Special Topic: NF-κB, Immunity and Cancer

G Protein–Coupled Receptor Connectivity to NF-κB in Inflammation and Cancer

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Complex intracellular network interactions regulate gene expression and cellular behavior. Whether at the site of inflammation or within a tumor, individual cells are exposed to a plethora of signals. The transcription factor nuclear factor-kappaB (NF-κB) regulates genes that control key cellular activities involved in inflammatory diseases and cancer. NF-κB is regulated by several distinct signaling pathways that may be activated individually or simultaneously. Multiple ligands and heterologous cell-cell interactions have an impact on NF-κB activity. The G protein–coupled receptor (GPCR) superfamily makes up the largest class of transmembrane receptors in the human genome and has multiple molecularly distinct natural ligands. GPCRs regulate proliferation, differentiation, and chemotaxis and play a major role in inflammatory diseases and cancer. Both GPCRs and NF-κB have been, and continue to be, major targets for drug discovery. A clear understanding of network interactions between GPCR signaling pathways and those that control NF-κB may be valuable for the development of better drugs and drug combinations.

Keywords inflammation, cancer, GPCR, nuclear factor-kappa B

INTRODUCTION

The transcription factor nuclear factor-kappaB (NF-κB) regulates multiple cellular and physiologic functions, including proliferation and cell death, which are involved in regulating an immune response but also contribute to the development of disease [1, 2]. The signaling pathway that regulates NF-κB activation and nuclear translocation interacts with other intracellular pathways in the cytoplasm, in the nucleus, and at the site of DNA binding in gene promoter regions among several other transcription factors [1, 3–5]. Cellular responses to biological processes involves a dynamic network of interconnected

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signaling pathways [6]. A major convergence point of multiple pathways are promoter regions of individual genes, where complexes of nuclear proteins and transcription factors such as NF-κB bind DNA and regulate expression. The interactions at these levels are likely communal, resulting in a widespread dynamic network connectivity with multiple alternative pathways.

NF-κB has been implicated as playing a major role in inflammatory disease [7, 8] and in the development and progression of cancer [9–13]. The intracellular pathway that regulates NF-κB therefore has been a major target for drug development [14, 15]. NF-κB consists of five family members: p65 (RelA), RelB, c-Rel, p50, and p52, which in the active form are either homodimers or heterodimers [1]. All dimers bind a consensus DNA sequence. The most common and well-characterized forms are the heterodimers p50 with p65, and p52 with RelB. The p50/p65 heterodimer is the most abundant, ubiquitously expressed, and the main NF-κB family member responsible for regulating inflammatory responses and is termed the canonical pathway. The p52/RelB heterodimer regulates the noncanonical pathway involved in lymphoid development and noninflammatory responses [2].

Several extracellular and intracellular stimuli lead to NF-κB activation. There are several well-characterized extracellular receptors that are known to activate NF-κB, including members of the tumor necrosis factor receptor (TNFR) family, the interleukin-1 receptor (IL-1R)/Toll-like receptor (TLR) family, the T-cell receptor (TCR) and B-cell receptor (BCR), among others. The pathways that lead to NF-κB activation are well described [3, 16]. The majority of signaling converges on the IκB kinase (IKK) complex, which consists of α, β, and γ subunits. The IKK complex (IKKα, IKKβ, IKKγ) is activated by phosphorylation of IKKβ at Ser177 and Ser181 by kinases such as transforming growth factor-β (TGF-β)-activated kinase-1 (TAK1). Once activated, IKKβ phosphorylates inhibitor of κB (IκB) proteins IkBα, β and γ. The IkB proteins bind to NF-κB and block nuclear translocation and DNA binding. Phosphorylation of IkB proteins by IKKβ results in ubiquitination and proteasomal degradation, allowing NF-κB to translocate to the nucleus where it regulates transcription.

