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REVIEW

Reciprocal crosstalk between dendritic cells and natural killer cells under the effects of PGE2 in immunity and immunopathology

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The reciprocal activating crosstalk between dendritic cells (DCs) and natural killer (NK) cells plays a pivotal role in regulating immune defense against viruses and tumors. The cytokine-producing capacity, Th-cell polarizing ability and chemokine expression, migration and stimulatory functions of DCs are regulated by activated NK cells. Conversely, the innate and effector functions of NK cells require close interactions with activated DCs. Cell membrane-associated molecules and soluble mediators, including cytokines and prostaglandins (PGs), contribute to the bidirectional crosstalk between DCs and NK cells. One of the most well-known and well-studied PGs is PGE2. Produced by many cell types, PGE2 has been shown to affect various aspects of the immune and inflammatory responses by acting on all components of the immune system. There is emerging evidence that PGE2 plays crucial roles in DC and NK cell biology. Several studies have shown that DCs are not only a source of PGE2, but also a target of its immunomodulatory action in normal immune response and during immune disorders. Although NK cells appear to be unable to produce PGE2, they are described as powerful PGE2-responding cells, as they express all PGE2 E-prostanoid (EP) receptors. Several NK cell functions (lysis, migration, proliferation, cytokine production) are influenced by PGE2. This review highlights the effects of PGE2 on DC–NK cell crosstalk and its subsequent impact on immune regulations in normal and immunopathological processes.
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INTRODUCTION

Dendritic cells (DCs) and natural killer (NK) cells have critical roles in immune regulation,[1] and both play key roles in the innate immune response against cancer and infections.[2,3] DCs are a heterogeneous group of cells that differ in their origin, anatomic location, phenotype and function. They have strong antigen-presenting capacity and potently stimulate naive, memory and effector T cells.[4] Several different phenotypic and functional subsets of DCs have been described.[5,6] Stimulatory DCs are involved in the initiation of immune responses, while tolerogenic DCs are required for the initiation and maintenance of immunological tolerance.[7] Immature DCs and some subsets of resting plasmacytoid DCs can behave as tolerogenic cells, characterized by their low expression of costimulatory molecules and their ability to inhibit the effector T-cell responses.[8] Immunosuppressive cytokines, including IL-10 and TGF-β1, and phagocytosis of apoptotic cells are both involved in the generation of tolerogenic DCs, which subsequently can induce an anergic state in $CD4^+$ memory T cells.[9,10]

In addition to their professional antigen presenting function, DCs are able to converse with NK cells.[11] Although initially described as lymphocytes involved in innate immunity, NK cells are now known to be involved in the regulation of adaptive immune responses.[12] NK cells play critical roles in host defense against tumors and pathogens through their cytotoxic activity and the production of cytokines, particularly IFN-γ, which is the most important proinflammatory cytokine involved in controlling many pathogenic organisms.[13] Like DCs, NK cells are subdivided into different functional subsets in humans and mice. In humans, $CD56^{bright}CD16^+$ cells and $CD56^{dim}CD16^-$ cells are two subpopulations of NK cells that differ in their cytotoxic activity, cytokine production and migratory capacity.[14] Moreover, two mature subsets of NK cells, $CD27^{high}$ NK cells and $CD27^{low}$ NK cells, with distinct NK receptor expression profiles and functions have been

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identified in humans and mice.[15] Similar to DCs, NK cells can acquire tolerogenic activity through the release of TGF-β1, which suppresses their own production of IFN-γ and increases their cytotoxicity against activated $CD4^+$ T cells.[16]

The bidirectional crosstalk between DCs and NK cells has led to increasing interest in both the regulatory and potentiating mechanisms of the innate immune responses and the subsequent adaptive immune responses both in normal and pathological settings.[17,18] Recently, much interest has been focused on functional DC–NK cell crosstalk and its role in immune regulation.[11,19] The reciprocal crosstalk between DCs and NK cells can occur in the periphery or in secondary lymphoid tissues. In both locations, DCs and NK cells interact with each other through cell–cell contact and membrane-bound ligands or by soluble agents synthesized by immune and non-immune cells. DC-derived cytokines have critical role in regulating NK cell phenotypes and function, and IL-12 produced by activated DCs induces NK cell release of IFN-γ.[20] NK cell proliferation and expression of the activation marker CD69 can be induced by human monocyte-derived DC.[21–24] Moreover, mature DCs can activate NK cell cytotoxicity and IFN-γ production.[2,21] Conversely, NK cells can regulate DC function. NK cells may induce death rather than the activation of DCs, particularly in immature stage, based on the DC subset and the stage. Activated NK cells are able to kill autologous immature DCs through the CD94/NKG2A inhibitory NK receptor.[25]

