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Article · October 2015

DOI: 10.18281/iti.2015.4.5

INTERNATIONAL TRENDS IN IMMUNITY VOL.3 NO.4 OCTOBER 2015
ISSN 2326-3121 (Print) ISSN 2326-313X (Online) http://www.researchpub.org/journal/iti/iti.html

COX-2 Dependent Modulation of Immune Activity in Lung Cancer

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***Abstract*— Cyclooxygenase-2 (COX-2) the enzyme at the rate-limiting step of prostanoid production is over expressed in human lung cancer. Both COX-2 and its product prostaglandin (PG) E2 underlie molecular and cellular immunosuppressive networks that contribute to the pathogenesis of non–small cell lung cancer through immune tolerance and escape from host immune surveillance. Human lung cancer cell-derived PGE-2 orchestrates an imbalance in IL-10 and IL-12 production by lymphocytes and macrophages. IL-10 and IL-12 are critical regulators of cell-mediated anti-tumor immunity. While IL-10 inhibits, IL-12 induces type 1 cytokine production and effective anti-tumor T cell-mediated responses. PGE-2 stimulates the generation of tolerogenic dendritic cells (DC) that promote T cell anergy. In this review we will discuss the role of COX-2 mediated immune suppression as it relates to: (1) the induction of cytokine imbalance in the tumor microenvironment (TME), (2) suppression of DC activities, (3) T regulatory (Treg) and myeloid derived suppressor cell (MDSC) induction and (4) the vaccination responses in lung cancer. These findings suggest that the COX-2 pathway sustains immune suppression in lung cancer. Since COX-2 inhibition has been associated with toxicity, exploitation of EP receptors and m-PGES that are downstream signaling targets of COX-2 perhaps would have a favorable toxicity profile. Further studies are needed to determine the role of EP2/EP4 receptor blockade in lung cancer progression. Discerning miRNAs that regulate PGE-2 production and signaling may provide additional strategies to down regulate COX-2/PGE-2 mediated immune suppression in lung cancer.**

***Keywords* — COX-2, PGE-2, IL-10, IL-12, Immune Suppression, Dendritic Cells, Vaccination, CTL, Lung Cancer**

I. Introduction

LUNG cancer causes more deaths than the next three most common cancers (colon, breast and prostate) combined. An estimated 160,000 Americans die from lung cancer annually. Approximately 220,000 new cases occur every year in the United States and worldwide more than 1.1 million people die from lung cancer annually [1]. Recent ground-breaking studies in lung cancer immunotherapy reveal robust anti-tumor activity and durable responses following PD-1/PD-L1 immune checkpoint blockade in previously treated patients with progressive locally advanced or metastatic NSCLC [2, 3]. Immune inhibitory molecules are upregulated on T cells in tumors with an overall effect of down regulation of anti-tumor activity. The programmed cell death protein 1 (PD-1; also known as CD279) is an inhibitory receptor that regulates immune responses. The PD-1 receptor interaction with the PD-L1 and PD-L2 ligands delivers inhibitory signals that regulate the balance between T cell activation and tolerance. Recent studies reveal responses in approximately 20% of NSCLC patients treated with inhibitors of the PD-1 checkpoint. This includes robust and durable responses in previously treated patients with progressive locally advanced or metastatic NSCLC [2-7]. These findings are encouraging as it clearly demonstrates that targeting the immune exhaustion pathway in lung cancer is effective in generating durable immune response and suggests that other immune suppressive pathways could also be exploited for immune related anti-tumor benefits.

An important immune suppressive pathway in the lung cancer TME is mediated by COX-2 and its product PGE-2. The initiation of prostanoid synthesis from arachidonic acid involves the enzyme referred to as COX, which has also been

Received October 17, 2015. Accepted October 25, 2015
This work was supported by the University of California Los Angeles/VAGLAHS Lung Cancer Program, Department of Veterans Affairs Medical Research Funds, National Institutes of Health (NIH) Grants (RO1CA95686, RO1 CA126944 and P50 CA90388), National Center for Advancing Translational Sciences, Grant UL1TR000124, and Tobacco Related DiseaseProgram Award Program of University of California (15RT-0207 and 20FT0082).

