Plasmacytoid Dendritic Cells Alter the Antitumor Activity of CpG-Oligodeoxynucleotides in a Mouse Model of Lung Carcinoma

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*J Immunol* published online 20 September 2010
http://www.jimmunol.org/content/early/2010/09/20/jimmunol.1000881
Plasmacytoid Dendritic Cells Alter the Antitumor Activity of CpG-Oligodeoxynucleotides in a Mouse Model of Lung Carcinoma

Rosalinda Sorrentino,* Silvana Morello,* Antonio Luciano,† Timothy R. Crother,‡ Piera Maiolino,*,† Eduardo Bonavita,* Claudio Arra,† Ian M. Adcock,§ Moshe Arditi,‡,† and Aldo Pinto*†

The effect of CpG-oligodeoxynucleotides (CpG) has been studied on a number of tumors. Although CpG may facilitate tumor regression in mouse models of melanoma, its activity in lung cancer is unclear. The aim of our study was to elucidate the effect of CpG (0.5–50 μg/mouse) in a mouse model of Lewis lung carcinoma cell-induced lung cancer. Lung tumor growth increased at 3 and 7 d after a single administration of CpG. This was associated with a greater influx of plasmacytoid dendritic cells (pDCs), immature myeloid dendritic cells, and greater recruitment of regulatory T cells. Depletion of pDCs using a specific Ab (m927) reversed the immune-suppressive environment and resulted in a decreased lung tumor burden, accompanied by a greater influx of active myeloid dendritic cells and CD8+ T cells, and a higher production of Th1- and Th17-like cytokines. Furthermore, the rate of apoptosis in the lungs of mice treated with CpG increased following the depletion of pDCs. CpG treatment alone does not lead to tumor regression in the lung. However, ablation of pDCs renders CpG a good adjuvant for lung cancer chemotherapy in this experimental model. The Journal of Immunology, 2010, 185: 000–000.

Lung carcinoma is one of the leading causes of death worldwide. Despite advances in treatment, the prognosis remains poor, with only 15% of patients surviving more than five years from time of diagnosis. Lung carcinoma is a non-immunogenic cancer (1, 2), which renders it more resistant to immune surveillance. Activation of the immune system could, therefore, represent a means to induce tumor regression (2, 3).

TLRs recognize conserved microbial and endogenous molecules and can function as an “alarm signal” for the immune system to initiate the appropriate host immune response (3–5). Increasing evidence has highlighted the involvement of TLRs in cancer. In the nineteenth century, William Coley discovered that repeated injections of bacterial toxins (later identified as LPS-Coley’s toxins) could serve as efficient antitumor adjuvants (1). Since then, numerous studies have been conducted on TLR ligands investigating their potential as antitumor agents. Indeed, a synthetic ligand for TLR4, monophosphoryl lipid A; a synthetic ligand for TLR7, imiquimod; and other nonstimulant compounds, such as CpG (15), which induces a potent antitumor activity. The local administration of CpG-oligodeoxynucleotides (CpG, a TLR9 ligand) has shown a substantial antitumor activity (6). The local administration of CpG-oligodeoxynucleotides (CpG, a TLR9 ligand) reduced tumor progression in animal models of melanoma and renal carcinoma (6), possibly through enhancement of an efficient Th1-like and T cytotoxic immunity (1, 6).

The administration of CpG into the lungs of normal or naive mice can lead to a significant accumulation of inflammatory cells and overproduction of proinflammatory cytokines (7). In contrast, the administration of CpG in animal models of asthma has opposite effects because CpG induces the activation of plasmacytoid dendritic cells (pDCs) (8), which play an important immune-regulatory role in the airways during chronic inflammation (9, 10). In addition, CpG can also promote an influx of T regulatory (Treg) cells (11) that may render the site of inflammation immune suppressive through the activation of STAT-3, which constrains CpG antitumor activity in melanoma (12). Indeed, the administration of CpG conjugated with a STAT-3 small interfering RNA resulted in greater tumor regression in an animal model of melanoma (13). Furthermore, a phase III clinical study of CpG in lung cancer was ineffective (6), reflecting the fact that CpG enhanced the proliferation of human lung cancer cell lines (14).

Dendritic cells (DCs) are professional APCs whose influence can determine the immune host response against any type of insult. DCs are usually present in large numbers at the tumor microenvironment, although they are in their immature state (6). One recent antitumor strategy is based upon the activation of DCs with immunostimulant compounds, such as CpG (15), which induces a potent antitumor cytotoxic adaptive immune response.

