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Many cancers are known to produce high amounts of PGE2, which is involved in both tumor progression and tumor-induced immune dysfunction. The key enzyme responsible for the biological inactivation of PGE2 in tissue is NAD-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH). It is well established that cancer cells frequently show down-regulated expression of 15-PGDH, which plays a major role in catabolism of the PGE2. Here we demonstrate that tumor-infiltrated CD11b cells are also deficient for the 15-PGDH gene. Targeted adenovirus-mediated delivery of 15-PGDH gene resulted in substantial inhibition of tumor growth in mice with implanted CT-26 colon carcinomas. PGDH-mediated antitumor effect was associated with attenuated tumor-induced immune suppression and substantially reduced secretion of immunosuppressive mediators and cytokines such as PGE2, IL-10, IL-13, and IL-6 by intratumoral CD11b cells. We show also that introduction of 15-PGDH gene in tumor tissue is sufficient to redirect the differentiation of intratumoral CD11b cells from immunosuppressive M2-oriented F4/80+ tumor-associated macrophages (TAM) into M1-oriented CD11c+ MHC class II-positive myeloid APCs. Notably, the administration of the 15-PGDH gene alone demonstrated a significant therapeutic effect promoting tumor eradication and long-term survival in 70% of mice with preestablished tumors. Surviving mice acquired antitumor T cell-mediated immune response. This study for the first time demonstrates an important role of the 15-PGDH in regulation of local antitumor immune response and highlights the potential to be implemented to enhance the efficacy of cancer therapy and immunotherapy. The Journal of Immunology, 2009, 182: 7548–7557.

A dvanced tumors manage to evade the immune system by subverting the innate immune response and inhibiting the generation of adaptive antitumor immune response. Generally, there is an assumption that the expression of tumor-derived factors and cytokines by advanced cancers leads to the formation of an immunosuppressive and tolerogenic microenvironment that promotes tumor growth and protects malignant cancer cells from the immune system (1–3). Therefore, overcoming tumor-mediated immunosuppression is necessary before immunotherapy can successfully be applied (4, 5). One of the most evident immunosuppressive factors in various cancers is PGE2.

PGE2, one of the major metabolites of cyclooxygenase-2 (COX-2), is overproduced in many human solid tumors, and has been associated with increased tumor angiogenesis, metastasis, apoptosis, and cell cycle regulation (6, 7). Tumor-secreted PGE2 also produces multiple effects on immune system, and its overproduction is often associated with tumor-induced immune dysfunctions (8–12). Inhibition of COX-2 activity/expression and PGE2 production in a tumor host represents an attractive target for regulation of antitumor immunity and enhancement of cancer vaccine effect. Indeed, recent studies demonstrate that COX-2 inhibition in tumor host stimulates antitumor immunity and augments the efficacy of a cancer vaccine (13–15). However, clinical application of specific COX-2 inhibitors especially in elderly patients has serious limitations and side effects such as cardiotoxicity (16, 17).

Importantly, intratumoral PGE2 levels can be regulated not only through its synthesis but also through its degradation. The key enzyme responsible for the biological inactivation of prostaglandins is NAD-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which catalyzes the oxidation of the 15(S)-hydroxyl group of prostaglandins and lipoxins resulting in the formation of 15-keto metabolites, which exhibit greatly reduced biological activities (18, 19). 15-PGDH controls the levels of biologically active PGE2 and lipoxins in tissues, which may explain an important role of this enzyme in the regulation of inflammation (20). Recent publications have shown that 15-PGDH has a tumor suppressor function in multiple cancer types including colon, lung, breast, and bladder cancers (21–23). However, both expression and catabolic activity of 15-PGDH in various cancer tissues are markedly down-regulated (24, 25).

In recent years the functional role of 15-PGDH in cancer biology has been studied extensively. However, its potential role in the regulation of antitumor immunity remains undefined. Here we demonstrate that expression of 15-PGDH gene in intratumoral myeloid CD11b cells is down-regulated. Targeted transfer of the 15-PGDH gene in mice with preestablished transplantable colon carcinomas resulted in inhibition of tumor growth. This therapeutic
effect is associated with major changes in the tumor microenvironment including inhibition of PGE2, and immunosuppressive cytokines, stimulation of APC differentiation/maturation, and attenuation of tumor-induced immune suppression.

Materials and Methods

Mice and tumor models
Female BALB/c and C57BL/6 male mice (all 6–8 wk of age) were obtained from the National Cancer Institute (Frederick, MD). The CT-26 murine colon carcinoma cell line was purchased from the American Type Culture Collection. RM-9 cells murine prostate tumor cells were kindly provided by Dr. T. C. Tompson (Baylor College of Medicine, Houston, TX). Tumor cells were maintained in vitro at 37°C in a 5% CO2 humidified atmosphere in complete culture media. To establish s.c. tumors, mice were injected with $5 \times 10^6$ CT-26 tumor cells into the left flank of BALB/c mice.

Reagents
Mouse GM-CSF and IL-4 were obtained from R&D Systems. Anti-CD11c, CD11b, CD16, CD32, CD8, I-A/d, and Ly-6C Abs were obtained from BD Pharmingen and anti-F4/80 Ab was from Serotec, BnaU kit and Ab against mouse arginase I were both obtained from BD Pharmingen. Ab against murine arginase IL, COX-2, and STAT6 were obtained from Santa Cruz Biotechnology: the 5-lipoxygenase Ab and PGE2 were purchased from Cayman Chemical and the Abs against STAT-1 and phospho-STAT1 were purchased from Cell Signaling Technology.