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DOI: 10.18281/iti.2015.4.5

termed PGH synthase or PG endoperoxide synthase [8]. Two isoenzymes have been identified: a constitutive form (COX-1) and an inducible isoenzyme (COX-2). COX-2 is up-regulated in response to a variety of stimuli, including growth factors and cytokines. Because COX-2 leads to enhanced PGE-2 production and subsequent cytokine imbalance *in vivo*, tumor expression of COX-2 has a key role in the generation of tumor-induced abrogation of T cell-mediated anti-tumor responses [9]. In this review we will discuss the role of COX-2 dependent modulation of the immune suppressive networks with a focus in lung cancer.

II. NSCLC COX2/PGE-2 INCREASES LEUKOCYTIC IL-10 BUT REDUCES IL-12 PRODUCTION

Our group was the first to demonstrate that NSCLC cells expressed functional COX-2 and define the pathway whereby tumor COX-2 expression and a high level of PGE-2 production modulated IL-10 and IL-12 cytokine balance in the lung cancer microenvironment [10, 11]. Human lung cancer cell-derived PGE-2 increased IL-10 but reduced IL-12 production by lymphocytes and macrophages [11]. Both IL-10 and IL-12 are important immune regulatory cytokines. While IL-10 inhibits important aspects of cell-mediated immunity, IL-12 induces type 1 cytokine production and effective anti-tumor cell-mediated responses. IL-10 over production in the tumor has been implicated in tumor-mediated immune suppression. In contrast, IL-12 is critical for effective anti-tumor immunity. The significance of increased T lymphocyte IL-10 production and subsequent responsiveness to tumor growth was assessed utilizing IL-10 transgenic mice. The IL-10 transgenic mice were unable to limit the growth of Lewis Lung (3LL) tumors. Administration of blocking IL-10 mAbs restored *in vivo* anti-tumor responses in the transgenic mice [12]. This demonstrates that a single alteration in the T cell cytokine profile can lead to dramatic changes in immune responses in a manner that is stimulus dependent. In separate but related studies transfer of T cells from IL-10 transgenic mice to control littermates transferred the IL-10 immunosuppressive effect and led to enhanced 3LL tumor growth [13]. In addition to changes in T cell-mediated immunity, professional APC from IL-10 transgenic mice were found to have significantly suppressed capacity to induce MHC alloreactivity, CTL responses, and IL-12 production. Tumor Ag pulsed DCs from IL-10 transgenic mice failed to generate anti-tumor reactivity against established 3LL tumors *in vivo*. These results demonstrate that increased levels of T cell-derived IL-10 severely impair anti-tumor immunity *in vivo*, due to defects in both T cell and APC function [13]. Consistent with the immune suppressive effects of IL-10 in lung cancer, IL-10 has been shown to downregulate CTL responses in cervical cancer through reduced HLA-1 expression that can be rescued by anti-IL-10 antibody [14]. In a recent study, IL-10 has been shown to cause immune suppression in B-cell non-Hodgkin lymphoma by inducing the development of immunosuppressive CD14(+)HLA-DR(low/-) monocytes that inhibited the activation and proliferation of T cells [15]. The findings from this study suggests that elevated IL-10 serum levels contribute to increased numbers of immunosuppressive CD14(+)HLA-DR(low/-) monocytes in B-cell NHL.

