Prostaglandin E2 Promotes Tumor Progression by Inducing Myeloid-Derived Suppressor Cells

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Abstract

A causative relationship between chronic inflammation and cancer has been postulated for many years, and clinical observations and laboratory experiments support the hypothesis that inflammation contributes to tumor onset and progression. However, the precise mechanisms underlying the relationship are not known. We recently reported that the proinflammatory cytokine, interleukin-1β, induces the accumulation and retention of myeloid-derived suppressor cells (MDSC), which are commonly found in many patients and experimental animals with cancer and are potent suppressors of adaptive and innate immunity. This finding led us to hypothesize that inflammation leads to cancer through the induction of MDSC, which inhibit immunosurveillance and thereby allow the unchecked persistence and proliferation of premalignant and malignant cells. We now report that host MDSC have receptors for prostaglandin E2 (PGE2) and that E-prostanoid receptor agonists, including PGE2, induce the differentiation of $Gr1^+CD11b^+$ MDSC from bone marrow stem cells, whereas receptor antagonists block differentiation. BALB/c EP2 knockout mice inoculated with the spontaneously metastatic BALB/c-derived 4T1 mammary carcinoma have delayed tumor growth and reduced numbers of MDSC relative to wild-type mice, suggesting that PGE2 partially mediates MDSC induction through the EP2 receptor. Treatment of 4T1-tumor–bearing wild-type mice with the cyclooxygenase 2 inhibitor, SC58236, delays primary tumor growth and reduces MDSC accumulation, further showing that PGE2 induces MDSC and providing a therapeutic approach for reducing this tumor-promoting cell population. [Cancer Res 2007;67(9):4507–13]

Introduction

Chronic inflammation has long been correlated with tumor onset and progression (1), and a causative relationship between inflammation and cancer is supported by several lines of evidence. For example, individuals with long-term chronic inflammatory diseases are at higher risk of developing cancers of the target organ (1–5), whereas patients and/or experimental animals taking nonsteroidal anti-inflammatory drugs are frequently protected against the onset and/or progression of both primary tumor (6, 7) and metastatic disease (8, 9). In addition, overexpression of certain proinflammatory cytokines can result in increased tumor growth and metastatic disease (10–13), and blocking these cytokines can result in reduced tumor growth (10, 11). Furthermore, many tumors secrete high levels of proinflammatory cytokines and/or agents, including prostaglandin E2 (PGE2; refs. 14–17), and therefore have the potential to induce a local proinflammatory microenvironment. Although these observations are consistent with the hypothesis that chronic inflammation contributes to tumor onset and progression, the precise mechanism(s) for this relationship remains unclear.

We (18) and others (19) have shown that the proinflammatory cytokine interleukin (IL)-1β induces accumulation and retention of a population of cells called myeloid-derived suppressor cells (MDSC). MDSC are found in many cancer patients and are potent inhibitors of T-cell activation that cause a global and profound immunosuppression (20–25). These findings suggest that MDSC may be involved in the linkage between inflammation and cancer and have led us to hypothesize that MDSC induced by inflammation inhibit immunosurveillance, thereby facilitating malignant transformation and tumor progression. We now show using the spontaneously metastatic BALB/c-derived 4T1 mammary carcinoma that PGE2 induces the accumulation and retention of immunosuppressive MDSC, thereby providing a mechanistic explanation for how inflammation leads to immunosuppression and tumor progression.

Materials and Methods

Mice and cells. BALB/c and BALB/c DO11.10 transgenic [Vβ8 T-cell receptor (TCR) specific for chicken ovalbumin peptide 323 to 339 restricted to I-A^d; ref. 26] mice were obtained from The Jackson Laboratory. BALB/c clone 4 transgenic mice (αβ TCR specific for influenza hemagglutinin peptide 518 to 526 restricted to H-2K^d; ref. 27) were provided by Dr. E. Fuchs (The Johns Hopkins University, Baltimore, MD). BALB/c EP2$^{-/-}$ mice (10 generations backcrossed to BALB/c) were provided by Dr. Richard Breyer (Vanderbilt University, Nashville, TN). Mice were bred and maintained in the University of Maryland Baltimore County animal facility according to the NIH guidelines for the humane treatment of laboratory animals. All animal procedures have been approved by the University of Maryland Baltimore County Institutional Animal Care and Use Committee. The BALB/c-derived 4T1 mouse mammary carcinoma was maintained as described (28).