Cell isolation
At specified time increments following the tumor inoculation, mice were euthanized in CO2 chamber and tumor cell suspensions were prepared from solid tumors by enzymatic digestion as follows. Tumors were isolated from the mice and sliced into 1- to 3-mm3 pieces with scissors. The minced tissue from one tumor was incubated at 37°C for 1 h in an L-15 medium (BioWhittaker/Cambrex) containing 2% FBS (HyClone), antibiotics (penicillin/streptomycin, HyClone), 50 U/ml collagenase I, 100 U/ml collage-

Flow cytometry
To block Fc receptors, 1 million cells were incubated for 5 min at 4°C with anti-CD16/CD32 mAbs (clones CT-17-1 and CT-17-2; Caltag Laboratorys). The cells were then incubated for 30 min on ice in 100 µl of PBS with 1 µg/ml of relevant Abs or matched isotype control Abs and then washed twice with cold PBS. Both fluorochrome-conjugated Abs and isotype control Abs were used for cell staining. Non-specific staining was prevented by blocking Fc receptors. To block Fc receptors, cells were incubated for 5 min at 4°C with anti-CD16/CD32 mAbs. Flow cytometry data were acquired using a FACScalibur flow cytometer (BD Biosciences) and were analyzed with CellQuest software (BD Biosciences).

Intracellular cytokine staining
CD11b myeloid cells were isolated from tumor tissue, plated down to a 6-well plate (Costar, Corning), and cultured in the presence or absence of 1 µg/ml LPS (Sigma-Aldrich) for 24 h. During the last 6 h of culturing, a monensin (3 mM) was added to these cell cultures. Cells were then harvested and triple-stained with directly conjugated Abs according to the manufacturer’s instructions. Non-specific staining was blocked with FcR blocking Ab (CD16/CD32). The following Abs were used in different combinations: FITC-conjugated anti-CD11c (clone H3; BD Pharmingen); PerCP-conjugated anti-CD11b (clone M1/70; BD Pharmingen), and allo-

Multiplex cytokine assay
Cell supernatants collected from cultured intratumoral CD11b cells or whole tumor cell suspensions were subjected to cytokine analysis using Multiplex assay. Cell culture supernatants were collected, filtered, and stored at −80°C and then assayed for presence of cytokines. Multiplex cytokine kit was obtained from Bio-Rad Laboratories, and assays were performed using a Luminex 200.

Adenoviral vector construction
Replication-deficient E1- and E3-deleted adenoviral recombinant vectors encoding the human hpgd gene (15-PGDH) under flt1 promoter (Ad-
PGDH) and control adenovirus encoding luciferase gene under the same promoter (control Ad) were constructed by using the pAdEasy system (Quantum Biotechnologies). Briefly, PCR was employed to generate a PCR product encoding the 15-PGDH gene using human adult normal colonic tissue cDNA as a template. To generate the pGL3-PGDH plasmid a PCR product encoding the 15-PGDH gene was cloned using HindIII and XbaI sites into the PGL3-Basic vector (Promega), which was digested. The firefly luciferase gene (Luc) was removed before cloning in the 15-PGDH. To generate the pShuttle-flt-PGDH plasmid, a fragment including the 15-PGDH and SV40 late polyadenylation signal was replaced by the pGL3-Luc using HindIII and SalI and was incorporated into pShuttle-flt plas-
mid downstream of the flt-1 promoter element. Recombinant Ad genome was generated by homologous DNA recombination in Escherichia coli B5183 between pShuttle-flt-PGDH and pAdEasy-1 plasmid (Quantum Biotechnologies). The newly generated genomes were confirmed by partial sequencing analysis, linearized with PacI, and transected into HEK293 cells using the SuperFect transfection reagent (Qiagen) to generate Adflt-

Luciferase assay
A luciferase gene reporter assay was performed to evaluate in vivo pro-

Quantitative real-time PCR
Total cellular RNA was isolated from intratumoral CD11b+ cells using RNeasy Plus Mini kit (Qiagen) according to the manufacturer’s instruc-
tions. Integrity of the RNA was analyzed in a 2100 Bioanalyzer (Agilent Technologies). cDNA for each RNA sample was synthesized in 20-

Western blotting
Tumor cells, or freshly isolated CD11b cells, were lysed with a radiomunoprecipitation assay buffer in the presence of protease and phosphatase inhibitors. Samples (30 µg of protein per lane) were subjected to electrophoresis in SDS-polyacrylamide gels, and then blotted onto polyvinylidene difluoride membranes. Membranes were blocked for 1 h at room temperature with 5% dry skimmed milk in TBS (20 mM Tris-HCl (pH 7.6), 137 mM NaCl plus 0.1% (v/v) Tween 20) and then probed with primary Abs of appropriate specificity overnight at 4°C. Membranes were washed and incubated for 1 h at room temperature with secondary Ab conjugated with DcI.
In vivo treatment protocol

To evaluate the in vivo effect of 15-PGDH gene delivery, mice with pre-established CT26 tumors (16–25 mm²) were intratumorally injected with 2 × 10⁸ TCID₅₀ of adenovirus encoding 15-PGDH or control adenovirus twice a week. Mice were sacrificed at the indicated time. To assess the effect of 15-PGDH-mediated therapy on survival of treated animals we also used the CT-26 tumor model. Tumor cells were s.c. injected into BALB/c mice. On day 7 after tumor inoculation, mice with 16- to 25-mm² tumors were randomly divided in three groups: 1) untreated, 2) control Ad, and 3) Ad-PGDH. Control adenovirus and Ad-PGDH were injected twice a week starting on day 7 (five times in total). Tumor growth was monitored by regular (twice a week) measurement of tumor size using a Vernier caliper.