III. COX-2 INHIBITION RESTORES ANTI-TUMOR ACTIVITY BY ALTERING THE BALANCE OF IL-10 AND IL-12 PRODUCTION

Based on the findings that PGE-2 is pivotal in the reciprocal regulation of IL-10 and IL-12, we determined the role of COX-2/PGE-2 on the altered cytokine balance in the tumor and the modulation of anti-tumor immunity utilizing specific genetic or pharmacological inhibition of COX-2 in the murine Lewis lung carcinoma (3LL) model [9]. 3LL is a weakly immunogenic murine lung cancer that produces PGs. The two isoforms of COX were evaluated in 3LL. The constitutive enzyme, COX-1, has been previously found to be present in most cells and tissues, whereas the inducible isoenzyme, COX-2, is expressed in response to a variety of stimuli. COX-1 and COX-2 genes are both expressed in 3LL cells [9]. We determined the effect of IL-1β stimulation on 3LL COX-2. COX-2 mRNA was induced in 3LL with maximal induction occurring 2 h after incubation with IL-1β. In contrast, COX-1 mRNA expression remained unchanged. High-level PGE2 (2-fold) production by 3LL cells after stimulation with IL-1β *in vitro* was COX-2 dependent as evidenced by genetic or pharmacologic inhibition of COX-2 in 3LL cells. Thus, the capacity for COX-2 induction in 3LL was similar to that previously described in human lung cancer [10, 11] and suggested that these tumor cells would be relevant for assessment of COX-2 on tumor growth *in vivo*. *In vivo* inhibition of COX-2 in 3LL tumor-bearing mice with either indomethacin or SC-58236 led to reduction in tumor growth (12-fold) and prolonged survival (median survival 50 days compared median of 30 days in control, $p<0.05$). Because drugs that inhibit COX have effects in addition to COX inhibition, we assessed the involvement of COX-2 expression on tumor growth kinetics of 3LL cells genetically modified with COX-2 anti-sense or control constructs. The 3LL COX-2 sense or control vector transfected tumors had *in vivo* growth rates comparable to the parental 3LL cells whereas the COX-2 anti-sense-transfected 3LL revealed a significant reduction in tumor growth (24-fold) that was comparable to tumor burden in mice receiving COX-2 inhibitors. Treatment of tumor bearing-mice with anti-PGE-2 mAb replicated the growth reduction seen in mice treated with COX-2 inhibitors [9]. Histologic evaluation of regressing tumors following COX-2 inhibition revealed marked lymphocytic infiltration of the tumor and reduced tumor growth. The histologic appearance suggested that an immune-mediated pathway was operative in mediating tumor reduction. To assess this possibility, we evaluated IL-10 and IL-12 cytokines following COX-2 inhibition *in vivo* and found a significant decrement in IL-10 and a concomitant restoration of IL-12 production by APCs. Because the COX-2 metabolite PGE-2 is a potent inducer of IL-10, it was postulated that COX-2 inhibition led to anti-tumor

responses by down-regulating production of this potent immunosuppressive cytokine. In support of this concept, transfer of IL-10 transgenic T lymphocytes that over express IL-10 under control of the IL-2 promoter reversed the COX-2 inhibitor-induced inhibition of tumor growth. These results demonstrate that abrogation of COX-2 expression promotes anti-tumor reactivity by restoring the balance of IL-10 and IL-12 *in vivo* [9]. The findings identify tumor COX-2 expression as a critical element in the development of immunosuppression and document that *in vivo* abrogation of this enzyme causes significant tumor reduction. Tumor-derived PGs play an important role in augmenting production of inhibitory cytokines such as IL-10 while suppressing endogenous production of cytokines including IL-12 that are necessary for effective host cell-mediated anti-tumor immune responses.

Consistent with our findings in lung cancer, Santiago Z *et al* in a recent report demonstrated a COX-2 dependent tumor growth through the evasion of immunity [16]. The authors showed that tumor growth of mutant BrafV600E mouse melanoma cells in immunocompetent hosts required tumor production of prostaglandin E2 that suppressed immunity by a cytokine signature consisting of increased IL-6, CXCL1 and G-CSF but reduced IFN1 expression that sustained tumor promoting inflammation. Genetic ablation of cyclooxygenases (COX) or prostaglandin E synthases in BrafV600E mouse melanoma cells, NrasG12D melanoma or in breast or colorectal cancer cells sensitized the tumor cells to immune control by increasing IFN1 but reducing IL-6, CXCL1 and G-CSF expression. Furthermore, COX-2 inhibition synergized with anti-PD-1 blockade in the eradication of tumors in the murine melanoma model. In addition the authors found that patient melanoma biopsies exhibited the conserved COX-2 inflammatory signature similar to murine melanoma that has important therapeutic implications [16].

