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Prostaglandin E$_2$ Induces FOXP3 Gene Expression and T Regulatory Cell Function in Human CD4$^+$ T Cells$^1$

Felicita Baratelli, Ying Lin, Li Zhu, Seok-Chul Yang, Nathalie Heuzé-Vourc’h, Gang Zeng, Karen Reckamp, Mariam Dohadwala, Sherven Sharma, and Steven M. Dubinett$^2$

Naturally occurring CD4$^+$CD25$^+$ regulatory T cells (T reg) are pivotal in suppressing immune responses and maintaining tolerance. The identification of molecules controlling T reg differentiation and function is important in understanding host immune responses in malignancy and autoimmunity. In this study we show that PGE$_2$ enhances the in vitro inhibitory function of human purified CD4$^+$CD25$^+$ T reg cells. Moreover, PGE$_2$ induces a regulatory phenotype in CD4$^+$CD25$^+$ T cells. PGE$_2$-treated T cell-mediated inhibition of anti-CD3-stimulated lymphocyte proliferation did not require cell contact. Phenotypic analysis revealed that PGE$_2$ diminished CD25 expression in both CD4$^+$CD25$^{dim}$ T cells and CD4$^+$CD25$^{bright}$ T reg cells. PGE$_2$ exposure induced the T reg cell-specific transcription factor forkhead/winged helix transcription factor gene (FOXP3) in CD4$^+$CD25$^+$ T cells and significantly up-regulated its expression in CD4$^+$CD25$^+$ T reg cells. Similarly, 24-h incubation with supernatants from cyclooxygenase-2-overexpressing lung cancer cells that secrete high levels of PGE$_2$ significantly induced FOXP3 in CD4$^+$CD25$^+$ T cells. Finally, PGE$_2$ up-regulated FOXP3 at both mRNA and protein levels and enhanced FOXP3 promoter activity. This is the first report indicating that PGE$_2$ can modulate FOXP3 expression and T reg function in human lymphocytes. The Journal of Immunology, 2005, 175: 1483–1490.

\[\text{Natually occurring regulatory T (T reg)}\]

CD4$^+$CD25$^+$ T reg cell population has been found to be significantly increased in PBL and within the tumor-infiltrating lymphocytes of epithelial cancers, including non-small cell lung cancer (NSCLC) (17–23). Given that COX-2 overexpression and PGE$_2$ up-regulation were highly associated with the progression of this malignancy (16), we hypothesized that PGE$_2$ could contribute to the tumor-induced immunosuppression through modulation of T reg cell function. In this study we demonstrate that PGE$_2$ enhances the inhibitory capacity of in vitro purified human CD4$^+$CD25$^+$ T reg cells and induces a suppressive regulatory phenotype in CD4$^+$CD25$^+$ T cells. The PGE$_2$-dependent acquisition of T reg cell function was correlated with induction of FOXP3 gene and protein expression. The enhanced inhibitory activity of PGE$_2$-treated CD4$^+$CD25$^+$ T reg cells was also associated with significant up-regulation of FOXP3. This report highlights novel roles for PGE$_2$ in controlling the generation and function of T reg cells.

\[\text{Materials and Methods}\]

\[\text{Cell isolation and culture}\]

Human CD4$^+$CD25$^+$ T reg cells and CD4$^+$CD25$^-$ T cells were purified from enriched buffy coat from healthy volunteers using the T reg cell isolation kit (Miltenyi Biotec) and the AutoMACS cell sorter (Miltenyi Biotec). An institutional review board approval was obtained, and all donors signed informed consent. Cell purity assessed by flow cytometry was $\geq$92%. Before being used in different experimental conditions, purified CD4$^+$CD25$^+$ and CD4$^+$CD25$^-$ T cell fractions were preincubated for 24 h in X-Vivo 15 (BioWhittaker), 10% FBS, and 1% human serum AB (Gemini) (24) with or without 16,16-dimethyl-PGE$_2$ (Cayman Chemical; 13 or 26 $\mu$M). In some experiments, CD4$^+$CD25$^-$ T cells (2 $\times$ 10$^5$) were preincubated for 18–24 h with 2 ml of undiluted tumor supernatant obtained from H157 (human squamous cell carcinoma: American Type Culture Collection) NSCLC, genetically modified to express COX-2 sense (S), COX-2 antisense (AS), or control empty vector (CV) (25). A 2.0-kb cDNA fragment of human COX-2 (provided by Dr. H. Hershman, University of California, Los Angeles, CA) was cloned in S and AS orientations in the retroviral vector pLNCl (BD Clontech) as previously described (25). For each cell line, an $\sim$10-fold higher level of COX-2 expression and PGE$_2$ production was noted in COX-2 S compared with parental controls or CV (25). To neutralize the activity of the PGE$_2$ contained in the COX-2 S supernatant, anti-PGE$_2$ (10 $\mu$g/ml; provided by J. Portanova, Searle, St.