Arginase activity

Arginase activity was measured in tumor-infiltrated CD11b cells using the QuantiChrom arginase kit from BioAssay Systems.

PGE₂ production

Analysis of PGE₂ production was performed using an ELISA kit and protocol developed by Cayman Chemical. Briefly, freshly isolated intratumoral CD11b cells from Ad-PGDH-treated or control tumor-bearing mice were cultured for 24 h in absence or presence of LPS (E. coli 0127:B8, 1 μg/ml). Cell culture supernatants were collected, filtered, and stored at −80°C and then assayed for presence of PGE₂.

Statistical analysis

The statistical significance between values was determined by the Student’s t test. All data were expressed as the mean ± SD. Probability values of p<0.05 were considered nonsignificant. Significant values of p ≤ 0.05 are expressed with an asterisk. The flow cytometry data shown are representative of at least three separate determinations.

Results

Catabolism of PGE₂ in tumor-infiltrated CD11b cells is altered

Due to increased expression of COX-2, tumors are able to produce high amounts of PGE₂. Here we tested whether intratumoral CD11b myeloid cells alone are able to secrete PGE₂. As shown in Fig. 1, CD11b cells isolated from CT-26 tumors expressed both major PGE₂-forming enzymes: COX-2 and mPGES1 (Fig. 1, A and C, respectively), and they were able to secrete the substantial amounts of PGE₂ (Fig. 1A). It is well established that colon cancer cells have down-regulated expression of major PGE₂-catabolizing enzyme 15-PGDH (22, 24). Analysis of gene expression in CD11b cells isolated from CT-26 colon tumors revealed dramatic down-regulation of the 15-PGDH gene expression (Fig. 1D). Furthermore, similar reduction of expression of the 15-PGDH gene was also observed in intratumoral CD11b cells derived from RM-9 prostate tumors (Fig. 2D). Freshly derived myeloid cells from prostate tumors also were able to produce substantial amounts of PGE₂ (Fig. 2A), mostly due to expression of COX-2 (Fig. 2B) and mPGES1 (Fig. 2C). Collectively, our obtained results demonstrate that intratumoral CD11b cells derived from both colon or prostate tumors show increased expression of COX-2, mPGES1, and produce substantial amounts of PGE₂. At the same time, expression of 15-PGDH in these cells is significantly reduced.

Ad-PGDH promotes in vitro and in vivo growth inhibition of murine colon carcinoma

Down-regulated expression of 15-PGDH in both tumor and intratumoral myeloid cells prompted the restoration of its expression in the tumor microenvironment. To restore the 15-PGDH gene expression in tumor tissue, we first constructed a replication-deficient E1- and E3-deleted adenoviral recombinant vector encoding the 15-PGDH gene (Ad-PGDH) under the specific flt1 promoter. Fig. 3A shows that in vitro transduction of CT-26 colon carcinoma cells with Ad-PGDH but not with control adenovirus led to enhanced expression of the 15-PGDH protein in tumor cells. Intratumoral administration of Ad-PGDH in tumor-bearing mice promoted expression of the 15-PGDH protein, which could be detected by Western blotting (data not shown).

To evaluate the in vivo antitumor effect of Ad-PGDH, we established CT-26 tumors in mice through s.c. inoculation. Starting 7550 15-PGDH AND REGULATION OF ANTITUMOR IMMUNE RESPONSE

![FIGURE 1. Catabolism of PGE₂ in CD11b cells, isolated from murine CT-26 colon carcinoma, is altered. CT-26 tumor cells (5 × 10⁵ cells/mouse) were injected s.c. into BALB/c mice. On day 14 after tumor injection, mice were sacrificed and excised tumors were digested with collagenase cocktail. CD11b cells were isolated from tumors and spleens with magnetic beads and then they were analyzed for production of PGE₂ by ELISA (A) and expression of COX-2 (B), mPGES1 (C), and 15-PGDH (D) by quantitative RT-PCR. Total RNA was isolated from purified pooled CD11b cells (three to five mice per group). Assays were done in triplicates. Average means ± SD are shown.](http://www.jimmunol.org/)

![FIGURE 2. Catabolism of PGE₂ in CD11b cells, isolated from murine RM-9 prostate tumor, is altered. RM-9 tumor cells (3 × 10⁵ cells/mouse) were injected s.c. into C57BL/6 mice. On day 14 after tumor injection mice were sacrificed and excised tumors were digested with collagenase cocktail. CD11b cells were isolated from tumors with magnetic beads, and then they were analyzed for production of PGE₂ by ELISA (A) and expression of COX-2 (B), mPGES1 (C), and 15-PGDH (D) by quantitative RT-PCR. Total RNA was isolated from purified pooled CD11b cells (three to five mice per group). Average means ± SD are shown.](http://www.jimmunol.org/)
on day 7, when tumor size reached 16–25 mm², mice were given four intratumoral injections of adenovirus encoding 15-PGDH (Ad-PGDH) or control adenovirus (control Ad). As shown in Fig. 3B, adenovirus-mediated delivery of the 15-PGDH gene in tumor tissue resulted in significant inhibition of tumor growth.