IV. TUMOR COX-2 DEPENDENT SUPPRESSION OF DENDRITIC CELL FUNCTION

Anti-tumor immune responses require the coordinate activities of lymphocyte effectors and professional APCs. DCs are professional APCs that are pivotal participants in the initiation of T-cell responses [17]. DCs acquire Ag in the periphery and subsequently transport it to lymphoid organs, where they prime specific immune responses [17]. DCs play a multitude of roles in the development of an antigen-specific immune response. Through the expression of both MHC class I and MHC class II molecules, DCs are able to interact with and activate naive CD8+ T cytotoxic and naive CD4+ T helper lymphocytes, respectively [18, 19]. For a naive T lymphocyte to become an effector cell necessitates several signals. The first signal is through the interaction of the T cell receptor (TCR) of the naive T lymphocyte with the peptide bound to the MHC molecule on the APC. The second signal is initiated by DC:T cell interactions through co-stimulatory molecules such as CD80 and CD86 on the DC with CD28 on the T cell. In the absence of co-stimulatory signaling, the T lymphocyte will become anergic. The third signal originates from DCs and lead to specific immune responses by T-cell differentiation through cytokine signaling. The differentiation of multiple T helper subsets and naive CD4+ T helper cells into activated effector T helper cells is orchestrated by DC-derived cytokines. The fourth signal derived from DCs instructs T cells to migrate to certain tissues by inducing the expression of specific chemokine receptors and integrins in T cells upon interaction with antigen-pulsed DCs [20].

The levels of expression and interplay between positive and negative co-stimulatory molecules in both DCs and T cells will decide whether T cells become activated or anergic. Antigen uptake in the absence of inflammatory signals renders phenotypically immature DCs, expressing low levels of MHC-II and co-stimulatory molecules. In addition, antigen presentation in the absence of effective positive co-stimulation can lead to T-cell anergy and tolerance [21, 22]. These DCs are considered “tolerogenic” in comparison to “immunogenic” DCs capable of inducing potent specific immune responses. DCs can switch from immunogenic to tolerogenic depending on the microenvironment cues. Further work is required to define the key cellular and molecular events that enable DC to mediate an immunogenic or toleregenic response to antigens.

Optimal function of APCs is a critical factor for effective anti-tumor control [23]. However, suppressed DC function has been reported in tumor-bearing models and patients [24-26]. The TME can adversely affect immune activation by reducing DC maturation and function [23]. Tumor-derived cytokines that are known to mediate DC dysfunction include IL-10, vascular endothelial growth factor, macrophage colony stimulating factor, and IL-6 [27-29].

We determined the role of tumor COX-2 expression on the maturation and activity of DCs and its effect on host anti-tumor immune responses. We have demonstrated that bone marrow-derived DCs pulsed with 3LL-specific peptide MUT1 and MUT2 antigens can reduce tumor volume and extend survival in mice after intra-tumoral therapy [13]. In contrast, DCs propagated in tumor supernatant (TSN) and pulsed with MUT1 and MUT2 3LL tumor specific peptide antigens did not reduce tumor volume but promoted tumor growth. Because tumor COX-2 expression down regulates anti-tumor immunity [9], we postulated that the immunosuppressive effect on DCs were mediated by COX-2-dependent soluble products in the TSN. To test this hypothesis, we evaluated genetic or pharmacological inhibition of murine 3LL lung cancer COX-2 expression *in vitro* for its subsequent effect on DC anti-tumor activity *in vivo*. The anti-tumor efficacy of DCs propagated in culture media (CM) was maintained after culture in SN from COX-2-inhibited tumor cells compared with DCs in 3LL TSN. Because PGE2 affects lymphocyte and APC function [9, 10], we determined the role of PGE-2 present in the TSNs in down-regulating DC mediated anti-tumor immune responses. Neutralizing antibody mediated blockade of PGE-2 *in vitro* blocked the immunosuppressive effect of the TSN and yielded fully functional DCs with potent anti-tumor activities *in vivo* [30]. The DCs expressed detectable levels of the PGE receptor subtypes 2, 3, and 4.