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$^3$Abbreviations used in this paper: T reg cell, regulatory T cell; AS, antisense; COX-2, cyclooxygenase-2; CV, control empty vector; FOXP3, forkhead/winged helix transcription factor gene; NSCLC, nonsmall cell lung cancer; S, sense.

Louis, MO) was added, and a mouse IgG (10 μg/ml; Sigma-Aldrich) was used as a control Ab. T cell activation was performed by incubation of both CD4^+ CD25^- T cells and CD4^+ CD25^+ T reg cells in plate-bound anti-CD3 (1 μg/ml)-coated, 96-well plates (BD Biosciences) from 24 to 120 h. Alternatively, T cells were activated in the presence of PMA (20 ng/ml; Sigma-Aldrich) and ionomycin (1 μg/ml; Sigma-Aldrich).

**Flow cytometric analysis**

CD4^+ CD25^- T cells and CD4^+ CD25^+ T reg cells, pretreated with or without PGE2, and cultured with or without anti-CD3 stimulation, were resuspended in PBS/2% FBS and labeled with human anti-CD25 PE (Miltenyi Biotec) and human anti-CD4 FITC Abs or with control isotype (all from BD Biosciences) at the indicated time points. Acquisition was performed using a Life Science Research flow cytometer or a SCAN X instrument (BD Biosciences) using CellQuest software (BD Biosciences) at University of California-Los Angeles Jonsson Comprehensive Cancer Center Flow Cytometry Core Facility. Data analysis was performed with CellQuest software.

**In vitro proliferation assay**

T cell proliferation was assessed by BrdU incorporation using a BrdU ELISA colorimetric assay (Roche). Briefly, magnetic bead-purified human CD4^+ CD25^- T cells or CD4^+ CD25^+ T reg cells were pretreated with PGE2 (13 or 26 μM) for 24 h. PGE2 was removed by washing before starting the proliferation assay. To rule out the possibility that residual PGE2 would be transferred to the second culture, we performed preliminary experiments in which CD4^+ CD25^- T cells were treated with PGE2 (26 μM; Cayman Chemical) for 24 h. PGE2 was removed by washing before the second culture was added.

**Statistics**

The p values were calculated using unpaired Student’s t test. Values ≤0.05 were considered significant.

**Results**

**PGE2 enhances CD4^+ CD25^- T regulatory function and confers T regulatory activity in CD4^+ CD25^+ T cells**

Previous studies have focused on the direct effect of PGE2 in mediating suppression of lymphocyte proliferation, highlighting the role of calcium-dependent mechanisms (29, 30). In the current study we first investigated whether exogenous PGE2 was able to augment the suppressive activity of CD4^+ CD25^- T reg cells. Purified CD4^+ CD25^- T reg cells demonstrated significant enhancement of their inhibitory function after incubation with PGE2, as shown in Fig. 1A. Treatment of CD4^+ CD25^- T reg cells with escalating doses of PGE2 (13 or 26 μM) inhibited T cell proliferation in a dose-dependent pattern (Fig. 1A). The T cell proliferation was measured as OD values of BrdU incorporation, obtained from seven different donors were 1.04 ± 0.17 (CD4^+ CD25^-), 0.68 ± 0.16 (CD4^+ CD25^+), and 0.34 ± 0.14 (CD4^+ CD25^- + PGE2, 26 μM). During the 5-day culture period of the assay, the T cell proliferative response by light microscopy.

Wells containing stimulator T cells not treated with PGE2 displayed numerous homogeneously distributed cell clusters, indicative of ongoing proliferation. In contrast, wells containing stimulator T cells pretreated with PGE2 consistently showed significantly fewer proliferating clusters (data not shown). The OD values of BrdU incorporation were considered significant.