Given these previous studies, we wanted to determine whether CpG could affect lung tumor growth in a mouse model of lung carcinoma. In this study, we show that CpG administration exacerbated lung tumor growth in mice, due to an increased immune-suppressive environment, which correlated with a greater presence of pDCs and immature myeloid DCs (mDCs), and a higher re-

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The online version of this article contains supplemental material.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1000881
ruitment of Treg cells. The ablation of pDCs using a specific Ab reversed this tumor-promoting phenotype, and allowed an effective Th1-, Th17-like, and T cytotoxic immune response in the lungs of tumor-bearing mice.

Materials and Methods

Mice

Female specific pathogen-free C57BL/6J mice (6–8 wk) were purchased from Harlan Laboratories (Milan, Italy), fed a standard chow diet, and housed under specific pathogen-free conditions at Istituto Nazionale Tumori, Fondazione G. Pascale. All animal experiments were performed under protocols that followed the Italian and European Community Council for Animal Care (DL.no 116/92).

Cell culture

Lewis lung carcinoma cells (LLC1) were provided by G. Shurin (Pittsburgh, PA). Cells were cultured in RPMI 1640 containing 10% FBS, L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) in an atmosphere of 5% CO2 at 37°C.

Experimental protocol

Mice were injected i.v. with $2 \times 10^5$ of LLC1 (day 0), and 10 d later CpG (type B in PBS; Vincibiochem, Milan, Italy) was administered by the i.p. route. CpG was injected once, and mice were sacrificed at day 3 or 7. Lung, spleen, and mediastinal lymph nodes (LNs) were isolated. In some experiments, pDC-depleting Ab (m927 Ab [rat IgG], 500 μg/mouse; i.p.) (16) was administered on day 11 or on days 11, 13, and 15 before mice were sacrificed. The depleting Ab was injected i.p. on day 11 for experiments at 3 d post-CpG treatment and on days 11, 13, and 15 for experiments at 7 d post-CpG treatment. pDCs were depleted 1 d after CpG or PBS treatment. This time point was chosen because we observed a higher influx of pDCs into the LNs and spleen 24 h after CpG administration (data not shown). m927 Ab depleted lung pDCs by ~95% compared with IgG (data not shown). m927 Ab was injected every 2 d because the turnover of pDCs into the lung was ~48 h (data not shown).

Lungs, LNs, and spleens were isolated and digested with 5–10 U/ml collagenase and 20 μg/ml DNase I (both Sigma-Aldrich, Rome, Italy). Cell suspensions were passed through 70-μm cell strainers, and RBCs were lysed. Cell suspensions were used for flow cytometric analysis of different cell subtypes. Bronchoalveolar lavage (BAL) fluid was collected using 0.5 ml PBS containing 0.5 mM EDTA, and cell counts were performed. In addition, lungs were homogenized and cytokines were measured.

Flow cytometry analysis

The composition of lung inflammatory cells was determined by flow cytometry (BD FACS Calibur, Milan, Italy) using the following Abs: CD11c FITC, CD11c PE, CD11b PeCy5.5, Gr1 PE, CD3 PeCy5.5 or PerCP, CD4 PE or FITC, CD8 PE, F4/80 PE, B220 PE, CD19 PeCy5.5, Siglec H PerCP, MHC II PE, CD80 PE, CD25 PE, FoxP3 PeCy5.5 (eBioscience, San Diego, CA), annexin V FITC, and propidium iodide (Bender Med-Systems, Milan, Italy).

FIGURE 1. CpG significantly increased tumor lesions in LLC1-implanted mice after 3 d. A, Experimental protocol: CpG (0.5–5–50 μg/mouse) was administered i.p. 10 d after i.v. injection of LLC1 (2 × 10^3 cells/mouse). Lung histology was assessed 3 d after CpG administration. B, H&E staining of lung sections in naive, PBS-, and CpG-treated mice (original magnification ×20). C, Ratio tumor lesions/lung, analyzed as tumor area compared with total lung section area and D, immunohistochemistry analyses of Ki-67 and survivin (E) expression, in the lungs of naive mice and 3 d after administration of PBS or CpG. Ki-67- and survivin-positive cells were calculated by means of Image J Software and expressed as Ki-67- or survivin (FITC)-positive staining/μm² lung cryosections. F, TLR9 expression was increased in PBS-treated mice compared with naive animals. CpG treatment did not further modulate TLR9 expression. Data represent mean ± SEM, n = 12. Experiments were performed in three different experimental days. *p < 0.05; **p < 0.01; ***p < 0.005, as determined by one-way ANOVA and Student t test.
TNF-α, IL-6, IL-12p40, IL-10, IFN-γ, IL-17A, and IgG2b were measured in homogenate and BAL by using commercially available ELISAs (R&D Systems and eBioscience, London, U.K.; Bethyl Laboratories, Montgomery, TX).