V. Tumor COX-2 expression reduces DC functional activity in vitro

Based on the *in vivo* results, we determined the role of tumor COX-2 expression and PGE-2 production on murine bone marrow derived DC phenotype and function *in vitro*. We found that DC phenotype, cytoplasmic and nuclear RelB protein levels, alloreactivity, antigen presentation capacity, and production of IL-12 and IL-10 were markedly altered by tumor COX-2 expression. Consistent with limited *in vivo* anti-tumor capacity, DCs cultured in TSN showed a decreased immune stimulatory molecule expression that correlated with marked limitation in DC functional activities [30].

Recognition of target cells by CD8+ CTL requires the presence of cell surface peptide epitopes in the context of MHC class I molecules. In the majority of cases, these epitopes are generated from endogenously expressed proteins by breakdown in the cytosol via a large multi-catalytic protease complex, the proteasome. Thereafter, the resultant peptides are moved from the cytosol into the endoplasmic reticulum by the TAP, a heterodimeric complex composed of TAP1 and TAP2 subunits. TAP therefore forms a major link between antigen generation and presentation. Indeed, TAP inactivation reduces both MHC class I loading and the surface expression of antigen-loaded complexes [31]. We assessed DC capacity to process and present the ovalbumin antigen in an *in vitro* assay using the B3Z T-cell hybridoma that recognizes the ova peptide SIINFEKL. Consistent with the reduction in the number of TSN-cultured DCs expressing TAP, these DCs had a reduced capacity to process and present the ovalbumin antigen. Tumor COX-2 inhibition resulted in maintenance of DC TAP and Ag presenting properties *in vitro* [30].

DC maturation has been associated with RelB activation. Failure to develop functional antigen presenting activity in the TME is accompanied by decreased RelB translocation. TSN-cultured DCs demonstrated a COX-2-dependent reduction of both cytoplasmic and nuclear RelB. The decrease in RelB also coincided with down-regulation of DC cell surface molecules and activity. Whereas PGE-2 caused a significant reduction in DC surface immune stimulatory molecules, anti-PGE-2-treated TSN partially blocked the alteration in DC phenotype suggesting that other tumor-derived factors such as vascular endothelial growth factor may also contribute to these effects. The alterations in DC phenotype in TSN cannot be attributed to granulocyte colony-stimulating factor, IL-6, or IL-10 because 3LL cells do not produce detectable levels of these cytokines [30].

Consistent with the reduction in anti-tumor capacity of TSN-cultured DCs, there was a marked decrement in IL-12 production that could be replicated by adding PGE-2 to the CM. Our results are in agreement with earlier studies showing that PGE-2-promoted maturation results in DCs that produce only low amounts of IL-12 and bias the development of naive Th cells toward the production of Th2 cytokines [32]. In apparent contrast to our findings are those indicating that PGE-2 enhances tumor necrosis factor α-induced DC maturation [33, 34]. However, these studies focus on specific culture conditions for human monocyte-derived DCs that, for example, include cytokines in addition to PGE-2 such as tumor necrosis factor α, IL-1β, and IL-6. PGE2 became a component in DC maturation protocols after it was demonstrated that the expression of CCR7, a DC maturation marker on *in vitro* propagated monocyte-derived DCs was enhanced by maturation in the presence of PGE2. Although PGE2 is believed to play an important role in DC migration and lymph node homing, it is also considered responsible for some immune inhibitory properties of DCs [35]. It has been shown that DC expression of CCR7 can be induced in the absence of PGE2 [36]. The advantage of maturation protocols without PGE2 is that the DCs produce higher levels of the type-1 polarizing factor, IL-12p70 [36, 37]. Furthermore, PGE2-matured DCs have been shown to induce Th2 cells [38, 39], and PGE2 regulates the production of the immune inhibitory cytokine, IL-10 [38]. In particular, the induction of indoleamine-2,3-deoxygenase (IDO) [40], the increased secretion of IL-10 and the diminished secretion of IL-12 suggest that PGE2 induces a tolerogenic immune response. The findings of reduced DC production of IL-12 in the presence of PGE-2 are consistent with earlier studies documenting a role for paracrine or autocrine PGE-2 release modulating IL-12 production and functional activities in murine bone marrow-derived DCs [41]. Collectively, these findings suggest that the tumor COX-2 metabolite PGE-2 is an important modulator of DC IL-12 secretion and can limit the initiation of Th1 responses required for anti-tumor immunity. In contrast to IL-12, IL-10 production by TSN-cultured DCs was significantly enhanced in a PGE-2-dependent manner. This is in agreement with earlier findings that have shown PGE-2 to be a potent inducer of macrophage and lymphocyte IL-10 secretion [10, 11]. DC production of IL-10 has been demonstrated to limit the maturation of monocyte-derived DCs and their capacity to initiate Th1 responses [42]. IL-10 inhibits IL-12 production in immature DCs, but, in addition, it prevents DC development when present at early maturational stages, inducing tolerogenic DCs [34]. IL-10-exposed DCs have a Th2-driving function that may contribute to their immunosuppressive activity *in vivo* [43]. Also of importance, PGE2 has been associated with enhanced Treg differentiation, function [44] and attraction via secretion of CCL22 [45], though some studies also suggest a role for PGE2 in the induction of Th17 cells [46]. These studies demonstrate that inflammatory cytokines are crucial in influencing DC function.