**Real-time PCR for FOXP3**

For quantitative real-time analysis, RNA was extracted, and cDNA was prepared with a kit (In Vitro Life Technologies) according to the manufacturer’s instructions. Human FOXP3 mRNA expression was quantified using the SYBR Green quantitative PCR kit (Finnzymes) in the iCycler (Bio-Rad) and was corrected with human β-actin housekeeping control amplifications. Amplification was conducted in a total volume of 20 μl for 40 cycles of 15 s at 95°C, 20 s at 60°C, and 30 s at 72°C. Samples were run in triplicate, and their relative expression was determined by normalizing the expression of each target to β-actin and then comparing this normalized value to the normalized expression in a reference sample to calculate a fold change value.

**For FOXP3, primers were:** forward, 5'- CAA GAT TTT CAT GGC AG-3'; reverse, 5'- ATT GAG TGT CCG CTG CT-3'.

**FOXP3 primers were synthesized by Integrated DNA Technologies. For β-actin, primers were:** forward, 5'- CAA GAT TTT CAT GGC AG-3'; reverse, 5'- CAC CTC CCG TCC GAT TT-3'.
reg cells (Fig. 1B). The T cell proliferations, expressed as OD values of BrdU incorporation, obtained from three different donors were 1.26 ± 0.39 (CD4+CD25+ and CD4+CD25− cells, precultured with or without PGE2, were mixed with autologous CD4+ cells in anti-CD3-bound, 96-well plates for 5 days. B, PGE2 induces T reg cell activity in CD4+CD25− T cells. CD4+CD25+ and CD4+CD25− cells were precultured with increasing concentrations of PGE2 before a 5-day proliferation assay. C and D, IL-7, but not IL-2, overcomes suppression of T cell proliferation. IL-7 (C) or IL-2 (D) was added on day 1 to the proliferation assay described above. E, PGE2 mediates T reg cell activity without the requirement for cell contact. Both untreated and PGE2-treated CD4+CD25+ and CD4+CD25− cells were separated from dendritic cell-activated CD4+CD25− T cells by Transwell inserts. In all experiments, cell proliferation was measured by BrdU incorporation. Results are expressed as the mean (±SD) of one representative experiment of at least three performed in triplicate from different donors.

FIGURE 1. A, PGE2 enhances T reg cell function. Purified CD4+CD25+ and CD4+CD25− cells, precultured with or without PGE2, were mixed with autologous CD4+ cells in anti-CD3-bound, 96-well plates for 5 days. B, PGE2 induces T reg cell activity in CD4+CD25− T cells. CD4+CD25+ and CD4+CD25− cells were precultured with increasing concentrations of PGE2 before a 5-day proliferation assay. C and D, IL-7, but not IL-2, overcomes suppression of T cell proliferation. IL-7 (C) or IL-2 (D) was added on day 1 to the proliferation assay described above. E, PGE2 mediates T reg cell activity without the requirement for cell contact. Both untreated and PGE2-treated CD4+CD25+ and CD4+CD25− cells were separated from dendritic cell-activated CD4+CD25− T cells by Transwell inserts. In all experiments, cell proliferation was measured by BrdU incorporation. Results are expressed as the mean (±SD) of one representative experiment of at least three performed in triplicate from different donors.

IL-7 (≥2 ng/ml) and a high concentration of IL-2 (≥100 IU/ml) demonstrated the capacity to induce proliferation of T reg cells (data not shown) in agreement with previous reports (31, 32). Interestingly, when CD4+CD25− responder T cells were separated by Transwell inserts from PGE2-treated stimulator cells (CD4+CD25− or CD4+CD25+), both PGE2-treated populations maintained the capacity to inhibit T cell proliferation (Fig. 1E). These data taken together suggest that the PGE2-induced T regulatory function does not require cell contact. We have previously found that PGE2 is a potent inducer of lymphocyte IL-10 gene transcription (15), and PGE2 has also been reported to increase TGF-β expression (33–35). However, neither anti-IL-10 nor anti-TGF-β neutralizing Abs were able to reverse the PGE2-induced inhibitory activity mediated by CD4+CD25− or CD4+CD25− T reg cells (data not shown). These findings suggest the contribution of additional soluble factors other than IL-10 or TGF-β to PGE2-induced cell-contact independent T regulatory cell function.
Down-regulation of CD25 expression in PGE₂-treated CD4⁺ T cells