**Immunohistochemistry**

Left lung lobes were fixed in OCT medium (Pella, Milan, Italy), and 7-µm cryosections were cut. H&E staining was performed and used to measure the tumor burden. Tumor lesions were analyzed by using serial lung cryosections and expressed as tumor lesions/lung (area in mm²), as determined by the ratio of the tumor lesions area compared with the total lung area (Image J Software, National Institutes of Health, Bethesda, MD). Anti–Ki-67 (Dako, London, U.K.) and anti-TLR9 (eBioscience) were used to evaluate tumor proliferation and TLR9 expression, respectively. The diaminobenzidinic acid system was used to detect complexes. Rat IgG was used as an isotype control (eBioscience). Data were expressed as Ki-67– or TLR9-positive cells/mm² lung section determined by using Image J Software (National Institutes of Health).

**Immunoblot analysis**

Lungs were homogenized in radio-immunoprecipitation assay buffer and then analyzed by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were immunoblotted with anti–Bcl-2, caspase-3 Abs (Santa Cruz Technologies, Santa Cruz, CA), or an anti-tubulin Ab (Sigma-Aldrich).

**Statistical analysis**

Results are expressed as means ± SEM. Changes observed in treated groups compared with controls were analyzed using one-way ANOVA, followed by Bonferroni’s posttest and Student t test. The p values, **p < 0.05; ***p < 0.005; ****p < 0.0001, as determined by one-way ANOVA, followed by Bonferroni’s posttest and/or Student t test.

**Results**

CpG administration increased lung tumor growth

To investigate the role of CpG in lung carcinoma, we used a mouse model by which LLC1 were i.v. injected into the tail vein of C57BL/6j mice (Fig. 1A). We used LLC1 because they have a high tropism for the lungs when injected i.v. (17, 18) and they can mimic human lung carcinoma (19). Ten days post-LLC1 inoculation, CpG (0.5–50 µg/mouse) was administered i.p. Mice were sacrificed 3 d after the single administration of CpG at day 10 (Fig. 1A).
The systemic administration of CpG induced a significant dose-dependent increase in the growth of lung carcinoma in these mice (Fig. 1B, 1C). The increase in tumor growth was measured by assessing the tumor area in H&E-stained sections (Fig. 1B, 1C) and by the number of cells positive for Ki-67 (Fig. 1D), a tumor-cell proliferation marker (20). The tumor lesions were significantly increased in a dose-dependent manner following the administration of CpG (0.5 μg/mouse, 0.34 ± 0.15; 5 μg/mouse, 0.505 ± 0.12; 50 μg/mouse, 0.79 ± 0.19) compared with PBS-treated (0.178 ± 0.02) lung tumor-bearing mice (Fig. 1C). In addition, the nuclear detection of Ki-67 in lung cryosections was dose dependently increased in the lungs of tumor-bearing mice treated with CpG (0.5 μg/mouse, 28.8 ± 7.8; 50 μg/mouse, 62.7 ± 15.9) compared with PBS (20.5 ± 2.6) or naive-normal control nontumor mice (4.8 ± 0.2; Fig. 1D). The overproliferation of cells was particularly high at the bronchioles, which are the preferential zone from where human lung cancerous cells start spreading (1, 6).

The alveolar structure was also completely altered in mice treated with the highest dose of CpG (50 μg/mouse; Fig. 1B). To confirm tumor growth in the lungs, we also evaluated the expression of survivin, a proliferation and antiapoptotic marker (21, 22). We observed a greater increase in cells staining for survivin when mice were treated with CpG, especially with the higher dose (50 μg/mouse), than in PBS and naive mice (Fig. 1E). Moreover, the levels of vascular endothelial growth factor were increased in a dose-dependent manner after CpG administration compared with PBS (data not shown).

