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Induction of a CD4+ T Regulatory Type 1 Response by Cyclooxygenase-2-Overexpressing Glioma

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PGE₂, synthesized by cyclooxygenase-2 (COX-2)-overexpressing tumor, is known to contribute to cellular immune suppression in cancer patients, but the mechanism remains unclear. We report the mechanism of a CD4+ T regulatory type 1 (Tr1) induction by CD11c+ mature dendritic cells (DCs) that phagocytose allogeneic and autologous COX-2-overexpressing glioma. A human glioma cell line, U-87MG, and primary cultured glioblastoma cells (MG-377) overexpressed COX-2. We did not detect IL-10R expression in these gliomas, and rIL-10 did not suppress their COX-2 expression. Exposure to COX-2-overexpressing glioma induced mature DCs to upregulate IL-10 and decreased IL-12p70 production. These DCs induced a Tr1 response, which is characterized by robust secretion of IL-10 and TGF-β with negligible IL-4 secretion by CD4+ T cells, and an inhibitory effect on admixed lymphocytes. Peripheral CD4+ T cell populations isolated from an MG-377 patient also predominantly demonstrated a Tr1 response against MG-377 cells. Selective COX-2 inhibition in COX-2-overexpressing gliomas at the time of phagocytic uptake by DCs abrogated this regulatory response and instead elicited Th1 activity. COX-2 stable transfectants in LN-18 (LN-18-COX2) also induced a Th1 response. The effect of a COX-2 inhibition in LN-18-COX2 is reversible after administration of PGE₂. Taken together, robust levels of PGE₂ from COX-2-overexpressing glioma, which is unresponsive to IL-10 within the local microenvironment, may cause DCs to secrete high levels of IL-10. These results indicate that COX-2-overexpressing tumors induce a Tr1 response, which is mediated by tumor-exposed, IL-10-enhanced DCs. The Journal of Immunology, 2004, 173: 4352–4359.

Effects of PGE₂ on DC function. We observed that exposure to COX-2-overexpressing glioma cells induced potent secretion of IL-10 in DCs and impaired their ability to produce IL-12. These DCs lost the ability to induce a Th1 response and instead elicited an IL-10 and TGF-β secreting Th phenotype. The induced CD4+ T cells potently suppressed admixed lymphocyte proliferation, and the suppressor effector function was blocked by IL-10 neutralization. Notably, the selective inhibition of COX-2 in COX-2-overexpressing gliomas at the time of phagocytic uptake by DCs abrogated this regulatory response. In this report we propose a novel mechanism...
of Tr1 induction in patients with COX-2-overexpressing malignant tumor.

Materials and Methods

Tumor cells

A human primary cultured malignant glioma (MG-377) was established from a surgical specimen of a patient with newly diagnosed glioblastoma at Cedars-Sinai Medical Center after institutional review board-approved consent was obtained. MG-377 and human glioma cell lines, U-87MG (American Type Culture Collection, Manassas, VA) and LN-18 (provided by Dr. E. Van Meier, Emory University, Atlanta, GA) were maintained at 37°C in 5% CO₂ in DMEM with 10% heat-inactivated FBS, 2 mM glutamate, 10 mM HEPES, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Plasmid and transfections

The full-length COX-2 cDNA was isolated from pSG5-COX-2 plasmid (provided by Dr. R. Kulmacz, University of Texas Medical School, Houston, TX) by EcoRI and Xbal digestion. COX-2 expression plasmid (pTracer-COX2) was constructed by inserting COX-2 cDNA into an EcoRI and XbaI site on pTracer-CMV2 (Invitrogen Life Technologies, Carlsbad, CA), which contains the selection marker zeocin. Empty plasmid, pTracer-CMV2 (LN-18-E/P), or pTracer-COX2 was transfected to LN-18 (LN-18-COX2) with Lipofectamine 2000 (Invitrogen Life Technologies) and Plus Reagent (Invitrogen Life Technologies), then stable transfecants were established by selection with zeocin (400 μg/ml; Invitrogen Life Technologies).