Because T reg cells are identified as a CD4⁺CD25bright T cell subset (36), we analyzed whether the PGE₂-treated CD4⁺CD25dim starting population acquired the same phenotype of naturally occurring, thymic-derived CD4⁺CD25bright T reg cells. As shown in Table I, CD25 was differentially expressed in magnetic bead-purified CD4⁺CD25dim and CD4⁺CD25bright T cells. Indeed, with (Fig. 2A and Table II) or without PGE₂ preincubation (data not shown), CD4⁺CD25dim up-regulated CD25 as early as 24 h after stimulation with anti-CD3 plate-bound Ab (Fig. 2A). These T cells maintained high levels of CD25 expression throughout a 96-h culture period (Fig. 2A). However, when anti-CD3 Ab-activated T cells were pretreated with PGE₂, CD25 expression declined in both CD4⁺CD25dim T cells (Fig. 2B, bottom left, and Table II) and CD4⁺CD25bright T reg cells (Fig. 2B, bottom right, and Table III), compared with their counterparts without PGE₂ exposure (Fig. 2, B and C, upper panels, and Tables II and III). Despite decreased CD25 expression, suppressive T reg function was maintained, as shown in Fig. 1. Other markers indicative of the T reg phenotype, including glucocorticoid-induced TNFR family-related protein, CTLA-4, human latency-associated peptide of TGF-β1, lymphocyte activation Ag 3, PD-1, CD103, CD122, and CCR4, were either not altered or were down-regulated by PGE₂ (data not shown).

PGE₂ induces FOXP3 in CD4⁺CD25⁻ T cells and up-regulates its expression in CD4⁺CD25⁺ T reg cells

Because FOXP3 is specifically expressed in naturally occurring T reg cells and programs their development and function (1–5), we determined whether PGE₂ had an impact on the induction of FOXP3 gene expression in both CD4⁺CD25⁺ T reg and CD4⁺CD25⁻ T cells. As expected, purified, peripheral blood-derived, CD3-activated CD4⁺CD25⁺ T reg cells expressed high levels of FOXP3 mRNA compared with CD4⁺CD25⁻ T cells (Fig. 3A). Furthermore, a 24-h preincubation with PGE₂ (26 μM) resulted in >4-fold up-regulation of FOXP3 (Fig. 3A). When CD4⁺CD25⁻ T cells were cultured under the same stimulatory conditions in the presence of PGE₂ (26 μM), FOXP3 mRNA expression was also significantly induced, consistent with the acquisition of a T reg cell suppressive function (Fig. 3B). Thus, PGE₂ facilitated the differentiation of CD4⁺CD25⁻ T cells into a T cell population endowed with inhibitory properties and FOXP3 mRNA expression.

Table 1. CD25 expression in CD4⁺CD25dim and CD4⁺CD25bright following magnetic bead purification

<table>
<thead>
<tr>
<th>CD4⁺CD25dim</th>
<th>CD4⁺CD25bright</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Positive (MFI)</td>
<td>% Positive (MFI)</td>
</tr>
<tr>
<td>24.2 ± 19 (51 ± 7.7)</td>
<td>94 ± 2.7 (247 ± 113.8)</td>
</tr>
</tbody>
</table>

*CD25 surface expression and PGE₂-mediated modulation of CD25 in CD4⁺ PBL. Data summarize (n = 8) the CD25 expression of CD4⁺CD25dim and CD4⁺CD25bright T cell populations following cell purification (Miltenyi Treg Cell Isolation kit). Results are expressed as percentage of CD25 expression within the CD4⁺ population. Values in parentheses indicate the mean fluorescence intensity (MFI) of CD25 expression based on density plot analysis.

PGE₂, we used COX-2 gene-modified NSCLC cells as an experimental model for the tumor site, which is often enriched for PGE₂. For this purpose we genetically modified NSCLC cell lines to express COX-2 S, COX-2 AS, or empty CV, as described in Materials and Methods and as we have previously reported (25). Using this in vitro model we preincubated CD4⁺CD25 T cells with tumor supernatant from H157 COX-2-gene-modified NSCLC cell lines. FOXP3 gene expression was quantified in CD4⁺CD25 T cells after 24-h activation with anti-CD3 plate-bound Ab. A 24-h exposure to H157 COX-2 S supernatant significantly induced FOXP3 gene expression in CD4⁺CD25 T cells. In contrast, COX-2 AS and CV supernatant had no effect on FOXP3 gene expression. Addition of anti-PGE₂ Ab to COX-2 S supernatant blocked the induction of FOXP3 mRNA expression to the levels demonstrated in the AS or CV supernatants (Fig. 3C). Control Ab did not impact the COX-2 S tumor supernatant-mediated induction of FOXP3 mRNA expression (Fig. 3C).
PGE₂ up-regulates FOXP3 mRNA and protein expression and induces FOXP3 promoter activity