NK cells can also enhance DC maturation and immunostimulatory capacity.[26] Several studies have reported that NK cells efficiently promote human monocyte-derived DC differentiation and maturation and markedly augment their capacity to produce proinflammatory cytokines and stimulate T-cell responses.[21,27,28] In contrast to $CD2^+$ NK cells, activated $CD2^-$ NK cell subset produce IFN-γ, inducing DC maturation[29] and stimulating T-cell responses[30] in an animal model of *Mycobacterium bovis* infection. Direct contact with DCs and NK cell-released cytokines, including TNF-alpha and IFN-γ, are both involved in these effects.[22,31,32] Thus, DCs and NK cells appear to guide each other's functions both in the periphery and secondary lymphoid organs through cell–cell contact and the release of soluble factors, including cytokines. Other soluble factors, especially prostaglandin E2 (PGE2), have emerged as a potential regulator of DC–NK crosstalk during immunity and immunopathology. PGE2, the most well-known and well-studied PG, can profoundly modulate the various aspects of the immune and inflammatory responses.[33–35] PGE2 is produced by many immune and non-immune cells and acts on all the components of the innate and adaptive immune responses.[36]

PGE2 PRODUCTION BY DCS BUT NOT BY NK CELLS

A fundamental aspect of DC function is their ability to produce various endogenous mediators, including cytokines and other inflammatory mediators, including PGs[37] and leukotrienes.[38] Among the PGs, PGE2 is one of the main inflammatory lipid mediators produced in large amounts by many cell types, including macrophages, DCs, fibroblasts, endothelial cells and some types of malignant cells.

PGE2 is a lipid mediator synthesized by COX from an arachidonic acid precursor. The COX enzyme has two isoforms, COX-1 and COX-2, with different physiological functions and different susceptibilities to inhibition by non-steroidal anti-inflammatory drugs (NSAIDs).[39] COX-1 is constitutively expressed in most cells and is involved in regulating normal physiological functions, such as immune responses, blood pressure, gastrointestinal integrity and fertility, whereas COX-2 expression is undetectable in the resting state but can be markedly upregulated following stimulation of immune and stromal cells. The rate-limiting enzyme in PGE2 synthesis is COX-2. In DCs, COX-2 can be induced by bacterial lipopolysaccharide,[40] mimicking bacterial infection, or CD40 triggering,[41] which may occur during physiological interactions between APC and T cells during antigen presentation. Pro-inflammatory cytokines, especially TNF-alpha, can also induce COX-2-derived PGE2.[42]

Substantial research has focused on the ability of different subsets of DCs and other immune cells to synthesize PGE2 in response to inflammatory stimuli. We and other groups have reported that mouse bone marrow-derived DCs express both isoforms of COX enzymes (COX-1 and COX-2) and produce large amounts of PGE2 but not PGD2.[40,43,44] Similar data were obtained with immature and mature human monocyte-derived DCs.[45,46] Immune cells that produce large amounts of PGE2 are considered to be the most powerful modulators of inflammatory processes and immune function.[33] Although COX expression and PGE2 production by activated and non-activated human and murine DCs have been amply demonstrated, no studies have examined the ability of NK cells to synthesize arachidonic acid-derived PGs, particularly PGE2. The expression of COX-2-derived PGE2 has been demonstrated in $FOXP3^+CD4^+CD25^+$ adaptive regulatory T cells.[47] Other immune cells, such as B lymphocytes, are unable to produce PGE2. However, they are an important target of PGE2 immunomodulatory effects.[48,49]

AUTOCRINE AND PARACRINE EFFECTS OF PGE2 ON DCS AND NK CELLS

PGE2 is predominantly produced by APCs and has marked autocrine and paracrine effects on their phenotype and function.[50,51] The biological effects of PGE2 on immune and inflammatory cells are exerted by four G protein-coupled receptors on the plasma membrane, also known as E prostanoid (EP) receptors (EP1–4).[52] The presence of PGE2 EP receptors on many immune and stromal cell types reflects the ubiquitous nature of PGE2 function.[52,53]