Western blot

Recombinant TRAIL (300 ng/ml; PeproTech, Rocky Hill, NJ), TNF-α (20 ng/ml; BioSource International, Camarillo, CA), IL-10 (10 ng/ml; R&D Systems, Minneapolis, MN), and NS-398 (10 μM; selective COX-2 inhibitor; Cayman Chemical, Ann Arbor, MI) were used to treat sample cells. Samples were extracted with buffer containing 1% Triton X-100, 150 mM NaCl, 50 mM Tris (pH 7.5), and 1 mM PMSF and were subjected to SDS-PAGE with 20 μg of general protein loading into each lane on a 7.5% polyacrylamide gel. Electrophoretic transfer to nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ) was followed by immunoblotting with IgG1 mouse mAb of anti-COX-2 (BD Pharmingen, San Diego, CA), unconjugated CCR7 (R&D Systems), and IL-10R (BD Biosciences). The signal was detected by an ECL detection system (Amersham Biosciences). The signal was detected by an ECL detection system (Amersham Biosciences).

Preparations of DC and lymphocytes

Human PBMCs were suspended in X-VIVO 15 serum-free medium (Cambrex, Santa Rosa, CA) and allowed to adhere to a 24-well culture plate at 37°C for 4 h. The nonadherent PBMCs were collected as general lymphocytes. The adherent PBMCs were subsequently cultured in X-VIVO 15 (1 ml/well), GM-CSF (20 ng/well; BioSource International) and IL-4 (10 ng/well; BioSource International) were added on days 0, 2, and 4. The group without GM-CSF and IL-4 were also cultured. The nonadherent PBMCs were also cultured with general lymphocytes (106 cells/well) with TRAIL or TNF-α (10 ng/ml) for 24 h. The group without GM-CSF and IL-4 were also cultured. The nonadherent PBMCs were also cultured with general lymphocytes (106 cells/well) with TRAIL or TNF-α (10 ng/ml) for 24 h.

Coculture with glioma cells and DC

Glioma cells were plated onto a six-well plate (1 × 10⁵ cells/well) with serum-free X-VIVO 15. Glioma cells pretreated with NS-398 (10 μM) for 8 h in fully supplemented DMEM were treated with TRAIL (300 ng/ml), TNF-α (20 ng/ml), and NS-398 (10 μM) for 24 h. The group without NS-398 pretreatment was treated with TRAIL and TNF-α. Subsequently, iDCs were put into wells (3 × 10⁶ cells/well) and cocultured with glioma cells for 16 h (cocomultured DCs). PGE₂, (0.1 μM; Sigma-Aldrich) was used to treat glioma-DC coculture. MG-377 was treated with autologous and nonstimulated lymphocytes (10⁶ cells/well) for 48 h for restimulation. Cocultured cells were stained with micorode-conjugated human anti-COX-2 Ab (Milltenyi Biotec), then CD4⁺ lymphocytes were isolated with a MiniMACS cell isolation unit. ELISPOT assay was performed using a dual human IFN-γ/IL-10 ELISPOT kit and a polynivilidene difluoride-bottomed, 96-well plate (Cell Sciences, Norwood, MA). Samples (1 × 10⁶ cells/well) were plated onto an IL-10 or IFN-γ capture Ab-coated well and cultured for 20 h. Spot forming was analyzed by an Alpha Imager Spot-Reading System (Alpha Innotech, San Leandro, CA).

Lymphocyte proliferation assay

The isolated CD4⁺ lymphocytes (1 × 10⁵ cells/well/100 μl) were cocultured with autologous nonstimulated lymphocytes (4 × 10⁵ cells/well/100 μl) in a 96-well plate with rat IgG2a Ab anti-human IL-10 (500 ng/ml; clone JES5-19F11; BD Pharmingen) or rat IgG2a isotype control (BD Pharmingen). After 24 h culture, a lymphocyte proliferation assay was performed to incubate cells for 30 h at 37°C with cell proliferation reagent WST-1 (10 μl/well; Roche, Indianapolis, ID), which is a colorimetric assay for mitochondrial dehydrogenase activity.