Expression 15-PGDH gene in our adenoviral construct is driven by the flt1 promoter. Flt-1, a receptor for vascular endothelial growth factor (VEGFR1), is known to display a high expression in endothelial cells and tumor cells, as well as in CD11b myeloid cells (26–29). To evaluate the distribution of the 15-PGDH gene after adenovirus-mediated delivery, we intratumorally injected CT-26 tumor-bearing mice with the adenovirus encoding Renilla luciferase gene under flt1 promoter (Ad-Luc). Forty-eight hours later, mice were sacrificed, and promoter-specific luciferase activity was determined in the whole tumor cell population, isolated tumor-infiltrated CD11b cells, and in the CD11b-negative tumor cell population. Fig. 3C shows that the highest flt1 promoter activity was observed in the CD11b-negative tumor cell population, whereas intratumoral CD11b cells demonstrated intermediate flt1 promoter activity, which was lower than tumor cells but significantly higher than control levels. These data suggest that adenoviral-mediated therapy with the 15-PGDH gene under the flt1 promoter could target both tumor cells and nontumor CD11b myeloid cells.

Introduction of the 15-PGDH gene in tumor tissue promotes significant changes in the cytokine profile of tumor-associated CD11b cells and stimulates APC differentiation

The PGE₂ overproduction has a major impact on both intratumoral immune and inflammatory cells favoring Th2 cytokine milieu, inhibiting APC differentiation and promoting immunosuppressive microenvironment (8–10). Since the 15-PGDH enzyme is known to biologically inactivate PGE₂, we examined whether introduction of the 15-PGDH gene in tumor tissue may have an impact on the cytokine secretion by myeloid cells. To address this hypothesis, we isolated intratumoral CD11b cells from treated or control tumor-bearing mice. Fig. 3D shows a purity of freshly isolated tumor-infiltrated CD11b cells (98%). Cells were cultured for 24 h and then cell supernatants were collected and assayed for PGE₂ and cytokines. Additionally, CD11b cells were analyzed by flow cytometry for intracellular cytokine production. As shown in Fig. 4A, left panel, adenoviral-mediated delivery of the 15-PGDH gene resulted in the 4-fold inhibition production of PGE₂ by tumor-associated CD11b cells. Importantly, the PGDH-mediated inhibition of PGE₂ secretion by tumor-infiltrated CD11b cells was associated with reduction of the IL-10 and IL-6 (Fig. 4A, central and right panels, respectively) in cellular supernatants. Similarly, intracellular expression of both IL-10 and IL-6 cytokines was markedly

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**FIGURE 3.** Adenoviral delivery of the 15-PGDH gene inhibits growth of preestablished CT-26 tumors. A, In vitro transduction of tumor cells with Ad-PGDH results in 15-PGDH protein expression. One hundred PFU/cell of Ad-PGDH or control Ad were added to semiconfluent cultures of CT-26 murine colon carcinoma cells. Forty-two hours later cells were collected, washed, and lysed. Expression of 15-PGDH protein in tumor cell lysates was evaluated using Western blotting as described in **Materials and Methods.** B, Intratumoral delivery of PGDH gene in tumor-bearing mice promotes inhibition of tumor growth. CT-26 murine colon carcinoma cells (5 × 10⁵) were inoculated s.c. in BALB/c mice at day 0. When tumors reached 20–25 mm² size (day 7), mice were randomly divided in three groups. Each group included five mice. The first group of tumor-bearing mice was injected with PBS (untreated), the second group was injected with control adenovirus (control Ad), and the third group was injected with adenovirus encoding 15-PGDH (Ad-PGDH). Adenovirus (2 × 10⁸ TCID₅₀) was administered intratumorally twice a week starting on day 7 after tumor inoculation (four injections in total). Tumor size (width × length, mm) was measured with calipers twice a week. Kinetics of tumor growth in mice are shown. C, In vivo flt1 promoter activity. BALB/c mice were s.c. injected with 2 × 10⁵ CT-26 tumor cells. On day 10 after tumor cell inoculation, 2 × 10⁸ TCID₅₀ of adenovirus encoding Renilla luciferase gene under flt1 promoter was administered into tumor site. Forty-eight hours later, mice were sacrificed and excised tumors were digested with collagenase cocktail as described above. Luciferase activity was determined in cell lysates obtained from whole tumor cell population, isolated tumor-infiltrated CD11b, and in CD11b⁻ cells. Averages ± SD are shown. Luciferase activity values were normalized to Renilla luciferase activity. D, Purity of isolated CD11b myeloid cell population. Tumor-infiltrated myeloid cells were isolated from excised CT-26 tumors with magnetic beads as described in **Materials and Methods.** Cells were stained with CD11b-PerCp Ab and analyzed by flow cytometry.
decreased in tumor-infiltrated CD11b cells isolated from PGDH-treated mice (Fig. 4B). In these mice we also observed reduction of proinflammatory cytokine IL-1β (Fig. 4C) and simultaneous up-regulation in the production of eotaxin and RANTES (Fig. 4C).