Effects of PGE2 on DC maturation, activation and migration

PGE2 has long been considered a major product and modulator of activated macrophages,[36] but has become a key regulator of DC biology.[34,54,55] Cytokine-producing capacity, Th-cell polarizing ability, and chemokine expression, migration and APC functions of DC have been reported to be

regulated by PGE2 in both autocrine and paracrine manners. Depending on the nature of maturation signals and tissue localization, PGE2 has different and sometimes opposite effects on DC biology.[35] In peripheral tissues, PGE2 seems to act as a potent activator of DCs. PGE2 stimulates the surface expression of C–C chemokine receptor type 7, a chemokine that promotes DC migration to secondary lymphoid organs.[56] When DCs migrate to secondary lymphoid organs, PGE2 has an inhibitory role and impedes the maturation of DCs, their expression of MHC class II molecules and their ability to activate T cells.[37,40] PGE2 exhibits both pro- and anti-inflammatory effects on DCs.[57] Depending on the maturation stage, PGE2 exhibits differential effects on immature and mature DCs. With immature DCs, PGE2 appears to cooperate with the pro-inflammatory cytokines IL-6, TNF-α and IL-1beta to promote the development of pro-inflammatory subsets of DCs.[58,59] Rieser et *al.*[60] reported that, when given in combination with other pro-inflammatory factors, PGE2 can stimulate DC pro-inflammatory phenotypes and promote IL-12 production. Conversely, through stimulating the production of IL-10 by mature DCs,[37] PGE2 can act as an anti-inflammatory factor. We have previously demonstrated that PGE2 can exert inhibitory activity, reducing the maturation of DCs and their ability to present antigen.[40] Jing et *al.*[61] reported that PGE2 acts as an anti-inflammatory molecule by inhibiting DC CCL3 and CCL4 inflammatory chemokine release, preventing excess accumulation of activated immune cells. These inhibitory effects of PGE2 on DC biology corroborate the well-known suppressive effects of PGs on the immune response.[33,36]

The existence of four EP receptors and their various isoforms coupled to their distinct intracellular signaling could explain the complex and sometimes opposite effects of PGE2, especially on DC function. We are the first group to describe the coexpression of all EP receptors in murine DCs and to show that PGE2 suppresses DC functions mainly through an EP2 and EP4 receptor-dependent mechanism.[50] Other investigators have clearly demonstrated that the EP2 and EP4 receptors mediated most of the effects of PGE2 on DC phenotype, maturation, migration and function.[44] Collectively, these data suggested that (i) targeting PGE2 EP2/EP4 receptor signaling may be a powerful mechanism for modulating DC activity and that (ii) the seemingly contradictory actions of PGE2 should be considered in developing rational protocols based on DCs aimed at treating immunological disorders, ranging from autoimmune disorders to cancers.

Effects of PGE2 on DC-derived cytokines and T-cell polarization

Cytokines are produced by different cell types and act in a coordinated manner on hematopoiesis, immune responses, and inflammation. Another established function of PGE2 is the regulation of cytokine production by immune cells, especially DCs.[43] We previously reported that PGE2-primed DCs produced high levels of IL-10, which is a prototypically anti-inflammatory cytokine that down-regulates IL-12 production and APC activity in DCs.[37] As PGE2 is an environmentally bioactive molecule, its activity may be prolonged and sustained by other endogenously produced factors, mainly IL-10. Similar to PGE2, IL-10 is thought to play a major role in decreasing antigen presentation and inhibiting Th1-mediated immune responses. By triggering IL-10 synthesis, which inhibits various aspects of cell-mediated immunity,[62] PGE2 induces the development of a tolerogenic subset of DCs.[63,64] In addition, cytokine secretion profiles and the differentiation of T helper lymphocytes are modulated by PGE2. In fact, PGE2-primed DCs induce the differentiation of naive T cells into Th2 cells, which produce high levels of IL-4 and no IFN-γ.[65] These results support the crucial role of PGE2 in biasing the immune response toward a Th2 cytokine profile. This finding has been confirmed in BALB/c mice, which are characterized by a Th2-dominant immune response *in vivo*, which was shown to be dependent on PGE2.[66] The modulatory effects of PGE2 on DC-derived cytokines and T helper lymphocyte differentiation have been shown to be primarily mediated by EP2 and/or EP4 receptor-dependent mechanisms.[67,68]

Effects of PGE2 on NK cell activity

NK cells are a population of innate leukocytes that play an important role in the host immune response against tumors, virus-infected cells and bacterial infections[20] and that are able to recognize and kill tumor cells. Several lines of evidence have support a marked effect of PGE2 on NK cell biology. This lipid mediator can act directly on NK cells to inhibit their cytotoxic activity and their ability to produce cytokines, particularly IFN-γ.[69–71] PGE2 has been reported to inhibit NK cell IFN-γ production and cytotoxicity through downregulating the activating receptors NKG2D and 2B4.[18] In addition, Joshi *et al.*[72] reported that the suppressive effects of PGE2 on NK cell function may be mediated by inhibiting IL-15-induced IFN-γ production.