Human lung cancerous tissues have a high expression of TLR9 (23, 24). Immunohistochemistry staining showed that TLR9 expression increased in PBS-treated mice compared with naive mice. Administration of CpG did not further modulate TLR9 expression (Fig. 1F).

To determine whether CpG could induce lung inflammation in tumor-bearing mice as described for normal mice (7), we evaluated...
the number of cells in the BAL of PBS- and CpG-treated mice. BAL cell numbers were higher in PBS-treated tumor-bearing mice (1.6 ± 0.5) compared with CpG-treated tumor-bearing mice (0.5 μg/mouse, 0.6 ± 0.25; 50 μg/mouse, 0.6 ± 0.25; Supplemental Fig. 1). Furthermore, BAL IFN-α levels were increased in CpG-treated mice (data not shown). These results indicate that lung inflammation was attenuated by CpG treatment in lung tumor-bearing mice.

CpG administration increased pDCs, but not mDCs, to the lungs of tumor-bearing mice  

To explain why CpG treatment enhanced the lung carcinoma burden in our model, we determined the identity and numbers of leukocytes recruited to the lungs 3 d after CpG treatment. We digested the lungs and performed flow cytometry analyses for mDCs and pDCs. mDCs were identified as CD11c high, CD11b positive, and F4/80 negative. pDCs were determined as CD11c negative/low, B220 positive, and Siglec H positive. pDCs were also assessed by gating for CD11c low and Gr-1 intermediate cells (data not shown), as previously reported (25). The percentage of mDCs was only slightly increased in the lungs of mice injected with the higher dose of CpG (50 μg/mouse) compared with PBS-treated tumor-bearing mice (Fig. 2A, 2B). However, the expression of MHC II was significantly increased on these (CD11c+CD11b+) cells in a dose-dependent manner after CpG administration (Fig. 2C; isotype control mean fluorescence intensity [MFI]: 174.43 ± 43.13). Furthermore, the expression of CD80, another DC maturation marker (25), was significantly decreased on CD11c+CD11b+ cells in the lungs following CpG administration (Fig. 2D; isotype control MFI: 159.17 ± 29). These results demonstrated that the mDCs detected in the lungs were only partially mature (MHC II+ but CD80−), which suggests that CpG induces a tolerogenic/immature state in lung mDCs.

The immature state of mDCs was also described in a virus infection model by Kool and Lambrecht (26), who demonstrated that the mDCs detected in the lungs were only partially mature (CD11c+CD80+). These mDCs were responsible for the tolerogenic/immature state of mDCs.

Depletion of pDCs increased lung inflammation in CpG-treated tumor-bearing mice

To better understand the role of pDCs in our experimental model, we depleted pDCs using a specific depleting Ab (16), m927, given 1 d (i.p.) after CpG administration (day 11) in LLC1-implanted mice. The injection of m927Ab or IgG control 1 d after CpG administration was based upon preliminary data, which showed that there was a high influx of pDCs to the LNs and spleen 24 h after CpG injection (data not shown), implying a recruitment of pDCs from the circulatory system to the lungs at 3 d (Fig. 2E, 2F).

Mice were sacrificed at day 13 (3 d post-CpG injection; Fig. 3A). The administration of the IgG control did not alter the morphology of the lungs previously observed when CpG was administered alone in LLC1-implanted mice (Fig. 3B versus Fig. 1B). In contrast, the injection of m927 increased the influx of inflammatory cells to the lungs of tumor-bearing mice (H&E representative panels in Fig. 3B). This was confirmed by the BAL cell counts, which had a 100-fold increase in BAL cell numbers in animals treated with 50 μg/mouse of CpG (0.9 ± 0.3 versus 112.6 ± 29.7, p < 0.001; Fig. 3C).