Furthermore, a similar 15-PGDH-mediated inhibition of the IL-10 and IL-6 was observed in both LPS-stimulated and nonstimulated tumor-derived CD11b cells (data not shown).

PGE2 is one of the main tumor-secreted factors responsible for altered APC differentiation in the tumor microenvironment. We tested in vitro whether presence of PGE2 could inhibit GM-CSF-driven APC differentiation from bone marrow progenitor cells. Fig. 5A demonstrates that addition of PGE2 to the cell cultures of normal bone marrow cell progenitors substantially reduces the number of CD11c+ dendritic cells in a dose-dependent manner (Fig. 5A; 83% in control vs 40% in presence of 1 μg/ml PGE2).

We next asked whether conditioning of tumor microenvironment with 15-PGDH gene could influence the in situ differentiation/maturation of intratumoral APCs. First, we measured expression of the MHC class II molecule in tumor-infiltrated F4/80 and CD11b cells. Most tumor-infiltrated CD11b cells from control mice also coexpressed F4/80 (data not shown), and a much smaller portion of those cells was expressing the MHC class II molecule and CD11c. Fig. 5B shows that the treatment of tumor-bearing mice with Ad-PGDH resulted in increased expression of the MHC class II molecule by tumor-infiltrated F4/80 and CD11b cells (data not shown). When tumor-infiltrated CD11b cells were isolated from Ad-PGDH-treated mice, most of these cells (89%; Fig. 5C, right panel) became double-positive CD11c/F4/80 cells. Under similar conditions, tumor-infiltrated CD11b cells from control tumor-bearing mice produced significantly fewer CD11c+ (36%; Fig. 5C, central panel). Taken together, the obtained results indicate that the conditioning of the tumor microenvironment with the 15-PGDH gene improves differentiation of intratumoral myeloid APCs.

Expression of the 15-PGDH gene in tumor tissue inhibits IL-10 and stimulates IL-12 cytokine production in draining lymph nodes

To examine the effect of 15-PGDH gene delivery on cytokine production in draining lymph nodes, we isolated those lymph nodes

FIGURE 4. PGDH gene delivery changes cytokine profile of intratumoral myeloid cells. CT-26 murine colon carcinoma cells (5 x 10^5) were inoculated s.c. in BALB/c mice at day 0. When tumors reached 20-25 mm^2 size (day 7), mice were randomly divided in three groups. The first group of tumor-bearing mice was injected with PBS (control), the second group with control adenovirus (control Ad), and third group was injected with adenovirus encoding 15-PGDH (Ad-PGDH). Each group consisted of five mice. The following day after the last injection of adenovirus mice were sacrificed, tumors were excised, and tumor-infiltrated CD11b cells were isolated with magnetic beads. CD11b cells (1 x 10^6/ml) were plated in 6-well cell culture plates and cultured in humidified CO2 incubator at 37°C. Twenty-four hours later cell supernatants were collected, filtered, and assayed for presence of PGE2 and cytokines by ELISA and Multiplex assay, respectively. Collected CD11b cells were used for analysis of intracellular cytokine expression with flow cytometry. A, Left panel, PGE2 production by intratumoral CD11b cells. Concentration of PGE2 determined in CD11b cell supernatants by ELISA (central and right panels): Levels of IL-10 and IL-6 in CD11b cell supernatants. Concentration of cytokines was measured using Multiplex assay. Average means ± SD are shown. B, Expression of cytokines by tumor-infiltrated CD11b cells derived from Ad-PGDH-treated and control animals. CD11b cells were stained first for surface marker F4/80, then for intracellular IL-10 and IL-6 as described in Materials and Methods, and then analyzed by flow cytometry. C, Cytokines secretion by tumor-infiltrated CD11b cells. Concentration of IL-1β, eotaxin, and RANTES was measured using Multiplex assay. Average means ± SD are shown.
from PGDH-treated or control tumor-bearing mice, prepared single suspensions, and stimulated them with LPS. After 6 and 24 h of incubation, cell supernatants were collected and assayed for cytokine production. Additionally, lymph nodes were analyzed by flow cytometry for intracellular cytokine production. As shown in Fig. 6, adenoviral-mediated delivery of the 15-PGDH gene induced a change in IL-10 and IL-12 cytokine expression specifically by myeloid cells. This treatment inhibited expression of IL-10 (Fig. 6A), but stimulated IL-12 (Fig. 6A). This was associated with up-regulation in production of eotaxin, RANTES, IFN-γ, G-CSF, and chemokine KC (data not shown).

**Ad-PGDH administration attenuates the immunosuppressive characteristics of tumor-infiltrated myeloid cells**

The tumor-infiltrated CD11b cell population, which consists of myeloid-derived suppressor cell (MDSC) and tumor-associated macrophages (TAM), represents a major mediator in tumor-induced immune suppression. Tumor progression affects myelopoiesis inhibiting APC differentiation and promoting accumulation of immunosuppressive cells, which in turn inhibits the generation of adaptive antitumor immune responses and promotes tumor evasion (30–32). Recent publications suggest that tumors may promote MDSC-mediated immune suppression through overproduction of PGE2 (10, 11). Here we evaluated whether introduction of the 15-PGDH gene, which is directly involved in metabolism of PGE2, could attenuate tumor-induced immune suppression mediated by intratumoral CD11b cells. Accordingly, we isolated intratumoral CD11b cells from treated or control tumor-bearing mice and then analyzed these cells for ability to secrete IL-13 (Fig. 7A) and measured arginase I and II expression (Fig. 7B) and arginase activity (Fig. 7C), as well for activity of STAT6 (Fig. 7D). Obtained results indicate that the conditioning of tumor microenvironment with the 15-PGDH gene resulted in the significant 10-fold inhibition of immunosuppressive cytokine IL-13 production by intratumoral CD11b cells. These cells also had down-regulated arginase expression and activity. Interestingly, reduced phosphorylation of STAT6 was observed in both adenovirus-treated groups (control Ad and Ad-PGDH). Since COX-2 is a major enzyme responsible

