ± 2.2%; P < 0.05). Thus, 7.5 mg/kg was the minimal dose sufficient for induction of the substantial apoptosis of DA3-HA cells.

Effect of Chemotherapy on Function of T Cells. An adequate function of T cells is critically important for the success of immunotherapy. The main perceived limitation of the combination of chemotherapy and DC administration is immunosuppression induced by chemotherapy during the prolonged exposure to
the drugs. Here, we tested the effect of different doses of paclitaxel on the function of T cells in DA3-HA-bearing mice. T cell proliferation and IL-2 production in response to ConA, to immobilized anti-CD3 antibody, or to a combination of anti-CD3 and anti-CD28 antibodies were evaluated. Paclitaxel at the highest dose (15 mg/kg) induced a profound defect in T-cell function by day 7 after injection (Fig. 1). No defects in T-cell function were detected after the injection of 10 mg/kg or 7.5 mg/kg of the drug. Chemotherapy of cancer patients includes several cycles of treatment separated by 3–4 weeks. Repeated administration of drugs may cause much more serious defects of the immune system than does the single dose. To clarify this issue in our experimental system, paclitaxel was injected into mice three times with a 7-day interval. Mice were sacrificed 7 days after the second and third injections. Two injections of paclitaxel at a dose of 10 mg/kg caused profound decrease in T-cell response to all stimuli (Fig. 1). The dose of 7.5 mg/kg slightly reduced T-cell response to the combination of anti-CD3 and anti-CD28 antibodies. Three injections of the drug at the dose of 7.5 mg/kg resulted in a statistically significant, although moderate, decrease in T-cell response to all stimuli (Fig. 1). Thus, these data demonstrated that paclitaxel at the dose 7.5 mg/kg induced a substantial level of apoptosis in DA3-HA tumor with a moderate inhibition of T-cell function. This dosage was used in subsequent experiments.

![Fig. 2](image_url)

**Fig. 2** Effect of paclitaxel on antigen-specific T-cell response. T-lymphocytes were isolated from lymph nodes of HA transgenic mice using T-cell enriched columns. The presence of CD4/cloneotypic TCR cells in the total population of T-lymphocytes was evaluated using flow cytometry and double staining with anti-CD4 and anti-cloneotypic TCR (clone 6.5) antibodies. Lymphocytes from HA-TCR transgenic mice were adoptive transferred i.v. into naïve BALB/c mice (2.5 × 10^6 CD4/cloneotypic TCR cells per mouse). Three days later, mice were immunized with s.c. inoculation of 1 × 10^7 plaque-forming units of recombinant vaccinia virus encoding HA in 0.1 ml of PBS. Three days after immunization, mice were treated with 15 mg/kg or 7.5 mg/kg of paclitaxel. The treatment was repeated again, 1 week later, and mice were sacrificed 5 days after the last treatment. Each group included three mice. A, splenocytes were incubated for 4 days in U-bottomed 96-well plates. They were stimulated with 12.5 μg/ml specific HA-derived peptide. Proliferation of T cells was measured in triplicate using uptake of [3H]thymidine. B, T cells were isolated from the spleens of control mice or Paclitaxel-treated mice using T-cell enriched columns. T cells (2 × 10^5) and 2 × 10^5 irradiated splenocytes from naïve BALB/c mice were incubated in 200 μl of complete medium with or without 12.5 μg/ml specific HA-derived peptide. After 24 h of incubation, supernatants were collected, and the levels of IL-2 were evaluated using ELISA (PharMingen). C, T cells were isolated and stimulated with the specific peptide as described above. The levels of IFN-γ in supernatants were measured in ELISA (PharMingen); average ± SD from all tested mice are presented. *, statistically significant differences from the values of proliferation of T cells incubated without specific peptide (P < 0.05); #, statistically significant differences from control mice (P < 0.05).