FIGURE 4. The effect of pDC depletion on BAL cytokine levels at 3 d post-CpG administration. Depletion of pDCs using the m927 Ab resulted in increased TNF-α (A) and IFN-γ (B) levels in the BAL of CpG (0.5–50 μg/mouse)-treated mice. In contrast, IL-6 (C) and IFN-α (D) levels were reduced after depletion of pDCs. Data represent mean ± SEM, n = 9. Experiments were performed in three different experimental days. *p < 0.05; **p < 0.01; ***p < 0.005, as determined by one-way ANOVA, followed by Bonferroni’s posttest and Student t test.
Furthermore, the depletion of pDCs led to a dose-dependent increase in the percentage of CD11c⁺CD11b⁺ cells (mDCs) in the lungs after CpG treatment (Fig. 3D). Importantly, in contrast to data previously observed (Fig. 2), the depletion of pDCs resulted in the complete maturation of mDCs because they were both MHC II⁺ (Fig. 3E) and CD80⁺ cells (Fig. 3F). Altogether these results confirmed our hypothesis that the presence of pDCs in the lungs of tumor-bearing mice could drive the mDC phenotype to an immature state, thereby facilitating an anti-inflammatory effect.

Moreover, to evaluate the tumor environment after the depletion of pDCs at 3 d, we measured the expression of a number of proinflammatory cytokines (TNF-α, IL-6, IFN-α, and IFN-γ) in the BAL fluid (Fig. 4). The ablation of pDCs increased the release of TNF-α (Fig. 4A) and IFN-γ (Fig. 4B), but significantly reduced IL-6 (Fig. 4C) and IFN-α (Fig. 4D) release after CpG treatment. These data correlated with the increased inflammatory responses in the lungs of tumor-bearing mice depleted of pDCs.

The presence of pDCs enhanced the recruitment of Treg cells to the lungs of tumor-bearing mice after CpG treatment

Human and mouse pDCs can drive CD4⁺ T cells to the generation of CD4⁺CD25⁺Foxp3⁺ cells, which are identified as Treg cells (29, 30). The role of Treg cells in a tumor microenvironment can be translated into anergy and immune suppression, favoring the immune escape of tumor cells (31). To evaluate whether the increase of pDCs in the lungs could lead to the recruitment of Treg cells, we measured the influx of CD4⁺CD25⁺Foxp3⁺ cells by flow cytometry. Treg cells were identified as CD4⁺CD25⁺Foxp3⁺ cells. The percentage of lung Treg cells was increased in the lungs of mice treated with the higher dose of CpG (Fig. 5A, 5B) and were higher in the proximal LNs when the lower dose of CpG (0.5 μg/mouse) was injected (data not shown).

Depletion of pDCs significantly decreased the percentage of Treg cells in the lungs of 50 μg/mouse CpG-treated mice compared with the PBS mice (0.5 ± 0.1 versus 1.8 ± 0.5, p < 0.01; Fig. 5B). As a consequence of the decreased immune-suppressive environment, we observed an influx of CD3⁺CD8⁺ cytotoxic lymphocytes into the LNs (Fig. 5C) and the spleens (Fig. 5D) of pDC-depleted mice that received CpG. However, we were not able to detect any relevant changes for CD3⁺CD8⁺ T cells in the lungs of pDC-depleted mice (data not shown), possibly because the time of sacrifice (3 d post-CpG treatment) was not sufficient to allow the migration of these lymphocytes from the lymphoid organs to the damaged tissue.

**FIGURE 5.** Depletion of pDCs decreases lung Treg cells and increases CD8⁺ cells in the LNs and spleen. A, Representative dot plots of CD4 FITC-, CD25 PE-, and Foxp3 PeCy5.5-positive (+) Treg cells in the lungs of pDC-depleted (m927 Ab-treated, 500 μg/mouse) or IgG-treated mice. B, Depletion of pDCs by m927 Ab decreases CpG (50 μg/mouse)-induced Treg cells. Depletion of pDCs by m927 Ab increases CpG (50 μg/mouse)-induced CD3⁺CD8⁺ cells in the LN (C) and spleen (D) compared with IgG-treated mice. Data represent mean ± SEM, n = 9. Experiments were performed in three different experimental days. *p < 0.05; **p < 0.005; ***p < 0.0001, as determined by one-way ANOVA, followed by Bonferroni posttest and Student t test.
Our previous results indicate that the absence of pDCs could favor the influx of CD8+ T cells in the lymphoid organs, but not in the lungs. We attributed this absence to the time mice were sacrificed. Thus, we prolonged the depletion of pDCs to 7 d with m927 Ab by injecting every other day to maintain depletion of pDCs (Fig. 6A).