Reagents and antibodies. PGE2, Butaprost, AH6809, AH23848, and lipopolysaccharide (LPS) were from Sigma-Aldrich. Rabbit polyclonal anti-PGE2 antibody was from Abcam. The cyclooxygenase-2 (COX-2) inhibitor SC58236 was from Pfizer. Peptides ovalbumin$_{323-339}$ and hemagglutinin$_{518-526}$ were synthesized in the University of Maryland, Baltimore, Biopolymer Core Facility. N^W-hydroxyl-nor-L-arginine (nor-NOHA) was from Calbiochem. Fluorescently labeled anti-mouse monoclonal antibody (mAb) Gr1, CD11b, c-kit, CD3, B220, CD11c, Vβ8.1, 2, CD8, CD4, and FITC-goat-anti-rabbit IgG, FITC-rat IgG2a, PE-rat-IgG2a, and PerCP-rat-IgG2a isotype controls were from BD PharMingen. Anti–clonotypic mAb KJ1-26 to the DO11.10 TCR was from Caltag. Rabbit polyclonal antibodies to EP1 and EP3 were from Cayman Chemical; antibodies to EP2 and EP4 were from Alpha Diagnostics.

Flow cytometry. Cells were stained and analyzed on an Epics XL flow cytometer using Expo32 ADC software (Beckman Coulter) as described (29).
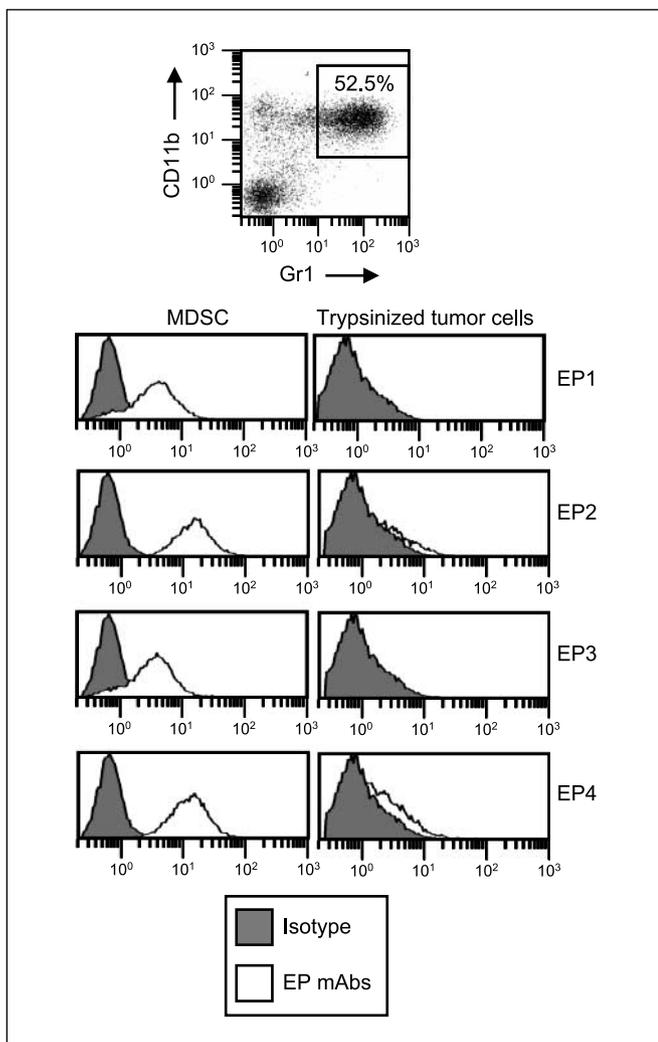
Figure 1. MDSC from 4T1 tumor-bearing mice express E-prostanoid (*EP*) receptors for PGE2. Splenocytes from 4T1 tumor-bearing BALB/c mice were triple labeled for Gr1-PE, CD11b-PerCP, and EP1-, EP2-, EP3-, or EP4-FITC or with appropriate isotype control mAbs. Gr1$^+$CD11b$^+$ cells were gated and analyzed by flow cytometry for expression of E-prostanoid receptors. Tumor cells were trypsinized to remove the E-prostanoid receptors and serve as a negative control.

Tumor inoculations, tumor growth, surgery, and COX-2 inhibitor treatment. Mice were inoculated s.c. in the abdominal mammary gland with 7,000 4T1 mammary carcinoma cells on day 0 and monitored for tumor progression as described. In some experiments, primary tumors were surgically removed as described (28). Mice treated with SC58236 were injected s.c. thrice a week with SC58236 (3 mg/kg) beginning on the same day they were inoculated with 4T1 (day 0) and continuing through day 21. SC58236 was prepared as a 100× stock in ethanol and diluted in Iscove's modified Dulbecco's medium for injection.

In vitro **induction of MDSC.** Bone marrow cells were harvested from the femurs of BALB/c mice and depleted for T cells, B cells, macrophages, granulocytes, neutrophils, and RBC using the mouse lineage cell depletion kit and LS columns from Miltenyi Biotec according to the manufacturer's directions. Resulting cells were >50% ckit$^+$ (CD117) as assayed by flow cytometry. Lineage-depleted bone marrow cells were cultured at 1×10^5/mL to 5×10^5/mL bone marrow medium (RPMI supplemented with 3% FCS, 1% antibiotic-antimycotic, 1% gentamicin Life Technologies), including granulocyte-macrophage colony stimulating factor (GM-CSF; 20 ng/mL) and IL-4 [10 ng/mL; both from Research Diagnostics (Flanders, NJ) according to the procedure of ref. 30], in T25 flasks, 6- or 24-well plates at 37°C in 5% CO$_2$ for 5