![Fig. 3](image_url)

**Fig. 3** Phenotype of DCs used in the study. DCs were generated from bone-marrow progenitors in 24-well plates using GM-CSF and IL-4 as described in “Materials and Methods.” On day 5, 1 μg/ml LPS was added to one-half of the wells. The other one-half was left intact. Cells were collected 24 h later, DCs were enriched on metrizamide gradient and then labeled with monoclonal antibodies as indicated. Typical results are shown.
immune response, we used a previously described model with adoptive transfer of transgenic CD4⁺ T cells with TCR specific for HA-derived peptide epitope SFERFEIFPKE (16, 17). Lymphocytes from HA-TCR transgenic mice were adoptively transferred i.v. into naïve BALB/c mice (2.5 × 10⁶ CD4⁺ clonotypic TCR⁺ cells per mouse). Three days later, mice were immunized with 5–6 days after the last injection, and splenocytes were isolated and stimulated with control or specific peptides as described in the text. The number of IFN-γ-producing lymphocytes was evaluated in quadruplicate in ELISPOT assay as described previously (10). A, average ± SD for each mouse has been calculated and shown; B, background values (stimulation with control peptide) were subtracted from the results of the stimulation with specific peptide, and average ± SD has been calculated for each group of mice.

Fig. 4 Effect of combined administration of paclitaxel and DCs on CD8-mediated immune response. DA3-HA tumor was established in BALB/c mice, and tumor-bearing mice were treated as shown. Mice in all of the groups had equal tumor size (0.5–0.6 cm in diameter) at the beginning of the treatment. All of the mice received three rounds of treatment with 6–7-day intervals. DCs (3 × 10⁶) were injected 32 h after paclitaxel (7.5 mg/kg). Mice were sacrificed 5–6 days after the last injection, and splenocytes were isolated and stimulated with control or specific peptides as described in the text. The number of IFN-γ-producing lymphocytes was evaluated in quadruplicate in ELISPOT assay as described previously (10). A, average ± SD for each mouse has been calculated and shown; B, background values (stimulation with control peptide) were subtracted from the results of the stimulation with specific peptide, and average ± SD has been calculated for each group of mice.

Response to the specific peptide was evaluated. Treatment of mice with paclitaxel at a dose of 15 mg/kg abrogated peptidespecific T-cell proliferation and IL-2 production and substantially decreased IFN-γ production (Fig. 2). This effect was less pronounced at the 7.5-mg/kg dose of paclitaxel. This dose did not affect peptide-specific T-cell proliferation (Fig. 2A). However, it substantially decreased peptide-specific IL-2 and IFN-γ production (Fig. 2, B and C). These data are consistent with the results of a study of nonspecific T-cell function and indicate that even a low dose of paclitaxel is capable of inducing a moderate decrease in antigen-specific T-cell function.
Effect of the Combined Treatment on the Induction of Tumor-specific Immune Response. Immature DCs are very effective in taking up apoptotic or necrotic cells. Mature activated DCs lose this ability. Therefore, it was important to ascertain that cells used in this study were indeed immature DCs. We have compared the phenotype of DCs generated from bone marrow progenitors and prepared for injection as described in "Materials and Methods" with that of mature activated DCs, which were generated by additional incubation of cells for 24 h with 1 μg/ml LPS. Cells were double stained with anti-CD11c antibody and antibodies against MHC class II, CD40, or CD86 (B7-2). DCs generated from bone marrow progenitors expressed the CD11c marker but no CD40. Less than one-half of CD11c+ cells expressed MHC class II (IAα) and only one-third of CD11c cells expressed B7-2 (Fig. 3). LPS markedly activated DCs. The proportion of CD11c+IAα+ cells was increased 1.5-fold, CD11c−B7−2− cells almost 2-fold, and CD11c−CD40− more than 20-fold (Fig. 3). These data indicate that the population of DCs generated in our experiments was indeed immature, and could be activated on exposure to one of the DC activation stimuli LPS.