Mice that were not depleted of pDCs (IgG treated) showed a massive tumor proliferation in the lungs at 7 d compared with the lungs at 3 d (Fig. 6B, 6C). This was confirmed using Ki-67 staining (20.47 ± 2.6 versus 150.7 ± 14; Fig. 6D versus Fig. 1D). Prolonged depletion of pDCs arrested tumor growth (1.17 ± 0.17 versus 0.29 ± 0.11; Fig. 6C) and tumor cell proliferation in mice treated with 50 μg/mouse of CpG (173.7 ± 17 versus 126 ± 4.5, p < 0.01; Fig. 6D). In support, H&E staining of these groups of mice showed that there was an increased influx of inflammatory cells into the lungs, but a reduced layer of cancerous cells (Fig. 6B).

We did not observe any difference between IgG- and m927 Ab-treated mice that received PBS at 7 d (Fig. 6C, 6D).

The administration of CpG induced a higher percentage of Treg cells in the lungs compared with PBS in IgG-injected mice (Fig. 6E, N). In contrast, the prolonged pDC depletion at 7 d still maintained a low level of Treg cells in the lungs of CpG-treated mice (Fig. 6E, n).
FIGURE 7. The absence of pDCs increases the cytotoxic effect on lung tumor cells after CpG administration. A. The presence of the pDC-depleting Ab m927 reduced Bcl-2 expression (28 kDa) in mice that received 50 µg/mouse of CpG. Depletion of pDCs resulted in increased IFN-γ (B) and IL-17A (C) expression in lung homogenate tissues from CpG-treated mice. Data represent mean ± SEM, n = 7. Experiments were performed in two different experimental days. *p < 0.05; **p < 0.01; ***p < 0.005, as determined by one-way ANOVA, followed by Bonferroni’s posttest and Student t test.

More importantly, the arrest of tumor growth at 7 d was correlated with the detection of CD8+ T cells in the lungs of pDC-depleted mice that had received CpG (50 µg/mouse) compared with PBS (Fig. 6F). To confirm that these cells were able to exert a cytotoxic effect on lung tumor cells, we measured the apoptotic rate of lung-derived cells after the depletion of pDCs for 7 d following CpG treatment. Lungs were excised and stained for annexin V and propidium iodide and analyzed by flow cytometry. IgG-treated mice did not show any difference in double-positive annexin V/propidium iodide cells after CpG treatment (Supplemental Fig. 3A, D). In contrast, the prolonged depletion of pDCs increased the number of apoptotic cells in mice that received the higher dose of CpG (Supplemental Fig. 3A, D). These data were further confirmed by the reduced expression of the anti-apoptotic protein Bcl-2 in m927 Ab-treated mice compared with IgG-treated mice after CpG injection (Fig. 7A). Concomitantly, we also observed increased cleavage of caspase-3, a hallmark of apoptosis (32), in the lungs of m927 Ab-treated mice compared with IgG-treated mice after CpG administration (Supplemental Fig. 3B).

To better identify the tumor microenvironment after the depletion of pDCs, we measured representative cytokines that are linked to an effective adaptive immunity (IFN-γ and IL-17A). There was no statistical difference in the expression of these cytokines in BAL fluid between IgG- and pDC-depleted mice after CpG treatment (Supplemental Fig. 4A, B). However, we observed an increased level of IFN-γ (Fig. 7B) and IL-17A (Fig. 7C) in the lung homogenates derived from pDC-depleted mice compared with IgG-injected mice after CpG (50 µg/mouse) treatment. Collectively, these data demonstrate that the absence of pDCs could favor CpG activity toward a Th1- and Th17-like cytokine milieu that is responsible for an immunosuppressive environment in the lungs of tumor-bearing mice, characterized by immature mDCs and an influx of Treg cells. In contrast, the depletion of pDCs after CpG administration led to a decreased tumor burden, which correlated with a greater influx of active CD8+ T cells, mDCs, and Th1- and Th17-like cytokines that favored an increased cancer cell apoptotic rate following CpG treatment.

CpG is currently being tested in multiple phases II and III human clinical trials as adjuvant to cancer vaccines and in combination with other therapies (1, 2). Basic research on animal models of melanoma demonstrated that CpG could be an efficient chemotherapeutic because it reduced tumor volume. To our knowledge, our study is the first to demonstrate that CpG facilitated cancer regression in the lungs of tumor-bearing mice.