Statistics

Student’s t test was used for statistical comparison of results.

Results

DCs efficiently phagocytose apoptotic glioma cells

The production of the cytokines TRAIL and TNF-α by DCs is known to induce tumor cell apoptosis, permitting phagocytic uptake of tumor cells and maturation of DCs in vivo (21). To reproduce this phenomenon efficiently in vitro, after induction of apoptosis in human glioma cells mediated by recombinant TRAIL and TNF-α, human PBMC-derived iDCs were cocultured with the glioma cell lines U-87MG (U-87MG-DG) and LN-18 (LN-18-DG) and a primary cultured glioblastoma, MG-377 (MG-377-DG). Several distinct stages of tumor cell uptake and processing by CD11c⁺ DCs were clearly visible under microscopic observation (Fig. 1, A–C). Induction of apoptosis in glioma by treatment with TRAIL and TNF-α caused a significant increase in the efficiency of phagocytosis by DCs compared with a control group that was not treated with TRAIL or TNF-α (Fig. 1D).
Cytoxisis by DCs (mean ± SD of: control group, 7.7 ± 0.58; treatment group, 72 ± 11.3; p = 0.01).

**IL-10Ra-deficient human gliomas overexpress COX-2 and produce high levels of PGE2**

COX-2 expression and production of PGE2 in U-87MG, LN-18, and MG-377 was confirmed by Western blot and ELISA, respectively. U-87MG and MG-377 expressed significant levels of COX-2, whereas expression was low in LN-18. TRAIL and TNF-α treatment of glioma cells in vitro increased COX-2 expression in U-87MG and MG-377 (Fig. 2A) and caused a significant increase in PGE2 production by U-87MG (p < 0.01) and MG-377 (p < 0.01; Fig. 2B). Treatment with NS-398, a selective COX-2 inhibitor, markedly reduced the level of PGE2 secretion from U-87MG (p < 0.01) and MG-377 (p < 0.01; Fig. 2B). It has been shown that IL-10 exhibits a potent suppressor effect against COX-2 expression (22), and a recent study revealed that the deficiency of IL-10Ra on tumor cell surfaces correlates with tumor COX-2 overexpression (23). In this regard, we observed the expression of IL-10Ra on glioma cells with FACS analysis and Western blot. Furthermore, to determine whether tumor COX-2 was inhibited in response to rIL-10 treatment, we used Western blot to observe COX-2 expression in glioma cells that had been treated with TRAIL/TNF-α/IL-10 or TRAIL/TNF-α. The expression of IL-10Ra was detected in LN-18 (Fig. 2, D and E). In contrast, IL-10Ra expression was significantly lower in U-87MG and MG-377 (Fig. 2, D and E). Consequently, overexpressed COX-2 in U-87MG and MG-377 was not inhibited in response to IL-10 treatment (Fig. 2E). These data indicate that IL-10 in the microenvironment fails to suppress COX-2 expression in IL-10Ra-deficient, COX-2-overexpressing glioma. There was no significant difference in the production of TGF-β, which has been recognized as a key mediator of glioma-derived, immunosuppressive cytokine, in each glioma in either setting (Fig. 2C).