Tumors were established by s.c. injections of 10⁶ DA3-HA cells. On day 6–7, when tumors reached 0.5 cm in diameter, mice were given injections of paclitaxel (7.5 mg/kg). Thirty-two to 36 h later, 3 × 10⁶ DCs were injected either into tumor site (t.s.) or i.v. Control groups included mice treated with either paclitaxel alone or DCs alone or left untreated. Treatment was repeated twice with 6–7-day interval. Five to 6 days after the last injection, mice were sacrificed, and immune responses were evaluated. CD8-mediated immune response was measured.
Antitumor Effect of Combined Treatment. Mice were split into four groups (six mice/group): untreated mice (control), mice treated with paclitaxel alone, mice treated with DCs alone, and mice treated with the combination of paclitaxel and DCs. Mice were treated with three cycles of chemotherapy and DCs starting on day 7, when tumor reached 5 mm in diameter. The last treatment was performed on day 21. DA3-HA is a spontaneously metastatic tumor. By the end of the 5th week, most of the mice in the control group either succumbed to metastatic disease or were sacrificed because of bulky disease (tumor size exceeding 450 mm²). Paclitaxel alone only marginally increased the survival of mice (Fig. 6). However, combined treatment increased median survival from 29 days in control group to 45 days (P = 0.012; Fig. 6). In clinical practice, chemotherapy is combined with the administration of glucocorticoids to alleviate the toxic effect of chemotherapy. The standard dose of treatment includes the administration of 24–60 mg dexamethasone over 12–24 h, together with taxane infusion. This treatment is repeated with each cycle of chemotherapy. Glucocorticoids are known to suppress the immune system. We investigated the effect of glucocorticoids by comparing the effect of combined treatment with and without comparable doses of dexamethasone (3 µg/mouse). Inclusion of dexamethasone in three cycles of the treatment with paclitaxel and DC administration did not affect immune response (Fig. 7).

All of the previous experiments were performed with DA3-HA to evaluate the generation of HA-specific immune response. Next we tested antitumor effect of the combined therapy on parental cell line DA3. The groups of mice and treatment regimen were used as described above. Each group included six mice. Paclitaxel alone slightly decreased tumor growth, which quickly resumed after the cessation of the treatment. Administration of DCs alone did not affect tumor growth. However, combined treatment significantly delayed the tumor growth. By day 40, tumor size in mice treated with a combination of DCs, dexamethasone, and paclitaxel was almost 3-fold smaller than that in all of the other groups (Fig. 8).

DISCUSSION

Successful treatment of advanced stage breast cancer is a significant clinical problem. It becomes increasingly clear now that neither cytotoxic therapy nor immunotherapy alone would be able to successfully solve this problem. Chemotherapy frequently fails because of the development of drug resistance (18), and immunotherapy alone is not able to maintain effective antitumor immune response in the presence of bulky tumor because of the effects of tumor-derived immunosuppressive...
factors (19). It appears that only a combination of different modalities may provide the necessary breakthrough in the treatment of this group of patients. In the present study, we have evaluated a potential utility of the combination of chemotherapy with the intratumoral administration of the most potent professional antigen-presenting cells, DCs. This approach is based on recent findings that DCs are able to take up apoptotic cells and to process and present antigens associated with these cells (3, 4, 6, 8). Because tumor cells are a rich source of TAAs, this would represent a good opportunity to load DCs with a large number of different TAAs. These DCs could induce immune response to a variety of TAAs. In contrast to a response to one or several defined peptide epitopes, this response would not require patients selection based on HLA and would be less sensitive to a variation in expression of certain TAAs.

During the last year, three groups have reported the successful results of combining apoptosis-inducing treatment of tumors with intratumoral administration of DCs. Candido et al. (9) demonstrated that injections of DCs into the tumor with a high rate of spontaneous apoptosis resulted in antitumor effect. We have demonstrated (10) that the combination of γ-radiation with local DC administration into the tumor site resulted in the induction of tumor-specific immune response and had significant antitumor effect. Tong et al. (11) have shown that the combination of chemotherapy and DC administration led to a potent antitumor effect. These studies have established proof-of-concept and have demonstrated the therapeutic potential of this approach.