Considerable research has reported that NK cells express all PGE2 EP receptors and that PGE2 acts on NK cells through the EP2 and/or EP4 receptor subtypes,[73–75] which are known to be powerful activators of the adenylate cyclase system.[70] Since the adenylate cyclase system is involved in inhibiting killing by NK cells and inducing the CD94/NKG2A inhibitory NK receptor following PGE2 signaling,[76] it is not surprising that PGE2 has an inhibitory effect on NK cell function. Thus, the EP2/EP4 receptors have emerged as pivotal regulators of NK cell activity, and targeting these receptors may prevent NK inhibition by PGE2.

Effects of PGE2 on DC–NK cell crosstalk

In the innate immune response, NK cell activity involves close interactions with activated APCs, especially DCs,[21] which are major producers of PGE2.[40] Given the synthesis of large amounts of PGE2 by such activated professional APCs and because NK cells expressed all known EP receptors,[73] it is not surprising that DCs can modulate NK cell activity through the paracrine effects of the endogenously released PGE2 during DC–NK cell crosstalk. In fact, DC-mediated NK-cell effector functions are influenced by PGE2. Recently, PGE2 has been

reported to inhibit DC-NK cell crosstalk by modulating DC secretion of the chemokines and cytokines that are involved in NK cell recruitment.[34,77] Moreover, PGE2-matured DCs fail to attract NK cells and show reduced capacity to stimulate NK cell IFN-γ production, and NK cell-dependent Th1 polarization and cytolysis activity can be inhibited following the DC-mediated effects of PGE2 on NK-cell responses. Conversely, the suppressive effects of PGE2 on NK cell function also have a crucial role in the DC-mediated immune response. Mailliard *et al.*[78] reported that PGE2 inhibition of NK cell IFN-γ production abolished NK cell 'helper' function in the DC-mediated induction of Th1 and CTL responses. Taken together, these data indicate that PGE2 may be a potent inhibitor of DC–NK cell crosstalk and thereby the innate and adaptive immune responses.[18] The efficient crosstalk between DCs and NK cells is analogous to that between activated DC and T lymphocytes. Thus, the inhibitory activity of PGE2 on DCs affects not only NK cells but also T-cell biology.

DCS AND NK CELLS UNDER THE EFFECTS OF PGE2 IN IMMUNOPATHOLOGIES

PGE2 has been described as a potent lipid mediator with diverse actions and is known to regulate many functions in all human biological systems.[79] Many clinical and pharmacological studies have reported that several immunological disorders, such as tumors, asthma and infectious diseases, are associated with high expression of COX-2 and PGE2 production.[80,81] Otherwise, the association of chronic inflammatory diseases with elevated levels of PGE2 may compromise the immunoregulatory function of DCs and NK cells and their subsequent functions in immune disorders.

Tumor pathology

In tumor pathology, complex interactions between stroma cells, tumor infiltrating cells and the tumor cells themselves result in elevated COX-2 expression and PGE2 production.[82] The overexpression of COX-2 and its major metabolite PGE2 has been reported to be linked to all carcinogenesis stages ranging from initiation to cancer progression.[83] Endogenously produced PGE2 suppresses multiple immune functions acting on most types of immune cells.[33,35] When overexpressed, COX-2-synthesized PGE2 acts as a tumor promotor, regulates tumor angiogenesis[84] and potently alters the phenotype and function of circulating and tumor infiltrating DCs, resulting in cancer-associated immunodeficiency.[85] Moreover, many tumors are known to be associated with impaired differentiation and antigen-presenting function of DCs with an immature phenotype.[86,87]

In addition to DCs, NK cell function may be modulated by tumor COX-2-derived PGE2. Pietra *et al.*[88] found that melanoma cells greatly hamper the anti-tumor activity of human NK cells by downregulating the surface expression of activating receptors, including NKp30, NKp44 and NKG2D. This tumor immunosuppressive effect has been shown to be primarily mediated by soluble factors, such as PGE2 and indoleamine 2,3-dioxygenase, which inhibit both T and NK cell functions. Moreover, NK cell cytotoxic activity can be increased by COX inhibitors, including indomethacin and celecoxib, by downregulating MHC class I expression in a syngeneic murine model of metastatic breast cancer.[89] Taken together, these data corroborate the well-known suppressive effects of PGE2 on DC- and NK cell-mediated anti-tumor immunity.[90,91]