days. PGE2 (2.6 μmol/L), Butaprost (5 μmol/L), anti-PGE2 antibody (6.25 μL/ 10 mL), AH6809 (10 μmol/L), and/or AH23848 (10 μmol/L) were added to some wells at the beginning of the 5-day culture. LPS (1 μg/mL) was added to some wells during the last 16 h of the 5-day culture. Half of the medium was replaced every 48 h with fresh bone marrow medium. Resulting cells were stained and analyzed by flow cytometry. Percentage increase in Gr1$^+$CD11b$^+$ cells was calculated as (100%) × [(Gr1$^+$CD11b$^+$ cells with drug) − (Gr1$^+$CD11b$^+$ cells without drug) / (Gr1$^+$CD11b$^+$ cells without drug)].

MDSC. MDSC were isolated from the spleens of 4T1 tumor-bearing mice by magnetic bead sorting using Gr1 mAb and LS columns according to the directions of the manufacturer (Miltenyi Biotec). Purified splenic MDSC were >90% Gr1$^+$CD11b$^+$.

MDSC suppression assay. Putative suppressor cells were irradiated (2,500 rad) and incubated with transgenic T cells plus antigen and T-cell proliferation assessed as described (29). The arginase inhibitor, nor-NOHA, was used at 500 μM. Transgenic T cells were similarly activated by peptide in the presence or absence of nor-NOHA (without MDSC). Data are expressed as the mean ± SD of triplicate cultures.

Statistical analysis. Student's *t* test for unequal variance was done using Microsoft Excell 2000.

Results

MDSC have receptors for PGE2. We have hypothesized that inflammation may enhance tumor onset and progression by inducing MDSC, which limit immunosurveillance, thereby facilitating tumor growth. Because PGE2 is secreted by many tumors and is a central factor in inflammation, we have reasoned that MDSC may be induced by PGE2. Therefore, we assayed MDSC for the four E-prostanoid receptors for PGE2, EP1, EP2, EP3, and EP4. BALB/c mice were inoculated on day 0 with 4T1 mammary carcinoma cells, and their spleens were removed on day 36 when their primary tumors were 9.9 mm in diameter. Following lysis of the RBC, the remaining splenocytes were triple stained for the two markers of mouse MDSC (Gr1 and CD11b), and the EP1, EP2, EP3, or EP4 receptor. Gr1$^+$CD11b$^+$ MDSC represent 52.5% of splenocytes, and these cells uniformly express all four E-prostanoid receptors (Fig. 1). Because trypsin cleaves the E-prostanoid receptors, trypsinized 4T1 cells served as a negative specificity control for the E-prostanoid antibodies. Therefore, MDSC express receptors for PGE2, making them potentially responsive to this prostaglandin.

E-prostanoid agonists induce MDSC differentiation; E-prostanoid antagonists block differentiation. To determine if PGE2 induces the differentiation of MDSC, bone marrow cells were extracted from the femurs of BALB/c mice; lineage depleted and the remaining cells were cultured with IL-4 and GM-CSF in the presence or absence of PGE2 or the EP2 agonist Butaprost. After 5 days of culture, the resulting cells were analyzed by flow cytometry for T cell (CD3), B cell (B220), stem cell (c-kit), dendritic cell (CD11c), and MDSC (Gr1$^+$CD11b$^+$) markers. Gr1$^+$CD11b$^+$ cells increase by 2- to 3-fold in cultures supplemented with PGE2 or Butaprost relative to medium-alone treatment (Fig. 2*A*). The accumulation of MDSC seems to be at the expense of dendritic cells, because dendritic cell differentiation is reduced when PGE2 or Butaprost are present.

The experiment of Fig. 2*A* indicates that MDSC induction is via the EP2 receptor. To determine if induction also occurs through the other PGE2 receptors, bone marrow cells were cultured with PGE2 or Butaprost as in Fig. 2*A*, and the EP4 antagonist AH23848 and/or the EP1 and EP2 antagonist AH6809 were added to some wells (Fig. 2*B*). Addition of either or both antagonists virtually eliminates the induction of Gr1$^+$CD11b$^+$ cells, indicating that PGE2 mediates MDSC differentiation through either the EP1, EP2, and/ or EP4 receptors.

To ascertain that the Gr1$^+$CD11b$^+$ cells induced by PGE2 and Butaprost have suppressive activity, bone marrow cells were induced as in Fig. 2B and the resulting cells were irradiated and cocultured with transgenic D011.10 T cells pulsed with ovalbumin peptide. Negative controls for the culture process included medium without PGE2 or Butaprost, or culture with LPS, an inducer of mature macrophages and dendritic cell (Fig. 2C and D). PGE2- and Butaprost-induced cells suppress T cell activation by 46% and 42%, respectively, whereas LPS-induced cells are not suppressive. Therefore, PGE2 induces the accumulation of Gr1$^+$CD11b$^+$ MDSC from bone marrow stem cells via EP1, EP2, and/or EP4 receptors.