To determine the mechanisms underlying PGE₂-dependent regulation of FOXP3, we analyzed the expression of FOXP3 mRNA and protein levels in untreated or PGE₂-treated (13 and 26 μM) Jurkat T cells in the presence or the absence of TCR-activating stimuli. We found that PGE₂ up-regulated FOXP3 mRNA levels in both TCR-activated and non-activated Jurkat T cells (Fig. 4, A and C). Consistent with our findings in PBL, PGE₂ induced the greatest increase in FOXP3 in TCR-stimulated Jurkat T cells. Western blot analysis showed a similar pattern of expression (Fig. 4B). Non-activated Jurkat T cells, in fact, expressed FOXP3 protein that was significantly up-regulated by both PGE₂ concentrations tested (13 and 26 μM). However, maximum FOXP3 up-regulation was obtained in Jurkat T cells stimulated with PGE₂ (26 μM) and activated with PMA plus ionomycin (Fig. 4B). Finally, in promoter reporter assays, Jurkat T cells transfected with a FOXP3 promoter construct (FOXP3-SubD) demonstrated significantly enhanced FOXP3 promoter activity in response to PGE₂ and costimulatory signals (Fig. 4C). This suggests that PGE₂ operates to induce FOXP3 at the level of mRNA transcription.

Discussion

Several in vitro studies have reported an immunosuppressive role of PGE₂ in T cell proliferation. Most studies focused on the direct effect of PGE₂ on the responding T cell in which elevated levels of intracellular cAMP were associated with reduced IL-2 production (36, 37). Several other pathways of PGE₂-induced immunosuppression have been described, including inhibition of polyamine synthesis, inhibition of intracellular calcium release, and suppression of p59 (Fyn) protein tyrosine kinase activity (38). However, the contribution of PGE₂ in modulating T regulatory cell activity has not previously been defined.

A specific subset of T reg cells bearing a CD4⁺CD25⁺ T cell phenotype has now been the focus of extensive investigation (1–5, 35). These T cells, endowed with distinct immunomodulatory properties, are acknowledged as fundamental in the prevention of autoimmunity and may be important in preventing effective immune responses in malignancy (17–22). Although naturally occurring CD4⁺CD25⁺ T reg cells develop directly from CD4⁺ precursors in the thymus, a body of work has now demonstrated the induction of cells with regulatory function in the periphery from naive CD4⁺ T cells in response to a variety of stimuli (6–12).

In this study we report a novel mechanism of PGE₂-induced immunosuppression that occurs through the modulation/induction of human T reg cell function. Our results show that PGE₂ not only enhances the suppressive capacity of in vitro purified CD4⁺CD25⁺ T reg cells, but also induces a CD4⁺CD25⁺ T cell population to develop T reg cell function. Analysis of this newly induced T reg cell-like population revealed that PGE₂ exposure conferred acquisition of FOXP3 expression consistent with the phenotypic feature seen in naturally occurring T reg cells. The original CD25⁺ population became CD25bright upon anti-CD3 activation. However, PGE₂ exposure markedly down-regulated the surface expression of CD25 in these CD3-stimulated CD4⁺CD25⁺ T cells that typically developed CD25bright expression upon stimulation. The down-regulation of CD25, the α-chain of the high affinity IL-2R, was also evident in the purified CD4⁺CD25⁺ T reg cells exposed to the same concentration of PGE₂. Constitutive expression of CD25 commonly has been used to define CD4⁺CD25⁺ T reg cells and IL-2 has been implicated as an important modulator of T reg activity, in vivo (39, 40). Our