VI. Tumor COX-2/PGE2–Dependent Promotion of FOXP3 Expression and CD4+CD25+ T Regulatory Cell Activities in Lung Cancer

Tumor-reactive T cells accumulate in lung cancer tissues but fail to respond, The presence in cancer tissue of Ag-specific, activated tumor infiltrating CD8(+) T cells proves that tumors express Ags capable of eliciting immune response. The tumor escape from immune-mediated clearance is therefore not attributable to immunological ignorance. However, tumor-infiltrating lymphocytes are defective in effector phase function, demonstrating that tumor-induced immune suppression likely underlies tumor escape [47-49]. Our studies have documented a COX-2-dependent immunosuppressive

network in the NSCLC microenvironment by increasing the frequency and activities of Tregs. High proportions of NSCLC tumor-infiltrating lymphocytes (TIL) are Treg cells [50] that help tumors evade immune responses [51]. Treg cells actively down-regulate the activation and expansion of self-reactive lymphocytes [52]. Given that many tumor-associated antigens recognized by autologous T cells are antigenically normal self-constituents, Treg cells engaged in the maintenance of self-tolerance may impede the generation and activity of anti-tumor reactive T cells [53, 54]. Thus, reducing the number of Treg cells or abrogating their activity within the TME may induce effective tumor immunity in otherwise non responding hosts by activating tumor-specific and non-specific effector cells [55-58]. Definition of the pathways controlling Treg cell activities will enhance our understanding of limitation of the host anti-tumor immune responses by these cells.

Foxp3, a forkhead transcription factor family member encoded on the X chromosome [59] is a marker for Tregs [60] and expressed in CD4+CD25+ T cells in the thymus and the periphery [61]. Forced expression of the Foxp3 gene can convert murine naive T cells to Treg cells that phenotypically and functionally resemble naturally occurring CD4+CD25+ Treg cells [59, 61, 62]. Furthermore, inoculations of CD4+CD25+ T cells prepared from normal mice can prevent autoimmune disease in Foxp3- deficient mice [62]. Collectively, these findings demonstrate that Foxp3 is a critical control gene for the development and function of natural CD4+CD25+ Treg cells. Consistent with this concept, Fontenot *et al.* have shown that CD4+CD25+ T cells from Foxp3-deficient mice lack regulatory activity [62].