CpG is a TLR9-specific ligand whose role in cancer is far from clear. Several reports suggest that CpG is beneficial against some cancers, but other studies suggest that because cancer is related to a chronic, nonresolved inflammatory response, the presence of CpG could induce tissue damage and subsequently lead to an increased cell transformation (3). However, Liu et al. (33) demonstrated that the activation of pDCs via CpG could induce NK cell-dependent tumor regression in melanoma animal models. In contrast, in our experimental model of lung cancer, the activation of pDCs through CpG had the opposite effect in that the activation of pDCs increased the recruitment of Treg cells and limited the inflammatory cell influx to the lungs, thereby establishing an immunosuppressive environment enabling tumor growth.

Although pDCs were initially described as IFN-producing cells, they can behave differently depending upon the local environment they encounter (31, 33). Tumor lesions are typically described as immunosuppressive (6), and under these conditions pDCs behaved as immune regulatory cells that altered the mDC phenotype to a Th1- and Th17-like pro-inflammatory pattern. This phenomenon has already been described in other models of lung disease, including asthma (9), virus infection (35), and cigarette smoke exposure (36). Overall, this dichotomy in potential CpG effects on pDC function may explain why CpG therapy has been successful, in part, in melanoma, but unsuccessful in a phase III clinical trial for lung cancer (6).

Interestingly, we found that IL-17A was highly expressed 7 d following depletion of pDCs in CpG-treated mice. The IL-17 family of cytokines has been found to be involved in both pro- and anti-inflammatory processes depending on the local context (37). IL-17A was recently described as being involved in tumor pro-

Discussion

In this study, we investigated the effects of CpG in a mouse model of lung carcinoma. We demonstrated that CpG increased tumor growth in the lungs of LLC1-implanted mice. This phenomenon was correlated to the increased presence of pDCs that were responsible for an immunosuppressive environment in the lungs of tumor-bearing mice, characterized by immature mDCs and an influx of Treg cells. In contrast, the depletion of pDCs after CpG administration led to a decreased tumor burden, which correlated with a greater influx of active CD8+ T cells, mDCs, and Th1- and Th17-like cytokines that favored an increased cancer cell apoptotic rate following CpG treatment.

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gression via the activation of STAT-3 (12, 13, 38) following lig-
igation of TLR9. This study was conducted in a mouse model of
melanoma, and this discrepancy with our results could be due to
a tissue specificity. In support of our data, IL-17–deficient mice
have increased lung tumor growth (39–41). This emphasizes the
need for further investigation of the role of IL-17 in lung carcinoma.

The presence of PGE2 (42) and the production of IgE (43) can
counter pDC activation via the inhibition of IFN type I. Because
lung cancer is a Th2-like pathology during which PGE2 and IgE
counter pDC activation via the inhibition of IFN type I. Because
We thank Dr. A. Montinaro and G. Forte for technical support.

The need for further investigation of the role of IL-17 in lung carcinoma.

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need for further investigation of the role of IL-17 in lung carcinoma.

Thus, because CpG can induce either a proinflammatory or a
tolerogenic response in this model of lung cancer, we feel that
careful transition into the effects of CpG monotherapy for lung
cancer must be performed. Our data indicate that CpG, in com-
bination with molecules capable of inhibiting the suppressive im-
mu ne environment in the lung cancer, might be more successful in
preventing tumor growth in the lungs. In support of our study,
previous reports found that the activation of STAT-3–dependent
signaling pathways by CpG can lead to the release of tolerogenic
cytokines, such as TGF-β and IL-10 (12, 13). Indeed, the com-
bination of CpG with a small interfering RNA for STAT-3 ren-
dered CpG more active against melanoma growth in mice (12, 13).
Similarly, our study opens a new therapeutic perspective for CpG
as an anticancer adjuvant, but only in association with other ther-
api es that alter the tolerogenic immune pattern in the lungs.

In conclusion, our study demonstrates that pDCs mediate the
activity of CpG and prevent its ability to mount an effective im-
mu ne response against lung cancer. The increase in pDCs re-
sulted in reduced numbers of mature mDCs, increased Treg cells,
and enhancement of the immunosuppressive environment in the lungs
of tumor-bearing mice. Depletion of pDCs allowed CpG to induce
a proinflammatory response that resulted in reduced tumor growth.
Thus, the depletion of the tolerogenic pDC component induced
by CpG could render CpG a good adjuvant for lung cancer che-
motherapy and allow an effective T cytotoxic immune response
against lung cancer.

Acknowledgments

We thank Dr. A. Montinaro and G. Forte for technical support.

Disclosures

The authors have no financial conflicts of interest.

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