Allergic diseases

The activation of NK cells is essential for the development of T cell-dependent adaptive immune response in allergic diseases. Several lines of evidence indicate that the development of asthma is related to the innate immune response, including NK cells and adaptive immune responses.[92] In the lung, NK cells are considered to be a potent regulator of Th1–Th2 cytokine production, and an increased number of more activated NK cells have been observed in patients with asthma,[93] suggesting that NK cells play an important role in the pathogenesis of asthma.[94,95] The contribution of NK cells in a mouse model of OVA-induced asthma has been clearly demonstrated.[96] NK cells may increase antigen-specific $CD8^+$ T-cell activity, and depletion of NK cells before exposure to an antigen alters the induction of T cell-dependent antigen-specific immune responses. NK cells have the capacity to lyse immature DCs, thereby limiting the number of DCs exposed to inflammatory stimuli and likely limiting the immune response.

In the respiratory system, many cell types can produce PGE2, including alveolar macrophages, airway epithelial cells and DCs. PGE2 produced by airway epithelial cells induces the differentiation of DCs with an anti-inflammatory phenotype characterized by reduced secretion of TNF-alpha and increased secretion of IL-10.[97] Airway epithelial cell-derived PGE2 reduces the pro-inflammatory activity of DCs and limits their activity through an EP4 receptor-dependent mechanism. PGE2 has well-established protective and beneficial effects in asthma.[98,99] This protective effect may be the result of both direct actions exerted by PGE2 on airway smooth-muscle proliferation and other anti-inflammatory mechanisms. Among these mechanisms is the inhibition of the release of pro-inflammatory leukotrienes[38] that are known to be major mediators in the pathogenesis of asthma and many allergic diseases. Differential involvement of the PGE2 EP receptors and signaling pathways has been observed in asthma. For example, PGE2-induced bronchodilation results from the direct activation of EP2 receptors on airway smooth muscle, whereas the induction of the airway constriction by PGE2 is mediated by the EP1/EP3 receptors and appears to be dependent on neural pathway activation.[100] These data suggest that selective agonists that activate EP2 without any effects on EP1/EP3 receptors appear to be clinically useful in the treatment of asthma.

Infectious diseases

NK cells are known to play a pivotal role in innate defense against viral infections.[101] In infectious diseases, *in vivo* studies identified a crucial role of the functional interaction between DCs and NK cells in controlling NK cell-dependent pathological processes. Using a murine model of cytomegalovirus

infection (the evolution of which is dependent on the NK cell activity), Andrews et *al.*[102] observed that the expansion $Ly49H^+$ NK cells is essential for the homeostasis of splenic DCs. Conversely, in infected mice, $CD8\alpha^+$ DCs are required for the expansion of $Ly49H^+$ NK cells in the late phase of infection.

EFFECTIVE APPROACHES FOR TARGETING THE EFFECTS OF PGE2 ON DC–NK CELL CROSSTALK: COX-2 AND EP RECEPTOR SIGNALING

COX-2/PGE2/EP receptor signaling is crucial for immune-mediated tumor suppression.[103,104] NSAIDs have a strong impact on several components of anti-tumor immunity because of their inhibitory effects on tumor-derived COX-2/PGE2 signaling. NSAIDs are the most popular medications for treating pain, fever and inflammation and have potent immunomodulatory effects on different immune cells, including tumor-associated macrophages, DCs, NK cells, T effector cells and T regulatory cells. NSAIDs act primarily through inhibiting COX activity, which in turn leads to decreased PGE2 production.[105] Although the anti-inflammatory activities of NSAIDs, which are the basis for their extensive clinical value, are well known, their long-term use is associated with gastrointestinal complications such as ulceration.[106] To decrease the risk of gastrointestinal toxicity, COX-2-selective inhibitors (Coxibs) have been developed as new anti-inflammatory agents. COX-2 has been targeted in many clinical studies as a potent candidate for anticancer drug development,[107] and the inhibition of PGE2 production by NSAIDs or Coxibs may, at least in part, powerfully increase anti-tumor responses.[108,109] In addition, various epidemiological and laboratory studies have indicated that NSAID usage might reduce the risk of cancer.[110,111] Thus, targeting downstream prostanoid synthetic enzymes might provide a new approach for inhibiting tumor progression.