EP2-deficient mice have retarded primary tumor growth and reduced MDSC accumulation. The experiments of Fig. 2 show that PGE2 induces MDSC differentiation through the EP1, EP2, and/or EP4 receptors *in vitro*. If PGE2 also induces MDSC *in vivo*, then EP2$^{-/-}$ mice should have fewer MDSC and delayed tumor growth relative to wild-type BALB/c mice. To test this hypothesis, EP2$^{-/-}$ and BALB/c mice were inoculated with 4T1 on day 0 and primary tumors were measured weekly beginning on day 8. Mice were also bled on day 20, and the level of MDSC in the blood was determined by immunofluorescence and flow cytometry. Primary tumors grew more slowly, and MDSC levels were reduced in EP2$^{-/-}$ mice relative to wild-type BALB/c mice (Fig. 3A and B, respectively). Because MDSC levels are frequently driven by tumor mass, EP2$^{-/-}$ mice may have fewer MDSC because their primary tumors are smaller. To address this issue, the percentage of MDSC in EP2$^{-/-}$ and wild-type BALB/c mice with 6 to 7 mm diameter primary tumors was compared. When EP2$^{-/-}$ and wild-type BALB/c mice have primary tumors of the same size, BALB/c mice have significantly more Gr1$^+$CD11b$^+$ MDSC in their blood (Fig. 3C), showing that primary tumor burden by itself does not dictate the level of MDSC. Therefore, elimination of the EP2 receptor reduces MDSC levels and retards primary tumor growth, indicating that PGE2 mediates MDSC accumulation *in vivo*.

MDSC induced via the EP2 receptor have heightened suppressive activity. To determine if MDSC induced through the EP2 receptor are qualitatively different from MDSC induced by other factors, we have compared the suppressive activity and mechanism of suppression of MDSC harvested from wild-type BALB/c and EP2-deficient mice. BALB/c and EP2$^{-/-}$ mice were inoculated on day 0 in the abdominal mammary gland with 7,000 4T1 cells; primary tumors were removed on day 22; and spleens

were removed on day 50 when the mice had extensive metastatic disease. Gr1$^+$CD11b$^+$ cells were isolated from spleens by MACS sorting. Sorted MDSC were cocultured with CD4$^+$ DO11.10 transgenic T cells (specific for ovalbumin peptide 323 to 339 peptide) or CD8$^+$ clone 4 transgenic T cells (specific for

A

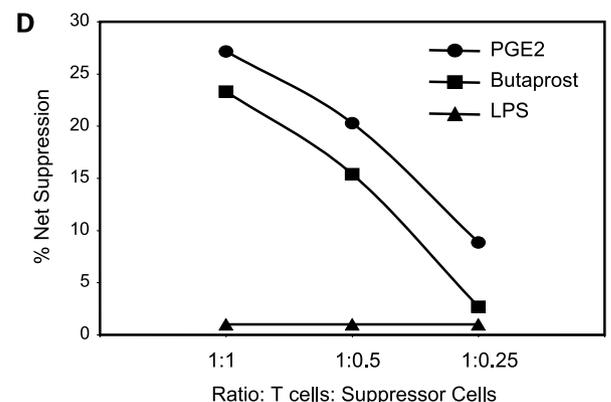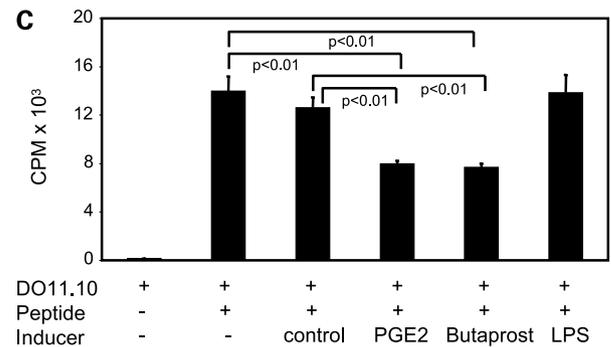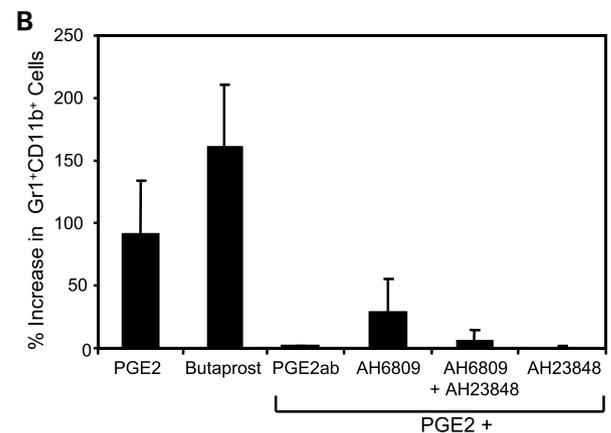|	Percent Positive Cells			
	Before Culture	After 5 Days of Culture		
		Medium	PGE2	Butaprost
B220 (B cells)	9.3	20.7	16.1	19.8
CD3 (T cells)	3.4	19	7.1	3.6
c-kit (stem cells)	52.5	7.7	7.5	6.9
CD11c (DC)	4.8	36.1	21.3	11.6
CD11b$^+$Gr1$^+$	20.3	21.1	46.4	61.5