**DCs mature after phagocytosis of glioma cells**

To determine whether the uptake of apoptotic glioma induced iDCs to mature, we observed CD86, HLA-DR, CD83, and CCR7 expression levels on the cell surface of DCs. CD86 and HLA-DR were increased COX-2, whereas expression was low in LN-18. TRAIL and TNF-α treatment of glioma cells in vitro increased COX-2 expression in U-87MG and MG-377 (Fig. 2A) and caused a significant increase in PGE2 production by U-87MG (p < 0.01) and MG-377 (p < 0.01; Fig. 2B). Treatment with NS-398, a selective COX-2 inhibitor, markedly reduced the level of PGE2 secretion from U-87MG (p < 0.01) and MG-377 (p < 0.01; Fig. 2B). It has been shown that IL-10 exhibits a potent suppressor effect against COX-2 expression (22), and a recent study revealed that the deficiency of IL-10Ra on tumor cell surfaces correlates with tumor COX-2 overexpression (23). In this regard, we observed the expression of IL-10Ra on glioma cells with FACS analysis and Western blot. Furthermore, to determine whether tumor COX-2 was inhibited in response to rIL-10 treatment, we used Western blot to observe COX-2 expression in glioma cells that had been treated with TRAIL/TNF-α/IL-10 or TRAIL/TNF-α. The expression of IL-10Ra was detected in LN-18 (Fig. 2, D and E). In contrast, IL-10Ra expression was significantly lower in U-87MG and MG-377 (Fig. 2, D and E). Consequently, overexpressed COX-2 in U-87MG and MG-377 was not inhibited in response to IL-10 treatment (Fig. 2E). These data indicate that IL-10 in the microenvironment fails to suppress COX-2 expression in IL-10Ra-deficient, COX-2-overexpressing glioma. There was no significant difference in the production of TGF-β, which has been recognized as a key mediator of glioma-derived, immunosuppressive cytokine, in each glioma in either setting (Fig. 2C).

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were highly expressed on not only single-cultured DCs, but also on U-87MG-DC, LN-18-DC, and MG-377-DC (Fig. 3). NS-398 treatment of these cocultured DCs did not change the expression level of CD86 or HLA-DR (Fig. 3). It has recently been reported that PGE₂ is an essential facilitator of APC maturation and migration into lymph nodes via expression of the E prostanoïd receptor 4 (EP4) on APCs (24). The migratory function of DCs from periphery to lymphoid organs is associated with their CCR7 expression, which is one of the maturation markers of DCs as well as CD83 (25). Our results, however, demonstrate that the addition of NS-398 to DCs and glioma cocultures did not restrict DC maturation, because robust up-regulation of CD83 and CCR7 was still observed (Fig. 3). These data indicate that iDCs can mature after phagocytosis of glioma cells, and maturation of glioma-exposed iDCs is independent of COX-2 activity in glioma cells.

**COX-2 overexpression in glioma induces high levels of IL-10 secretion from allogeneic DCs that, in turn, mediates a Tr1 induction**

We determined whether COX-2 overexpression by glioma affected DC functionality. Ligation of CD40 on DCs triggers IL-12 production (26). We therefore stimulated isolated CD11c⁺ cocultured or single-cultured DCs with NIH-CD40L cells for ELISA of IL-12p70 and IL-10. In an allogeneic model, U-87MG-DC without COX-2 inhibition secreted high levels of IL-10 (p < 0.01), whereas their ability to produce IL-12p70 was significantly inhibited (p < 0.01; Fig. 4A). In contrast, when U-87MG cells were cocultured with NS-398 at the time of phagocytic uptake by DCs, secretion of IL-10 and IL-12p70 by U-87MG-DC reverted to that of single-cultured DCs (Fig. 4A). Although statistical differences were observed in LN-18-DC IL-10 (p < 0.05) and IL-12p70 (p < 0.05) secretion levels, there was no significant change in IL-10 production in response to NS-398 treatment (Fig. 4A). Single-cultured DCs demonstrated only a negligible change in IL-10 and IL-12p70 production in response to NS-398 treatment (Fig. 4A), indicating that COX-2 activity in single-cultured DCs is negligible. The supernatants of cocultures with NIH-CD40L and all three types of glioma cell (U-87MG, LN-18, and MG-377) had no detectable IL-10 or IL-12p70 (not shown). These results indicate that COX-2 overexpression in U-87MG causes allogeneic DCs to secrete high levels of IL-10.