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** Delivery of the PGDH gene promotes in situ APC differentiation/maturation. A, PGE2 inhibits GM-CSF-driven differentiation of myeloid CD11c dendritic cells. Bone marrow cells from naive BALB/c mice were cultured in presence of recombinant GM-CSF (20 ng/ml). Endogenous PGE2 at two different concentrations was added to the cultures at the time of cell culture initiation. Seven days later cells were collected, washed, stained for CD11c and F4/80, and analyzed by flow cytometry. One representative experiment out of two is shown. B, Administration of Ad-PGDH increases number of MHC class II-positive myeloid cells in tumor. CT-26-bearing mice were treated with Ad-PGDH as described in Fig. 3. The next day following last adenovirus injection mice were sacrificed, tumors dissected, digested with collagenase cocktail and stained for I-Ad and F4/80 or CD11b. Average means ± SD are shown. C, Administration of Ad-PGDH increases number of CD11c+ DC cells in tumor site and draining lymph nodes. CT-26 tumorbearing mice were treated with Ad-PGDH as described in Fig. 2. The next day after the last adenovirus injection, mice were sacrificed. Draining lymph node-derived cells were stained for CD11c (left panel). Tumors from treated and control animals were dissected, digested with collagenase cocktail, and then CD11b cells were isolated with magnetic beads. CD11b cells were cultured for 24 h in complete culture medium; cells were collected, stained for CD11c and F4/80, and analyzed by flow cytometry. Results of one representative experiment out of two are shown.
for PGE2 production, we also measured its expression in the same cells and found that PGDH-mediated treatment did not affect expression of COX-2 (data not shown). Collectively, our results demonstrate that intratumoral delivery of 15-PGDH promotes significant changes in immunosuppressive tumor microenvironment, including the strong inhibition of secretion of immunosuppressive cytokines IL-13 and IL-10 and reduction of arginase expression/activity in tumor-recruited CD11b myeloid cells.

**Ad-PGDH-mediated therapy promotes tumor eradication and long-term survival of treated animals**

To evaluate whether Ad-PGDH-mediated therapy could promote tumor rejection and tumor-free survival, we designed the following experiment. CT-26 murine colon carcinomas were established in BALB/c mice by s.c. injections of tumor cells. On day 7, when tumors reached 16–25 mm^2 in diameter, mice were randomly divided in three groups: 1) control untreated (PBS only), 2) control Ad, and 3) Ad-PGDH. The adenovirus was injected twice a week starting on day 7 after tumor inoculation (stopped on day 20). To assess the treatment outcome, animals were studied for their long-term survival. As seen in Fig. 8A, all control tumor-bearing animals died within 25–43 days of tumor inoculation. Remarkably, 70% of treated mice in the Ad-PGDH group completely rejected tumors.

To examine whether this treatment led to generation of a specific T cell immune response, we isolated draining lymph nodes from surviving animals, prepared single-cell suspensions, and re-stimulated them in vitro with irradiated specific CT-26 or irrelevant 4T1 murine tumors. After 72 h of incubation, cell supernatants were collected and the concentration of IFN-γ was measured by Multiplex assay. Average mean ± SD is shown. B, Arginase activity. Whole-cell lysates were prepared from intratumoral CD11b cells derived from Ad-PGDH-treated or control tumor-bearing mice. Arginase activity was measured spectrophotometrically as described in Materials and Methods. C, Expression of arginases I and II in CD11b cells. Samples (30 μg of protein) were subjected to electrophoresis in 10% SDS-polyacrylamide gels, blotted onto 0.45-μm nitrocellulose membranes, and probed with anti-arginase I or II Ab. D, Expression of phosphorylated and total STAT6 in tumor-infiltrated CD11b cells was evaluated by Western blotting.

FIGURE 6. Introduction of the 15-PGDH gene in tumor microenvironment promotes switch of cytokine profile in draining lymph nodes. CT-26 tumors were established and treated with Ad-PGDH as described in Fig. 3. Draining lymph nodes were isolated next day after the last injection of Ad-PGDH or control Ad. Lymph node-derived cell suspension was plated in 6-well plates (1 × 10^6 cells/ml). After 6 and 24 h of incubation, cells were collected and analyzed by flow cytometry for intracellular cytokines IL-10 (A) and IL-12 (B).