Figure 2. PGE2 induces the differentiation of MDSC through E-prostanoid receptors. Bone marrow cells were harvested from the femurs of BALB/c mice, depleted for lineage cells [B cells, T cells, dendritic cell (*DC*), macrophages, granulocytes, and RBC], and cocultured for 5 d with E-prostanoid receptor agonists and/or antagonists. *A*, bone marrow cells were analyzed by flow cytometry for lineage markers before and after culture with PGE2 or Butaprost. *B*, bone marrow cells were analyzed by flow cytometry for Gr1 and CD11b after culture with PGE2 and/or Butaprost, anti-mouse PGE2 antibody, AH6809, AH23848, or AH6809 + AH23848. Percentage increase = (100%) × [(Gr1$^+$CD11b$^+$ cells + inducer) − (Gr1$^+$CD11b$^+$ cells without inducer)] / (Gr1$^+$CD11b$^+$ cells without inducer). Data are pooled from four (Butaprost) or five (PGE2) experiments. *C*, bone marrow cells were cultured in the presence of PGE2, Butaprost, or as a negative control LPS, as per *A* and *B*; the resulting cells were cocultured at a 1:1 ratio with DO11.10 transgenic splenocytes and ovalbumin$_{323-339}$ peptide, and T-cell activation was measured by incorporation of [^{3}H]thymidine. *D*, bone marrow cells treated with PGE2, Butaprost, or LPS were cocultured at varying ratios with D011.10 T cells and ovalbumin$_{323-339}$ peptide. Percentage net suppression = (% suppression in the presence of PGE2, Butaprost, or LPS − % suppression in the absence of inducer). Data in *A*, *C*, and *D* are from one of three independent experiments for each panel.
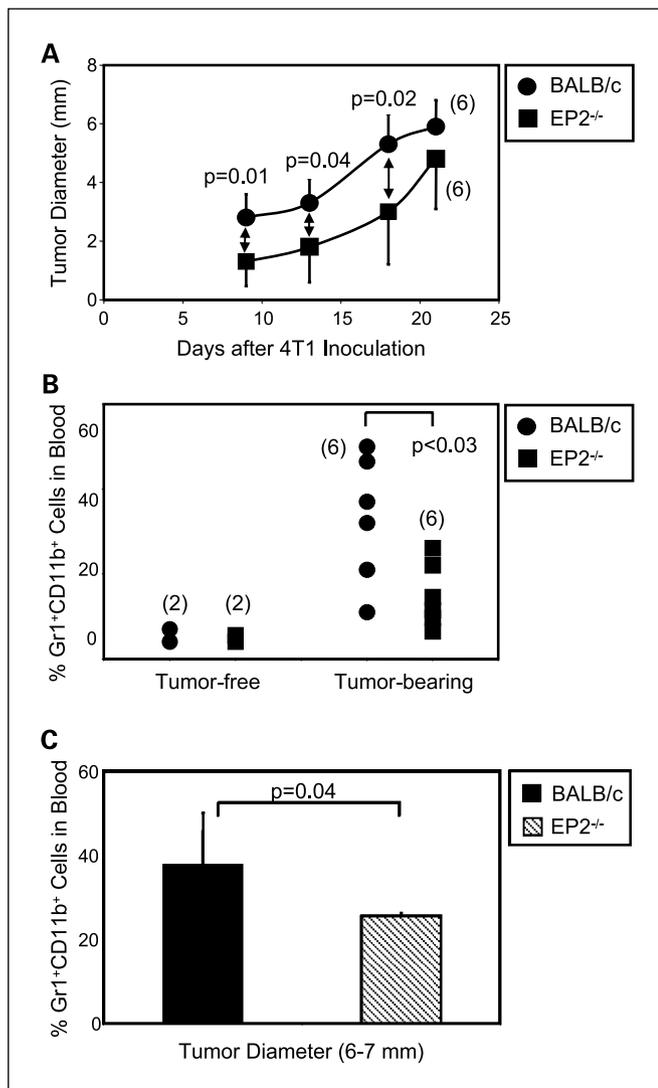
Figure 3. EP2-deficient mice have reduced primary tumor growth and fewer MDSC. BALB/c and EP2$^{-/-}$ mice were inoculated in the mammary gland with 7,000 4T1 mammary carcinoma cells on day 0. *A,* primary tumors were measured with a calipers. *Points,* average diameters of primary tumor as a function of days after tumor inoculation; *bars,* SD. *Numbers in parentheses,* number of mice per group. *B,* tumor-free and mice with 20-d-old 4T1 tumors were bled, and the percentage of Gr1$^+$CD11b$^+$ cells was determined by flow cytometry. Data are the pooled results of two independent experiments. *C, columns,* average of Gr1$^+$CD11b$^+$ cells for mice in *B* with primary tumors of 6 to 7 mm in diameter; *bars,* SD.