Following this observation, we determined whether glioma-exposed DCs induced a regulatory phenotype in CD4⁺ T cells. After isolation of CD11c⁺ DCs, autologous lymphocytes were stimulated with DCs, then restimulated with U-87MG or LN-18 cells. CD4⁺ T cells were isolated after restimulation and were assessed by means of an ELISPOT assay for IL-10 and IFN-γ and an ELISA for secreted IL-4 and TGF-β. In the ELISPOT assay, U-87MG-DC without COX-2 inhibition generated IL-10-secreting T cells (p < 0.01; Fig. 4B). Conversely, COX-2-inhibited U-87MG-DCs generated a Th1 phenotype, as evidenced by their enhanced IFN-γ production (p < 0.01; Fig. 4B). Statistically significant Th responses were observed when the lymphocytes were restimulated by U-87MG cells, but not by LN-18 cells (Fig. 4B), indicating that U-87MG-DCs induced a response only against U-87MG cells. Although LN-18-DCs also induced Th responses that react with LN-18 cells, the induced Th phenotype was polarized toward a Th1 response in both the presence and the absence of NS-398 (p < 0.05; Fig. 4B). Secretion of TGF-β was significantly enhanced in the Th phenotype stimulated with U-87MG-DC without COX-2 inhibition (p < 0.05; Fig. 4C). IL-4 secretion from isolated CD4⁺ T cells was not observed in either setting (not shown). CD4⁺ T cells stimulated with glioma cells without DC stimulation did not exhibit any detectable result in ELISPOT or ELISA (not shown). These data demonstrate that COX-2-overexpressing glioma modulates allogeneic DC function to induce an IL-10 and TGF-β secretory CD4⁺ Th response.

We determined whether these CD4⁺ T cell populations exhibited a suppressor effector function on autologous lymphocytes. To investigate this, we used a WST-1 mitochondrial dehydrogenase-based cell proliferation assay (27). In this experiment we found that CD4⁺ Th cells stimulated by U-87MG-DC without COX-2 inhibition demonstrated markedly inhibited proliferative responses on admixed autologous lymphocytes compared with those observed after stimulation with NS-398-treated U-87MG-DC (p < 0.001; Fig. 4D). Importantly, the suppressor function of this Th phenotype was blocked by IL-10 neutralization (p < 0.001; Fig. 4D). These data indicate that the CD4⁺ Th cell phenotype induced by U-87MG-DC is characteristically compatible with a Tr1.

**Peripheral CD4⁺ T cells in a patient with COX-2-overexpressing glioma predominantly demonstrated a Tr1 response**

We also observed the function of DCs in an autologous model. PBMC-derived autologous iDCs were cocultured with MG-377 cells (MG-377-DC). Similar to the allogeneic model, we found that IL-10 secretion by MG-377-DCs that had not been treated...
with NS-398 was significantly increased ($p < 0.01$), whereas IL-12p70 secretion was decreased ($p < 0.05$; Fig. 5A). In contrast, when MG-377 cells were treated with NS-398 at the time of phagocytic uptake by DCs, the secretion of IL-10 and IL-12p70 by MG-377-DCs reverted to that in single-cultured DCs (Fig. 5A).

After this observation, autologous lymphocytes were stimulated with isolated MG-377-DCs, then restimulated with MG-377 cells. In this analysis, MG-377-DCs without COX-2 inhibition induced generation of an IL-10 and TGF-$eta$ secretory Th phenotype (Fig. 5B). Of significance, however, was our finding that autologous CD4$^+$ cells without DC stimulation and stimulated with MG-377 cells did not exhibit any detectable result in ELISPOT assay or ELISA (not shown). We also found that CD4$^+$ Th cells stimulated with COX-2-inhibited MG-377-DCs demonstrated markedly increased proliferative responses on admixed lymphocytes ($p < 0.01$), whereas the CD4$^+$ Th phenotypes in other settings demonstrated a suppressor effector function that was blocked by IL-10 neutralization (Fig. 5C). These data indicate that peripheral CD4$^+$ T cells in a patient with COX-2-overexpressing glioma have already been primed toward a T1 phenotype. Significantly, a Th1 response against tumor cells can be elicited if tumor COX-2 is blocked at the time of phagocytic uptake by DCs.