Although Foxp3 expression plays a key role in Treg cell lineage commitment, the signals that regulate Foxp3 are only now becoming clear. COX-2 expression is constitutively elevated making the TME a rich source of PGE2. Treg cell activity is increased in the NSCLC TME, hence we postulated that TSN would induce Treg cell Foxp3 gene expression and modulate the activities of Tregs. We determined the role of tumor COX-2 expression and PGE-2 in TSN on Treg cell Foxp3 expression. Tumor-derived COX-2/PGE-2 induced expression of the Treg cell-specific transcription factor, Foxp3, and increased Treg cell activity [44]. Assessment of E-prostanoid (EP) receptor requirements revealed that PGE-2-mediated induction of Treg cell Foxp3 gene expression was significantly reduced in the absence of the EP4 receptor and ablated in the absence of the EP2 receptor expression. *In vivo*, COX-2 inhibition reduced Treg cell frequency and activity, attenuated Foxp3 expression in TILs and decreased tumor burden. Transfer of Treg cells or administration of PGE-2 to mice receiving COX-2 inhibitors reversed these effects. These findings were the first demonstration of tumor COX-2/PGE-2–dependent modulation of the Treg cell activity in lung cancer. The tumor induced effect on Treg cell activity is reversible when tumor COX-2 expression is inhibited genetically or pharmacologically. Taken together these studies demonstrate that inhibition of COX-2/PGE-2 suppresses Treg cell activity and enhances anti-tumor responses that have important therapeutic implications against lung cancer [44]. Consistent with our findings, a recent study demonstrated that elevated expression of Foxp3 in tumor-infiltrating Treg cells suppresses T-cell proliferation and contributes to gastric cancer progression in a COX-2-dependent manner [63]. In addition, recent investigations on Treg cell function in a genetically engineered mouse model of lung adenocarcinoma found that Treg cells suppressed anti-tumor responses in tumor-associated tertiary lymphoid structures (TA-TLSs). These TA-TLSs have also been described in human lung cancers. The TLSs in this model were spatially associated with >90% of tumors and facilitated interactions between T cells and tumor-antigen-presenting DCs. Costimulatory ligand expression by DCs and T cell proliferation rates increased in TA-TLSs upon Treg cell depletion, leading to tumor destruction. The authors proposed that Treg cells in TA-TLSs can inhibit endogenous immune responses against tumors, and targeting these cells might provide therapeutic benefit for cancer patients [64].

VII. COX-2 MODULATION OF MDSC ACTIVITY

Tumor production of immunosuppressive mediators recruits and/or activates myeloid derived suppressor cells (MDSCs). MDSCs, a subset of heterogeneous bone marrow-derived hematopoietic cells, are found in the peripheral blood of cancer patients and positively correlate to malignancy. Solid tumors contain MDSCs that maintain an immune-suppressive network in the TME. Tumor COX-2 also modulates MDSC activity through ARG1 in lung carcinoma [65]. MDSCs producing high levels of ARG1 block T-cell function by depleting arginine. Until recently, the mechanism by which ARG 1 in MDSCs is induced in cancer was unknown. Rodriguez *et al*., utilizing the mouse 3LL carcinoma model, showed that ARG1 expression is independent of T-cell-produced cytokines and that tumor-derived PGE2 maintains ARG1 expression in MDSCs [65]. 3LL tumor cells constitutively express COX-1 and COX-2, and produce high levels of PGE2. Genetic or pharmacological inhibition of COX-2, but not COX-1, blocked ARG1 induction *in vitro* and *in vivo*. Signaling through the PGE2 receptor E-prostanoid (EP) 4 expressed in MDSCs induced ARG1. Furthermore, blocking ARG1 expression with COX-2 inhibitors elicited a lymphocyte-mediated anti-tumor response [65]. These results demonstrate a new pathway of prostaglandin-induced immune dysfunction and provide a novel mechanism to help explain the anti-tumor benefits of COX-2 inhibitors, which-target the major immune-suppressive pathways mediated by MDSCs. Consistent with these findings, a recent study showed that COX-2 promotes metastasis in nasopharyngeal carcinoma by mediating interactions between cancer cells and MDSCs [66]. In another study, inhibition of tumor-derived PGE-2 blocks the induction of MDSCs and recovers natural killer cell activity that suggests favorable outcome of combining COX-2-targeted therapy and adoptive NK-cell transfer in patients with cancer [67].