FIGURE 7. Introduction of the 15-PGDH gene in tumor microenvironment reverses the immunosuppressive profile of tumor-infiltrated CD11b cells. A, Ad-PGDH-mediated treatment results in the inhibition of IL-13 production by tumor-infiltrated CD11b cells. CT-26 tumors were established and treated with Ad-PGDH or control Ad as described in Fig. 3. Forty-eight hours after the last injection of Ad-PGDH or control virus (control Ad), mice were sacrificed and tumor-infiltrated CD11b cells were isolated with magnetic beads. CD11b cells were added in 6-well plates (1 × 10^6 cells/ml). After 24 h of incubation, cell supernatants were collected and the concentration of IL-13 was measured by Multiplex assay. Average mean ± SD is shown. B, Arginase activity. Whole-cell lysates were prepared from intratumoral CD11b cells derived from Ad-PGDH-treated or control tumor-bearing mice. Arginase activity was measured spectrophotometrically as described in Materials and Methods. C, Expression of arginases I and II in CD11b cells. Samples (30 μg of protein) were subjected to electrophoresis in 10% SDS-polyacrylamide gels, blotted onto 0.45-μm nitrocellulose membranes, and probed with anti-arginase I or II Ab. D, Expression of phosphorylated and total STAT6 in tumor-infiltrated CD11b cells was evaluated by Western blotting.
Collectively, our data suggest that conditioning the tumor microenvironment with the 15-PGDH gene promotes remodeling of immunosuppressive cytokine milieu and stimulates the innate immune response with subsequent generation of the Ag-specific CD8 T cell-mediated antitumor immunity.

Discussion

Enhanced secretion of PGE2 by tumor cells is known to result in local immune suppression, which in turn favors further tumor growth. The PGE2 overproduction has a major impact on intratumoral immune and inflammatory cells, inhibiting APC functions, stimulating Th2 cytokine secretion, and promoting the immunosuppressive microenvironment. More specifically, PGE2 has been shown to prevent dendritic cell maturation, as well as its ability to produce IL-12 and present Ag. At the same time, PGE2 stimulates IL-10 secretion (8, 12, 33). COX-2 and PGE2 have recently been implicated in the induction of arginase I in CD11b MDSC (10). This enzyme plays a critical role in the inhibition of T cell immune responses by MDSC and tumor-associated macrophages. Also, PGE2 has been shown to promote the FoxP3 expression and inhibitory activity of T regulatory cells (34). These facts suggest that overproduction of tumor-secreted PGE2 contributes to developing tumor-induced immune dysfunction and also fuels cancer progression.

Importantly, intratumoral PGE2 levels are regulated not only by its synthesis, but also by its degradation. The key enzyme involved in inactivation of PGE2 is NAD+/H1001-dependent 15-PGDH. Recent studies identified a tumor suppressor activity of 15-PGDH in several cancers (21–23). This enzyme catalyzes the oxidation of the 15(S)-hydroxyl group of prostaglandins and lipoxins. The products, 15-keto-metabolites, exhibit greatly reduced biological activities, rendering this enzyme a key factor in the biological inactivation of these eicosanoids (19). Furthermore, 15-PGDH through catabolism of prostaglandins and lipoxins is also involved in the regulation of inflammation (19, 35). However, it appears that down-regulation of 15-PGDH expression is a common feature as opposed to the up-regulation of COX-2 expression in various types of cancers. This may provide complementary pathways to increase intratumoral PGE2 levels, thus subverting antitumor immune response and promoting tumor growth.

Here we show that isolated intratumoral CD11b cells are known to result in local immune suppression, which in turn favors further tumor growth. The PGE2 overproduction has a major impact on intratumoral immune and inflammatory cells, inhibiting APC functions, stimulating Th2 cytokine secretion, and promoting the immunosuppressive microenvironment. More specifically, PGE2 has been shown to prevent dendritic cell maturation, as well as its ability to produce IL-12 and present Ag. At the same time, PGE2 stimulates IL-10 secretion (8, 12, 33). COX-2 and PGE2 have recently been implicated in the induction of arginase I in CD11b MDSC (10). This enzyme plays a critical role in the inhibition of T cell immune responses by MDSC and tumor-associated macrophages. Also, PGE2 has been shown to promote the FoxP3 expression and inhibitory activity of T regulatory cells (34). These facts suggest that overproduction of tumor-secreted PGE2 contributes to developing tumor-induced immune dysfunction and also fuels cancer progression.

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Collectively, our data suggest that conditioning the tumor microenvironment with the 15-PGDH gene promotes remodeling of immunosuppressive cytokine milieu and stimulates the innate immune response with subsequent generation of the Ag-specific CD8 T cell-mediated antitumor immunity.
Obtained results clearly demonstrate that adenoviral-mediated delivery of the 15-PGDH gene directly in tumor site results in significant retardation of tumor growth. The antitumor effect was associated with substantially inhibited production of PGE\textsubscript{2}, Th2, and proinflammatory cytokines such as IL-10, IL-6, IL-1\textbeta, as well as the simultaneous up-regulation in production of eotaxin and RANTES by intratumoral CD11b cells. Our results also indicate that conditioning the tumor microenvironment with the 15-PGDH gene results in dramatically reduced production of immunosuppressive cytokine IL-13 by intratumoral CD11b cells. Secretion of IL-13 by tumor-recruited CD11b cells has recently been directly implicated in the active suppression of tumor-specific T cell immune response (36, 37). Consistent with this finding, we observed that administration of Ad-PGDH in tumor-bearing mice resulted in reduced decreased arginase expression, which is involved in mechanisms of immune suppression mediated by MDSC via enhanced arginine metabolism (10).