**Exposure to high levels of PGE$_2$ on DCs correlates with their T1 induction**

To further confirm the relevance of COX-2 overexpression in glioma cells and glioma-associated PGE$_2$ in mediating a T1 response by DCs, we engineered LN-18 glioma cells to overexpress COX-2 (LN-18-COX2). After confirmation of enhanced COX-2 expression by Western blot (Fig. 6A), we subjected iDCs to coculture with LN-18-COX2 (LN-18-COX2-DC) or control transfectant.
IL-12-deficient DC maturation in vitro, which elicits increased Th2 cytokine production and decreased IFN-γ secretion from admixed CD4+ T cells. Additionally, Harizi et al. (14) found that inducible COX-2-overexpressing DCs secreted robust levels of IL-10 and exhibited a diminished capacity to stimulate T cell proliferation. With regard to the role of IL-10, it is known that IL-10 is an anti-inflammatory cytokine and exhibits a potent suppressor effect against COX-2 expression, which is a proinflammatory signal (22).

In these contexts, it is likely that IL-10 enhanced by high levels of PGE2 stimulation on DCs negatively modulates inflammation when COX-2 is overexpressed at the inflammatory site. A recent report described a correlation between human glioma COX-2 expression and poor clinical outcome (19). It has been recognized that defects in the process of endogenous Ag presentation underlie the impaired cellular immunity seen in patients with glioma (29). These findings point to the potential relevance of COX-2 and PGE2 expressed by glioma in down-regulating tumoricidal immunity at the level of Ag presentation. In this report we demonstrated that exposure to COX-2-overexpressing glioma on iDCs elicits mature DCs that produce significant levels of IL-10 and decreased IL-12 secretion. Selective COX-2 inhibition in COX-2-overexpressing glioma reversed the changes in IL-10 and IL-12 secretion, indicating that COX-2 overexpression in glioma significantly affects IL-10 overexpression in glioma-exposed DCs. We also observed that the ability of rIL-10 to suppress COX-2 was negated in U-87MG and MG-377 cells, which are deficient in IL-10R expression, indicating that COX-2 overexpression is maintained in these gliomas even if the levels of IL-10 are increased in their microenvironments. It is possible, because IL-10 cannot suppress COX-2-overexpression in glioma, that stimulation of DCs with high levels of PGE2 are prolonged in a glioma-DC coculture, and the prolonged PGE2 stimulation may induce IL-10 overexpression in glioma-exposed DCs. Therefore, we propose that COX-2-overexpressing glioma which is unresponsive to IL-10 in COX-2 suppression, underlies IL-10 overexpression in glioma-exposed DCs.

The IL-10-enhanced DCs that had been exposed to COX-2-overexpressing glioma induced an IL-10 and TGF-β secretory Th response by DC, the effect of the COX-2 inhibitor was negated by addition of soluble PGE2 to the culture supernatant of COX-2-overexpressing glioma. This finding demonstrates a direct role for...
high levels of PGE2 in modulating DC function away from the generation of a Th1 and toward a regulatory phenotype.