### Table II. CD25 expression in CD4⁺CD25dim T cells treated with or without PGE₂

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>CD4CD25dim</th>
<th>CD4CD25dim + PGE₂</th>
<th>Percentage positive and MFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>64 (277)</td>
<td>40 (71)</td>
<td>24 h</td>
</tr>
<tr>
<td>II</td>
<td>59 (125)</td>
<td>41 (42)</td>
<td>48 h</td>
</tr>
<tr>
<td>III</td>
<td>75 (101)</td>
<td>55 (36)</td>
<td>72 h</td>
</tr>
<tr>
<td>IV</td>
<td>69 (144)</td>
<td>46 (66)</td>
<td>96 h</td>
</tr>
</tbody>
</table>

### Table III. CD25 expression in CD4⁺CD25bright Treg cells treated with or without PGE₂

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>CD4CD25bright</th>
<th>CD4CD25bright + PGE₂</th>
<th>Percentage positive and MFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>87 (179)</td>
<td>70 (60)</td>
<td>24 h</td>
</tr>
<tr>
<td>II</td>
<td>89 (78)</td>
<td>79 (41)</td>
<td>48 h</td>
</tr>
<tr>
<td>III</td>
<td>94 (66)</td>
<td>84 (44)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>81 (45)</td>
<td>68 (39)</td>
<td></td>
</tr>
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</table>
finding of PGE_2-mediated down-regulation of IL-2Rα is consistent with previous studies showing decreased IL-2 production and IL-2Rα expression in TCR-activated, PGE_2-treated CD4+ T cells (37, 38, 42, 43). Thus, the PGE_2-mediated down-regulation of IL-2Rα may limit typical autocrine and paracrine IL-2-dependent function, because Ab-blocking studies could not reverse the inhibitory function of both PGE_2-treated CD4+CD25+ and CD4+CD25− T reg cells. In contrast, it has also been shown that, as members of the IL-7R (44). The receptors for these cytokines in fact have been shown to preferentially produce IL-10 or TGF-β (data not shown). Neither of these cytokines appeared to significantly contribute to the in vitro regulatory function, because Ab-blocking studies could not reverse the inhibitory effect of either PGE_2-treated CD4+CD25+ or CD4−CD25− T cells (data not shown). Taken together, our data indicate that PGE_2-induced CD4+CD25+ T cells share common features of both thymic and peripheral T reg cells (4, 5, 47, 48).

The transcription factor FOXP3 exerts a key role in the development and function of T reg cells (1–5, 46). This separates FOXP3 from other T reg cell-associated markers such as CD25,
glucocorticoid-induced TNFR family-related protein, and CTLA-4, which may be more generally expressed by activated T cells (35, 36). In our study, PGE2-dependent T reg differentiation was indeed associated with induction of the transcription factor FOXP3, whose expression was also significantly up-regulated by PGE2 in purified CD4+CD25+ and CD4+CD25− T cells. Consistent with previous reports in which TGF-β was investigated (6–8), PGE2-mediated T reg cell differentiation and FOXP3 induction were evident predominantly in the presence of costimulation by either anti-CD3 or allogeneic dendritic cells (data not shown). Using a variety of approaches, several studies are now modeling the differentiation of cells with T regulatory function arising in the periphery (6–12, 48). For example, recent studies in mice and humans, have demonstrated that CD4+CD25− T cells treated with TGF-β in the presence of appropriate costimulation lead to T reg cell differentiation with the capacity for suppressive function in vitro (6, 7). It is unclear whether these extrathymic T reg cells represent altered states of differentiation or belong to a unique T cell lineage (48).

Our findings suggest a role for PGE2 in modulating T reg cell function and differentiation. In conditions of PGE2 overproduction, such as described in several malignancies (17–22), increased T reg cell differentiation and function could contribute to tumor-induced immunosuppression. Consistent with this concept, malignancies such as lung cancer that have previously been demonstrated to have high COX-2 expression and PGE2 production (16, 49) have also been noted to have increased T reg cell number and function within the PBL as well as the tumor-infiltrating lymphocyte populations (17–22). Extending these previous reports, we found that NSCLC cell lines overexpressing COX-2 were able to induce FOXP3 gene expression in CD4+CD25− T cells in a PGE2-dependent manner. We have recently reported that these relationships are operative in vivo in murine lung cancer models (50). Thus, taken together, our data indicate that regulation of PGE2 production may, in turn, modulate T reg cell development and function. These findings suggest new therapeutic strategies targeting COX-2/PGE2 in the prevention and treatment of cancer. This could include addition of COX-2 inhibitor treatment to immune-based therapies.

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Disclosures
The authors have no financial conflict of interest.
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44. R1748. R1748. R1748.