VIII. COX-2 Inhibition Augments Prophylactic Vaccination Responses in Lung Cancer

Tumor COX-2 reduces anti-tumor immunity by sustaining an immune suppressive network in the TME. Our group conducted studies to determine the role of COX-2 inhibition in augmenting the vaccination response to tumor challenge following either pharmacological or genetic inhibition of COX-2 in the murine 3LL lung cancer model [68]. Treatment of naive mice with the COX-2 inhibitor, SC-58236, skewed splenocytes toward a type 1 cytokine response, inducing IFN-γ, IL-12, and IFN-γ-inducible protein 10, whereas the type 2 cytokines IL-4, IL-5, and IL-10 remained unaltered. Fifty percent of mice receiving SC-58236 and an irradiated tumor cell vaccine completely rejected tumors upon challenge. Those mice that did form tumors following challenge demonstrated a reduced tumor growth. It is possible that if the COX-2 inhibitor was administered for the duration of the experiment or the number of irradiated tumor cells increased in the vaccine a greater percentage of mice would be protected from a tumor challenge. In contrast, all mice either vaccinated with irradiated tumor cells alone or receiving SC-58236 alone showed progressive tumor growth. Studies performed in CD4 and CD8 knockout mice revealed a requirement for the CD4 T lymphocyte subset for the complete rejection of tumors. To determine the role of host COX-2 expression on the vaccination responses, studies were performed in COX-2 gene knockout mice. Compared with control littermates, COX-2-/- mice showed a significant tumor growth reduction, whereas heterozygous COX-2+/- mice had an intermediate tumor growth reduction following vaccination. Depletion of IFN-γ *in vivo* abrogated the COX-2 inhibitor-mediated enhancement of the vaccination effect [68]. These studies demonstrate that COX-2 expression play an important role in modulating the generation and/or maintenance of the immune response to tumor Ags and suggest that the combination of COX-2 inhibition with vaccination strategies can enhance the generation of anti-tumor immunity. Consistent with our findings, COX-2 inhibition has been shown to promote enhancement of anti-tumor responses by transcutaneous vaccination with cytosine-phosphate-guanosine-oligo deoxy nucleotides and model tumor antigen in a melanoma model [69]. These findings provide a strong rationale for additional evaluation of the capacity of COX-2 inhibitors to enhance vaccination/immune activation strategies against cancer.

CONCLUSION

Results from preclinical studies demonstrate that tumor COX-2 expression promotes and sustains dysregulated inflammation in cancer that leads to hypo-responsiveness of the tumor to immune mediated destruction. Enhanced tumor COX-2 expression causes augmented PGE-2 production that impairs CTL and DC activities but increases the activities of immunosuppressive MDSC and Tregs in the cancer bearing host. COX-2 inhibition in murine tumor models have shown to reprogram the tumor niche and improve anti-tumor immune responsiveness with enhanced T cell and APC but reduced MDSC and Treg activities. Although the optimum biological dose of the COX-2 inhibitor celecoxib has been determined in lung cancer clinical trials [70], COX-2 inhibition has shown little survival advantage when used in combination with radiation, chemotherapy or targeted agents in patients with lung cancer. Perhaps the full anti-tumor benefits of COX-2 inhibition or PGE-2 blockade awaits a combination with immune approaches that are limited by COX-2/PGE-2 dependent immune suppressive mechanisms in the TME.

PGE-2 contributes to immune suppression thus understanding the coordination of genes (mPGES-1, mPGES-2 and cPGES) responsible for its synthesis and function will provide valuable information for the control of PGE-2 effects in the TME. Since aberrant miRNA expression occurs in various human cancers [71] dysregulation of specific miRNA networks may account for increased PGE-2 production and immune suppression. Discerning miRNAs that modulate PGE-2 production and signaling may provide additional strategies to down-regulate PGE-2 mediated immune suppression in lung cancer. MiRNAs are attractive as therapeutic targets because they regulate a large number of genes, thus, substituting or restoring expression of specific miRNA families identified to control expression of genes involved in the PGE-2 pathway may prove more effective than targeting individual genes to restore normal PGE-2 levels in the TME. PGE-2 mediates cellular responses by acting on a family of four G protein-coupled receptors (EP1–EP4) and determining their role in anti-tumor immune responses will provide further insights to circumvent PGE-2 mediated immune suppression in the TME.

ACKNOWLEDGEMENTS

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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