Chemotherapy is the most actively used modality in the treatment of advanced-stage cancer. It is well established that it is associated with significant immunosuppression (20–22). Because the success of combined treatment heavily depends on the adequate function of the immune system, this effect of chemotherapy may be critical for the success of combined treatment. Previous studies did not address this problem. In experiments with spontaneous apoptosis, no additional treatment was used (9). Local γ-irradiation of the tumor site used in our previous work has very little systemic immunosuppressive effect (10). In most of their experiments, Tong et al. (11) used two or three injections of the drugs every day or every other day with completion of the treatment within 3 or 4 days. In clinical practice, chemotherapy is a relatively long-term therapy that usually includes multiple cycles of the treatment separated by several weeks. This type of treatment may affect the immune system more profoundly than short-term administration of the same drug. Our experiments in the present study indeed confirmed this hypothesis. Paclitaxel at the dose of 10 mg/kg did not affect T-cell function after a single cycle of treatment. However, two cycles resulted in a profound decrease in T-cell response to stimulation. The same effect was observed at the dose of 7.5 mg/kg. Two cycles of treatments with the drug practically did not affect T-cell function. Three cycles, however, resulted in a significant decrease in T-cell function. These data indicate that repeated cycles of chemotherapy have a negative cumulative effect on the function of the immune system. To evaluate the effect of combined treatment, we have selected the dose of paclitaxel that induces a substantial level of apoptosis and moderate immunosuppression.

To accurately measure antitumor immune response, we used mammary adenocarcinomas that express HA. HA contains at least two known epitopes: matching MHC class I (Kd) and MHC class II (IEd). We hypothesized that, if combined treatment was able to induce tumor-specific immune response, it might manifest in peptide-specific responses. And, indeed, we detected specific CD8-mediated response in all nine of the mice treated with the combination of chemotherapy and DC administration, and we detected CD4-mediated response in four of six treated mice. Such a response was not observed if mice were treated either with chemotherapy alone or with DC administration alone. However, administration of DCs into the tumor site
Untreated or were treated with three rounds of paclitaxel (7.5 mg/kg) and 3 μg of dexamethasone (PAC+DEX), DCs alone (DCs), or a combination of paclitaxel, dexamethasone, and DC administration (DCs+PAC+DEX). Differences in tumor size between the first three groups and the last group became statistically significant by day 32.

Without chemotherapy resulted in increased background in the ELISPOT assay. Although the precise mechanism of this phenomenon is not clear, we may suggest that DCs became activated after administration into the tumor site and subsequent migration to the draining lymph nodes. Such activated DCs are able to present a variety of tumor-nonspecific antigens and stimulate T cells. It is important to note that i.v. injections of DCs were also ineffective. We have previously demonstrated that only a small proportion of DCs injected i.v. could reach the tumor site (10). Apparently, the number of DCs after i.v. injection was not sufficient to induce immune response. In clinical practice, chemotherapy is combined with the administration of glucocorticoids to alleviate the toxic effects of chemotherapy.

We tested the effect of dexamethasone on the induction of antitumor immune response. In our experimental model, short-term administration of dexamethasone did not have a negative effect on the induction of antitumor immune response.

Combined treatment had a clear antitumor effect, which manifested in the prolonged survival of the mice. An antitumor effect was observed not only in mice bearing tumor expressing HA (DA3-HA) but also in mice bearing parental tumor (DA3). The antitumor effect observed in our study was less dramatic than in the previously reported work (11) and did not result in tumor eradication. Evidently, repeated cycles of chemotherapy, which resulted in moderate immunosuppression, might decrease the efficacy of combined treatment.

Thus, in this study, we have demonstrated, for the first time, that the combination of DC administration with repeated cycles of chemotherapy and dexamethasone (conditions similar to real clinical practice) resulted in the induction of antitumor immune response despite the immunosuppression induced by such a treatment. This combined treatment led to a significant antitumor effect. Our results indicate that the clinical success of this method may depend on careful selection of the dosage of the drugs. The effect of the treatment may also be increased if we find the optimal ways to decrease chemotherapy-induced immunosuppression.

REFERENCES