hemagglutinin peptide 518–526) plus the appropriate peptide. Cultures were pulsed with tritiated thymidine on day 3 and cells were harvested and counted 16 h later. MDSC from both BALB/c and EP2$^{-/-}$ mice suppress the activation of CD4$^+$ and CD8$^+$ T cells at a 1:2 ratio of T cells to suppressor cells (Fig. 4*A*), and the suppressive effect diminishes with the inclusion of fewer Gr1$^+$CD11b$^+$ cells (Fig. 4*B*). However, MDSC from EP2$^{-/-}$ mice are less suppressive than MDSC from BALB/c mice. Therefore, MDSC from EP2$^{-/-}$ mice have significantly less suppressive activity on a per cell basis than MDSC from wild-type BALB/c mice, suggesting that MDSC induced via the EP2 receptor are qualitatively different from MDSC induced in the absence of the EP2 receptor.

MDSC induced in BALB/c mice by the 4T1 mammary carcinoma suppress the antigen-specific activation of CD4$^+$ T cells, and to a

lesser extent the activation of CD8$^+$ T cells, by producing arginase, which degrades arginine, an essential amino acid (29). To determine if 4T1-induced MDSC from EP2$^{-/-}$ mice inhibit by the same mechanism, nor-NOHA, an inhibitor of arginase, was added to some of the D011.10 and clone 4 transgenic T-cell cultures of Fig. 4. Nor-NOHA reverses the suppression CD4$^+$ and CD8$^+$ T cells by both BALB/c and EP2$^{-/-}$ MDSC, although it is more effective in reducing suppression of CD4$^+$ T cells than of CD8$^+$ T cells. Therefore, EP2-dependent and EP2-independent MDSC both inhibit T-cell priming by the production of arginase, although
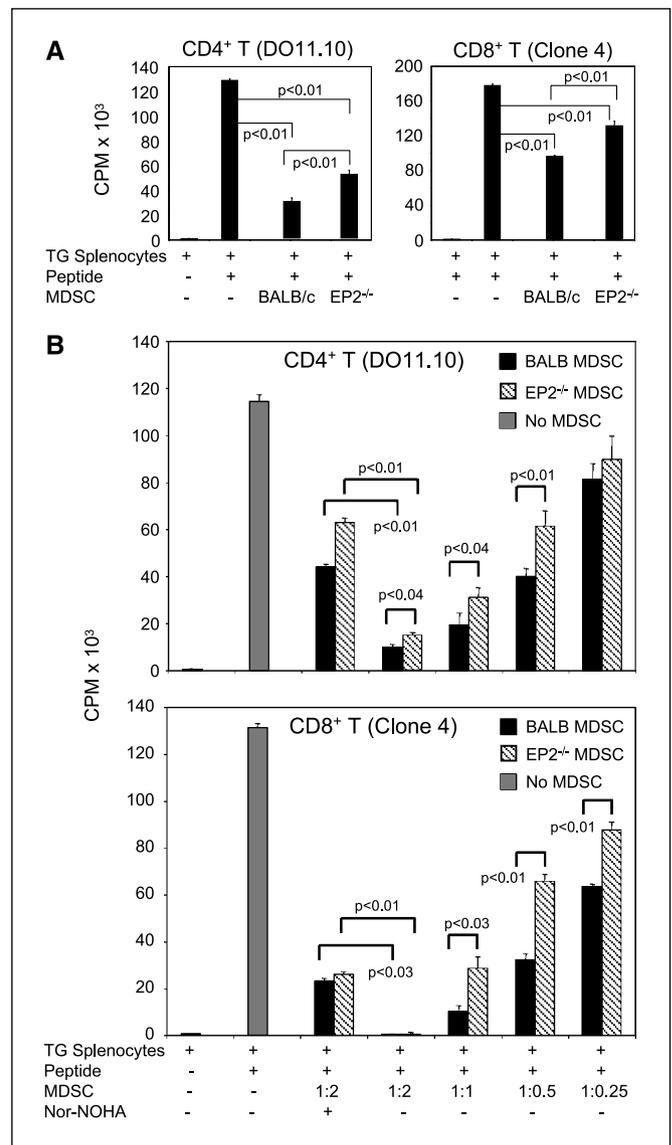

Figure 4. MDSC induced in EP2-deficient mice are less suppressive. CD4$^+$ DO11.10 or clone 4 transgenic splenocytes were stimulated with ovalbumin$_{323-339}$ or HA$_{518-526}$ peptide, respectively, in the presence or absence of MACS-sorted Gr1$^+$CD11b$^+$ MDSC from the spleens of tumor-bearing EP2$^{-/-}$ or BALB/c mice, and T-cell activation was measured by incorporation of [^{3}H]thymidine. Sorted MDSC were >90% Gr1$^+$CD11b$^+$. *A,* transgenic (*TG*) splenocyte to MDSC ratio was 1:2. *B,* transgenic splenocyte to MDSC ratio ranged from 1:2 to 1:0.25. The arginase inhibitor nor-NOHA (500 μmol/L) was added to some wells. Values for transgenic splenocytes with peptide were not significantly different from values for splenocytes with peptide and nor-NOHA (data not shown). Values for transgenic splenocytes with peptide were statistically significantly different from all other values at *P* < 0.01. Data for *A* and *B* are from one of two independent experiments.