We characterized the suppressive nature of Th cells generated with COX-2-overexpressing glioma-exposed DCs by documenting their robust secretion of IL-10 and TGF-β, which is a key mediator of Tr activity (30), and by demonstrating their inhibitory effect, mediated by IL-10, on autologous lymphocyte proliferation. Based on another observation, that these cells did not secrete detectable levels of IL-4, a cytokine linked to the generation of Th3 and CD4+CD25− T cell responses (9), we concluded that the inhibitory effect is compatible with that of a Tr1. The relevance of IL-10 secretory mature DCs to the generation of a Tr1 response is also supported by evidence that IL-10 secretory pulmonary DCs, which highly expressed CD80 and CD86, elicited a Tr1 response against respiratory allergen in vivo (31).

We also demonstrated that CD4+ Th cells isolated from PBMC of a glioblastoma patient predominantly displayed a Tr1 response against autologous glioma cells. This is an intriguing finding and points to the existence of an underlying bias toward a regulatory phenotype in circulating T cells from patients with malignant glioma. This is consistent with reports that have described CD4+ T cell populations isolated from glioma patients, which demonstrate markedly impaired tumoricidal responses (3). Based on our findings, we propose that this is secondary to a skew in endogenous Ag presentation away from tumoricidal Th responses and toward a Tr1 response, mediated by the in situ effect of PGE2 by COX-2-overexpressing glioma on tumor-exposed APCs. Th1 activity could be restored in these T cell populations by exposing them to DCs that had phagocytosed COX-2-inhibited glioma.

The ability of APCs to elicit Tr1 responses secondary to tumor COX-2 overexpression is contingent upon effective uptake of TAA by APCs. This requires either the presence of resident APC populations within the tumor microenvironment or trafficking of exogenous APCs into sites of tumor growth. Microglia have been recognized as the native APCs in the CNS, and although their presence within malignant gliomas is well established, the Ag-presenting function of microglia has been shown to be compromised, possibly due to down-regulation of cell surface MHC expression (32). Nevertheless, T cell reactivity against TAA have been demonstrated in the cervical lymph nodes of brain-tumor-bearing mice (33, 34). In this context, it is likely that peripheral APCs that traffic into the CNS and migrate to the cervical lymph nodes are responsible for effective Ag presentation to T cells. Although Schneider et al. (35) have described the presence of numerous CD11c+ macrophages that strongly expressed HLA-DR and were morphologically distinct from microglia within multiple human glioma specimens, it is more plausible that peripherally derived, intratumorally infiltrating DCs are the principal cells responsible for TAA presentation. In this regard, Yang et al. (36) have demonstrated the presence of OX-62-expressing DC populations within experimental rodent glioblastomas, strongly suggesting a role for endogenous tumor-infiltrating DCs as APCs in glioma. The relevance of DCs to glioma-associated Ag presentation is further supported by evidence that intratumoral inocation of ex vivo-cultured immature DCs results in the translocation of TAA-presenting DCs to regional lymph nodes and the generation of a therapeutically relevant cytotoxic immune response (37, 38).

Thus, in this report we propose that in situ processing of COX-2-overexpressing glioma by tumor-infiltrating DCs induces a tolerogenic T cell response by means of generating a Tr1 response. It is important to note, however, that the purely in vitro nature of our current study limits our ability to determine how relevant this immunosuppressive mechanism may be in vivo. Despite this limitation, our finding of an underlying suppressor bias in circulating CD4+ T cells in a patient with a COX-2-overexpressing glioblastoma as well as the potent induction of an IL-10 secretory phenotype in DCs that have been exposed to high levels of PGE2-secreting glioma cells indicate that COX-2 overexpression in tumor cells may represent an important immunevasive mechanism designed to stimulate Tr1 responses. The therapeutic significance of IL-12 supplementation in a DC-based, antitumor vaccine has already been demonstrated (39). In this regard, we note that high...
levels of DC IL-12 secretion and a Th1 response can be induced after COX-2 inhibition in COX-2-overexpressing tumor before Ag presentation. These findings support the relevance of using COX-2 inhibitors in clinical immunotherapy protocols with DC-based vaccines for patients with malignant tumor as a means of promoting Th1-directed tumor Ag presentation.

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