Tumors are known to skew differentiation of the dendritic cells to M2-oriented immunosuppressive macrophages and myeloid-derived suppressor cells (31, 38, 39). Here we demonstrate that overexpression of 15-PGDH in the tumor microenvironment is sufficient to redirect the differentiation of tumor-infiltrated CD11b cells from immunosuppressive M2-polarized F4/80\textsuperscript{+}CD11c\textsuperscript{−} TAM into M1-oriented CD11c\textsuperscript{+} MHC class II-positive myeloid APCs. Taken together, these data reveal that the intratumoral delivery of 15-PGDH gene promotes significant changes in immunosuppressive tumor microenvironment, including inhibition of IL-10 and IL-13 cytokine production, down-regulation of arginase expression in tumor-infiltrated CD11b cells, and stimulation of their differentiation toward dendritic cells.

To further address the role of 15-PGDH induced in immune activation, we analyzed regional lymph nodes derived from Ad-PGDH-treated mice for cytokine production. In response to intratumoral 15-PGDH gene administration we observed a substantial increase of IL-12 production with simultaneous reduction of IL-10 in draining lymph nodes. Similar to the tumor-infiltrated CD11b cells, lymph nodes isolated from 15-PGDH treated mice also displayed an up-regulation of eotaxin secretion. Eotaxin is a powerful chemokine that attracts eosinophils and promotes eosinophil-mediated tumor cell destruction via eosinophil degranulation (40). This suggests the involvement of 15-PGDH in eotaxin production through regulation of catabolism of eicosanoids.

Posttreatment analysis (2 and 4 mo after previous 15-PGDH administration) of lymph nodes and spleens from tumor-free mice revealed that Ad-PGDH-mediated treatment elicited a robust and persistent Ag-specific T cell memory immune response against the specific tumor. Taken together, obtained data indicate that conditioning the tumor microenvironment with the 15-PGDH gene results in attenuation of tumor-induced immunosuppression with the subsequent generation of adaptive antitumor immunity. This also underlines the importance of the eicosanoid balance in the regulating tumor immunity. One concern regarding the attenuation of immunosuppression as an antitumor treatment strategy is the risk of developing autoimmunity. In our study of mice bearing CT-26 tumors, we did not observe any apparent signs of toxicity, and there was no histological evidence of autoimmunity in the major organs (data not shown).

Importantly, expression of the 15-PGDH gene in our adenoviral construct was driven by the flt1 promoter. Several studies demonstrated that expression of VEGFR1/flt1 receptor and its promoter is inducible, and could be significantly enhanced under hypoxic conditions (41) or under exposure to tumor-induced oxidative stress (42). Hypoxia is one of the hallmarks of tumor microenvironment, and tumor-infiltrated cells including CD11b cells have been shown to express the VEGFR1/flt1 receptor (27, 42). Taking in account the high amounts of tumor-secreted VEGF in the tumor microenvironment and inducible nature of its cognate VEGFR1/flt1 receptor, this may have significant functional context for regulation of both angiogenesis and antitumor immune response in tumor host.

Our study supports the concept that 15-PGDH regulates both cancer inflammation and tumor-induced immune suppression. Cancer inflammation plays a significant role in tumor growth initiation and promotion. Recent studies also revealed an important link between cancer inflammation and cancer-associated immune suppression (36, 43–45). A significant portion of inflammatory cells in tumor tissue is represented by tumor-recruited CD11b cells, which play multiple roles in cancer progression. Our data and recent publications (46) demonstrate that intratumoral CD11b cells represent a major component of tumor-infiltrated inflammatory cell population. In murine tumors most of tumor-infiltrated CD11b cells express F4/80, which is a specific marker for monocyte/macrophages. These cells are highly immunosuppressive and potently inhibit T cell responses (47, 48). Importantly, CD11b\textsuperscript{+}F4/80\textsuperscript{+} cells could be differentiated into dendritic CD11c\textsuperscript{+} MHC class II-positive cells; however, the presence of high concentrations of PGE\textsubscript{2} prevents it. Recent studies demonstrate that tumor-infiltrated CD11b cells also represent a major part of tumor stroma and, consequently, the targeting of tumor stroma results in tumor rejection (46). Several recent studies revealed the active involvement of CD11b cells in neovascularogenesis (28), tumor angiogenesis (49), and in the process of tumor cell invasion and metastasis (50). Taken together, these facts emphasize the high plasticity of tumor-recruited CD11b cells and their multiple roles in cancer. Remarkably, introduction of the 15-PGDH gene in the tumor microenvironment attenuates the immunosuppressive characteristics of intratumoral CD11b cells (inhibition of IL-13 and IL-10 production and arginase expression) and promotes their differentiation into M1-oriented CD11c\textsuperscript{+} MHC class II-positive dendritic cells. This indicates the importance of 15-PGDH-mediated control of tumor-secreted PGE\textsubscript{2} balance in regulation of immune response. This also demonstrates that tumors may evade immune response through inhibition of 15-PGDH and alteration of intracellular PGE\textsubscript{2} catabolism in tumor-infiltrating myeloid cells, thus preventing its differentiation into APCs.

In summary, our findings suggest that 15-PGDH through enhanced catabolism of intratumoral eicosanoids regulates the generation of antitumor immune response. Enforced expression of the 15-PGDH gene in the tumor site could be sufficient to remodel the immunosuppressive tumor environment and promote activation of the immune system. This may provide a window of opportunity to enhance the efficacy of cancer immunotherapy and develop new strategies for cancer therapy.

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Disclosures
The authors have no financial conflicts of interest.

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