Although COX inhibitors (Coxibs and NSAIDs) appear to enhance the anti-tumor immune response and the therapeutic potential of cancer vaccines,[112,113] concerns regarding their potential toxicity during long-term usage might represent a major limitation bringing to their use into clinical practice.[114,115] Alternatively, the immunopharmacological use of potent and selective-EP receptor antagonists with decreased toxicity profiles in tumor settings may be an ideal approach for reversing tumor-mediated immune system suppression by preventing the direct effects of PGE2 on immune cells.[116] Selective antagonists for every EP receptor subtype have now been developed.[117,118] Some of them have been tested, and their anti-neoplastic activity and toxicity in experimental models of primary carcinogenesis have been evaluated. Encouraging results are summarized in table 1. Collectively, these data showed that antagonists of PGE2–EP receptor signaling, especially of EP2 and EP4, have promising anti-neoplastic activity with no toxicity in experimental models. However, their pertinent use in clinical settings requires further research with the aim of targeting PGE2–EP receptor signaling in immunocytes for enhancing anti-tumor immunity.

CONCLUDING REMARKS

The bidirectional interactions between DCs and NK cells result in reciprocal effects on both cell types and have a critical influence on the outcome of immune responses. Accumulating evidence is revealing that DC–NK cell crosstalk is markedly influenced by PGE2. However, it is important to know (i) the expression pattern of PGE2 and PGE2–EP receptors during DC–NK cell crosstalk; (ii) how PGE2 affects DC–NK cell crosstalk; and (iii) what subsets of both innate cell types should be targeted for the induction of efficient and specific immune responses. The relevant EP receptors and intracellular signaling pathways mediating the opposite and sometimes contradictory effects of PGE2 may differ depending upon the disease status of the host (asthma, cancer) and can also may vary over the course of the same immune disorder (asthma, for example). These data suggest that the seemingly contradictory actions of PGE2 should be considered in the development of rational protocols aimed at treating immunological disorders ranging from cancer to asthma. In addition, the relative contribution of each EP receptor in mediating PGE2 signaling depends on the cell subsets and their maturation-activation states. Since PGE2 has divergent effects (protective/suppressive) and innate immune cells do not all respond in similar manner, different and specific therapeutic approaches based on the interplay between PGE2 and DC–NK cell crosstalk will be required for each disease.

The general consensus is that the EP2 and EP4 receptors have emerged as pivotal regulators in mediating the effects of PGE2 in normal and pathological immune responses. Although COX-2 is responsible for the production of high levels of PGE2 during inflammatory conditions, targeting the EP2

Table 1 Anti-neoplastic activity of major EP receptor antagonists in experimental models

EP receptors	*Antagonists*	*Species*	*Diseases and results*	*Refs*
EP1	ONO-8711	Rat	Suppression of tongue carcinogenesis	119
	ONO-8711	Rat	Suppression of colon cancer development	120
EP2	PF-04418948	Human, mice, rat	Reduction of cutaneous blood flow	117
EP3	ONO-AE3-240	Human	Inhibition of growth in oral squamous cell carcinomas	121
EP4	AH23848	Mice	Inhibition of breast cancer metastasis	122, 123
	ONO-AE3-208	Mice	Inhibition of breast cancer metastasis	123
EP2/EP4	Frondoside A	Mice	Inhibition of breast cancer metastasis	124

Abbreviation: EP, E-prostanoid.

and/or EP4 receptors by using selective antagonists may offer more specificity than the current clinical usage of COX inhibitors and avoid inhibiting other COX metabolites, such as prostacyclin, which may be beneficial in the anti-tumor immune response. A clearer of understanding of PGE2/EP2/EP4 receptor signaling, using both agonists and antagonists, will be of great importance and should be considered in developing immunotherapeutic strategies to reinforce DC–NK cell crosstalk and the subsequent immune response. Further studies are required to answer the following outstanding questions regarding DC–NK cell crosstalk under the immunosuppressive effects of PGE2:

1. What are the primary mechanisms by which COX-2-derived PGE2 modulates NK-DC crosstalk and the subsequent immune response?
2. Is there any interplay between PGE2 and other immunosuppressive agents?
3. How can we reinforce NK cell- and DC-mediated immunity under the effects of PGE2?
4. What is the role of PGE2 in the connection between chronic inflammatory diseases and neoplastic transformation?

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