EP2-dependent MDSC are more suppressive on a per cell basis than EP2-independent MDSC.

Treatment with a COX-2 inhibitor reduces primary tumor growth and delays MDSC accumulation. The biosynthetic pathway for PGE2 involves the COX-2–catalyzed conversion of arachidonic acid to prostaglandin G2 (PGG2), and the subsequent modification of PGG2 by PGE synthase to PGE2. Because COX-2 is essential for PGE2 synthesis, drugs that specifically block COX-2 reduce PGE-2–mediated inflammation (31). To determine if *in vivo* interference with PGE-2 synthesis alters MDSC accumulation, wild-type BALB/c mice were inoculated on day 0 with 4T1 and concomitantly treated with the COX-2 inhibitor, SC58236. SC58236 treatment delays 4T1 primary tumor growth (Fig. 5A) and reduces MDSC accumulation (Fig. 5B). Therefore, inhibition of PGE2 biosynthesis in tumor-bearing mice retards tumor progression and MDSC differentiation, consistent with the concept that inflammation promotes tumor growth through the induction of immunosuppressive cells.

Discussion

The association of chronic inflammation with tumor onset and progression has been noted for many years, and inflammation resulting from infections is thought to be responsible for as many as 15% of the world's malignant cancers (32). Although it is likely that inflammation contributes to tumor progression through multiple and diverse mechanisms (1, 32, 33), the causative events connecting inflammation with cancer are not well understood. PGE2, one of the major products of inflammation, is thought to promote tumor growth by inducing neoangiogenesis (34) and by inducing tumor cell apoptosis (35). This report shows that in addition to these known effects, PGE2 also modulates tumor growth by inducing the accumulation of MDSC, which, in turn, suppress antitumor immunity. MDSC are present in many patients and experimental animals with cancer (20–25). They block adaptive immunity by inhibiting the activation of $CD4^+$ and $CD8^+$ T cells (29, 36–38) and innate immunity by inhibiting natural killer cells (39). In immunocompetent individuals, immunosurveillance protects against the development of premalignant cells and the proliferation of malignant cells (40). However, individuals with elevated MDSC are not fully immunocompetent (41); thus, immunosurveillance is not effective. Therefore, inflammation contributes to cancer onset and progression through the production of PGE2, which induces suppressor cells, and, which, in turn, counteracts the protective effects of immunosurveillance.

COX2 is produced by normal host cells in an inflammatory microenvironment and is also overexpressed and secreted by many tumor cells (42, 43). Therefore, both the onset and the progression of tumors could be enhanced by PGE2. Chronic, low-grade inflammation would facilitate the accumulation of MDSC, thereby blocking tumor immunity and providing a favorable environment for the expansion of premalignant cells, and the transition of these cells to a malignant phenotype. Once a tumor is established, tumor cell production of COX2 would maintain elevated MDSC levels, further blocking tumor immunity and allowing the malignant cells to proliferate without interference from the host's immune system.

The availability of EP2 knockout mice on the BALB/c background has allowed us to conclusively show that the EP2 receptor is involved in the induction of MDSC. Although knockout mice for the other E-prostanoid receptors are not available on a BALB/c background, the experiments of Fig. 2 indicate that MDSC
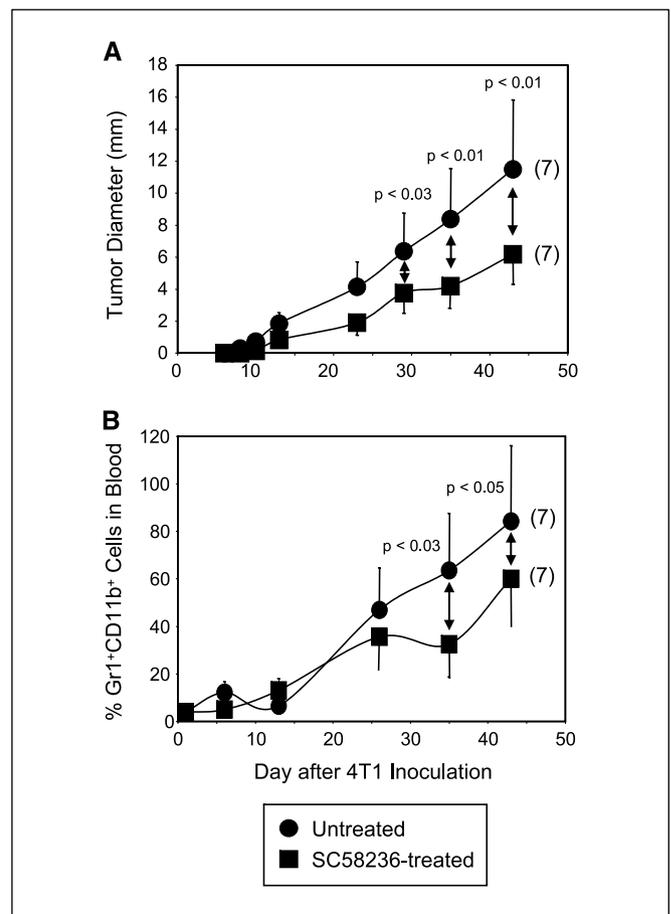