In terms of physiologic understanding, NF-κB activation cannot be viewed as an independent response. In a local microenvironment such as within an inflammatory autoimmune disease tissue or within a solid tumor, cells are surrounded by a mix of multiple paracrine and autocrine factors. G protein–coupled receptors (GPCRs), receptor tyrosine kinases, and cytokine receptors are just a few examples of cell surface molecules that have molecularly distinct signaling pathways.
Extracellular transmembrane receptors induce activation of diverse intracellular events that can directly interact with NF-κB signaling pathways within the cytoplasm or in the nucleus. In this review, we will explore GPCR involvement in regulating NF-κB activity.

**GPCR REGULATION OF NF-κB IN INFLAMMATION AND CANCER**

The GPCR superfamily makes up the largest class of transmembrane receptors in the human genome, consisting of approximately 800 full-length members [17] that have been divided into five main groups: glutamate, rhodopsin, adhesion, frizzled, and secretin [18, 19]. GPCRs have multiple molecularly distinct natural ligands including small molecules, lipids and peptides, and nucleotides among others [19] and are involved in regulation of several cellular activities including proliferation, differentiation, and chemotaxis. GPCRs are expressed in an array of tissues and cell types, and the restricted expression of some allows for selectivity of targeted drugs. The mechanisms of intracellular signaling by GPCRs make assay development for chemical library screening feasible, and therefore they are attractive drug targets. Perhaps more importantly, the structure of the receptor (all contain a seven transmembrane core region) makes them amenable to interaction with small-molecule agonists and antagonists. As a result, GPCRs have been a major focus of drug discovery for several diseases. GPCRs play a major role in development and progression of inflammatory diseases and cancer and have been, and continue to be, a major target for drug discovery for these diseases [20–24]. GPCRs play an important role in cancer development and progression affecting proliferation, metastasis, and angiogenesis [22]. Some of the ligands responsible include nucleotides, chemokines, leukotrienes, and prostaglandins. Interestingly, a number of these groups are directly involved in inflammatory diseases and in the link between inflammation and cancer [12, 25]. Given the number, diversity, and complexity of the GPCR superfamily and the major role NF-κB plays in inflammatory diseases and cancer, it is not surprising to find that their intracellular signaling pathways interact. Factors that activate NF-κB in tumors or inflamed tissues may frequently coexist with activating GPCR ligands such as chemokines, nucleotides, and prostaglandins to name a few. Signaling cascades activated by GPCR ligands can act “directly” on NF-κB activity by altering upstream signaling events [26–29] or “indirectly” through activation of other transcription factors that bind promoter regions next to NF-κB [30–32] (Fig. 1). GPCRs signal through three main
families of G proteins: Gi/Go, Gq, and Gs [33, 34]. Gq-coupled receptors activate phospholipase Cβ (PLCβ), which converts phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol-1,4,5-trisphosphate (InsP3) and diacylglycerol (DAG). DAG increases the activity of protein kinase C (PKC) [35, 36]. Upon ligand binding, GPCRs can also activate the G-protein α subunit Gs, and G-protein α subunit Gi. The Gs subunit causes elevated intracellular levels of cyclic 3′,5′ adenosine monophosphate, (cAMP), resulting in activation of signaling pathways through either protein kinase A (PKA) or exchange protein activated by cAMP (Epac) [37–39]. Both Gq-coupled GPCRs (activating PKC) and Gs-coupled GPCRs (activating PKA) interact with NF-κB in regulating inflammation and cancer.