Figure 5. 4T1 tumor-bearing BALB/c mice treated with SC58236 have reduced primary tumor growth and fewer MDSC. *A,* BALB/c mice were inoculated with 4T1 tumor cells on day 0 and simultaneously started on SC58236 treatment, which was continued through day 21. Primary tumors were measured with a calipers. *B,* mice from *A* were bled and their levels of $Gr1^+CD11b^+$ MDSC were determined by flow cytometry. *Numbers in parentheses,* number of mice per group. Data are representative of three independent experiments.

are probably induced through other E-prostanoid receptors as well (i.e., EP1 and EP4). Our findings that SC58236-treated and EP2 knockout mice have reduced, but not background, levels of MDSC is consistent with the concept that the EP2 receptor is not the only trigger for the induction of MDSC. This conclusion is also supported by the observation that low levels of $Gr1^+CD11b^+$ cells differentiate from bone marrow precursor cells in the absence of PGE2 or E-prostanoid agonists. Other factors, including vascular endothelial growth factor, IL-6 (20), GM-CSF (44), and transforming growth factor β (45), have also been implicated in MDSC induction. Because all of these factors are to some extent by-products of inflammation, it remains unclear if MDSC induction is mediated exclusively by inflammation or if there are non–inflammatory-dependent factors that also control MDSC levels.

Yang et al. (46) have reported that EP2-deficient mice have reduced tumor progression when inoculated with the C57BL6 3LL or MC26 tumors. They noted that dendritic cell function and levels were normal in tumor-bearing EP2-deficient mice, but were reduced in tumor-bearing wild-type mice, and concluded that dendritic cell abnormalities favored tumor progression by compromising tumor immunity. Because PGE2 induces MDSC at the expense of dendritic cell differentiation (Fig. 2A), and MDSC are known to accumulate in

mice with 3LL or MC26 tumors (47, 48), it is likely that at least some of the immunodeficiency observed by Yang et al. is due to MDSC.

At least two other cell types also facilitate tumor progression by interfering with immunosurveillance: CD4$^+$CD25$^+$ T regulatory cells and M2 macrophages. T regulatory cells promote tumor progression by blocking CD8$^+$ T-cell activation and thereby inhibit tumor immunity (49, 50). The suppressive activity of T regulatory cells is driven by expression of the forkhead/winged helix transcription factor (*FOXP3*) gene (51–53). PGE2 has been shown to induce expression of the *FOXP3* gene in CD4$^+$ T cells and to increase the expression of FOXP3 in existing T regulatory cells, thereby promoting immunosuppression (54, 55). Interestingly, MDSC have also been shown to induce T regulatory cells (56), raising the possibility that in addition to the direct effects of PGE2 on T regulatory cell differentiation, PGE2 may also indirectly activate T regulatory cells through its induction of MDSC. M2 macrophages, which produce high levels of arginase, favor tumor growth by enhancing angiogenesis, tissue repair, and tissue remodeling (57). Interestingly, PGE2 produced by tumor cells was recently shown to induce arginase production in cells expressing the macrophage marker CD11b, suggesting that tumor-secreted PGE2 also affects immunosurveillance by polarizing macrophages toward a tumor-promoting phenotype (58).

Interruption of the PGE2 pathway *in vivo* has been shown to delay and/or prevent tumor progression in many systems. For example, in mouse models, nonsteroidal anti-inflammatory drugs that inhibit COX-1 and/or COX-2 reduce spontaneous metastatic disease of transplantable tumors (8), delay primary tumor onset and tumor progression in transgenic (59) and transplantable (60, 61) models, and augment tumor immunity after vaccination (62, 63). Antagonists of prostaglandin receptors also reduce metastatic disease (9), and nonsteroidal anti-inflammatory agents limit tumor progression in patients (6). The antitumor effects of these agents have been attributed to various mechanisms, such as enhancing angiogenesis (34) and inducing tumor cell apoptosis (35). Our finding that PGE2 antagonists and COX-2 inhibitors reduce MDSC levels adds another mechanism by which these agents delay tumor progression, and provides additional reasons for developing new drugs that block the PGE2 pathway without having detrimental side effects.

Acknowledgments

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