PKC AND NF-κB IN INFLAMMATION AND CANCER

PKC is a family of nine serine/threonine kinases, seven of which are regulated by phosphotidylserine (PS) and DAG and can be divided into those that are calcium dependent (PKCα, PKCβ1, PKCβII, and PKCγ) and those that are calcium independent (PKCσ, PKCδ, PKCε, PKCη, and PKCθ) [35]. In addition there are two atypical PKCs, PKCλ and PKCζ. The isoforms have differing expression across tissues, substrate specificity, and have diverse biological functions such as regulation of apoptosis, proliferation, and migration.
Evidence suggests PKCs play a role in cancer development and progression [35, 36, 40] and a role in inflammation [41, 42]. Links between different PKC family members and NF-κB have been described for several tumor cells and cell lines [43–47] and in activated inflammatory cells [48–51]. PKCs can alter NF-κB activity in both inflammation and cancer directly by phosphorylation by interacting with upstream signaling molecules or indirectly by regulating other transcription factors that share promoter regions of individual genes. Post-translational modification of the NF-κB subunit p65 results in regulation of transcriptional activity [3]. A mechanism by which PKC regulates NF-κB is through phosphorylation of p65. The atypical PKC, PKCζ directly phosphorylates p65 at Ser 311 [52, 53]. Mice deficient in PKCζ show defective activation of NF-κB [52]. Phosphorylation of p65 at Ser311 by PKCζ inhibits binding to the NF-κB coactivator CREB binding protein (CBP), as well as its recruitment, and interaction with RNA polymerase II (Pol II) [53].

A number of direct links of signaling pathways that interact with NF-κB in the cytoplasm have been identified. CARMA1 and CARMA3 (CARD and MAGUK domain-containing protein 3) are scaffold proteins that have been shown to be important for signaling to NF-κB through IKK. CARMA1, which will be discussed in detail later, is expressed in hematopoietic/immune cells and plays a critical role in transmitting signals from antigen receptors (T-cell receptor [TCR] and B-cell receptor [BCR]) as well as FcεRI to NF-κB, and therefore is an important regulator of the adaptive as well as the innate immune response [54]. A related molecule, CARMA3, which is expressed widely in nonhematopoietic/immune cells, has recently been described as a link between GPCRs and NF-κB in both inflammation and cancer. Angiotensin II (AngII) is a proinflammatory peptide hormone that signals through the type I Ang II receptor (AT1R), a GPCR that is also expressed in multiple tissues. Like the pathway described for CARMA1 after antigen ligation and activation of the TCR or BCR in T cells and B cells respectively, AT1R activates NF-κB through CARMA3, Bcl10, and MALT1, which leads to activation of the IKK complex by ubiquitination of IKKγ[55]. Genetic deletion of CARMA3 in mice results in loss of GPCR-induced ubiquitination and activation of the IKK complex and activation of NF-κB [56]. Interestingly, activation of the innate immune response by TNF-α, or Toll-like receptor 4 (TLR4), are intact in CARMA3-deficient mice, indicating the response is specific to activation by GPCR ligation. Bcl10 and MALT1 have also been found to be important in GPCR signaling after binding the ligand lysophosphatidic acid (LPA) and endothelin-1 (ET-1) [29, 57]. LPA is a bioactive phospholipid that activates NF-κB.
upon binding to its receptor. The activation of NF-κB by LPA requires both Bcl10 and MALT1 adapter proteins. The activation of NF-κB by LPA has been further defined in ovarian cancer cell lines [58]. LPA-induced NF-κB activation in these cells requires Gi, Ras, PKCalpha, CARMA3, Bcl10, and MALT1, and it has been suggested that this signaling pathway is important in LPA-induced in vitro invasion assays for ovarian cancer cells.

Several other links between PKC and NF-κB have been made. The Raf kinase inhibitory protein-1 (RKIP-1) is a widely expressed kinase involved in several cellular functions including those involved in cancer development and progression [59]. RIPK-1 inhibits the MAP3K family member Raf and subsequently affects downstream transcription factors such as AP-1. RIPK-1 is also involved in a feedback mechanism for GPCR signaling. RIPK-1 binds G protein–coupled receptor kinase 2 (GRK2), inhibiting its ability to phosphorylate and inactivate GPCRs. PKC phosphorylates RIPK-1 enhancing its ability to bind and inhibit GRK2 resulting in increased GPCR signaling [60, 61]. In addition to regulating GPCRs and MAP3K, RIPK-1 inhibits NF-κB activation by antagonizing IKK activity [62]. RIPK1 associates with a number of kinases that regulate NF-κB signaling including NF-κB–inducing kinase (NIK), transforming growth factor beta-activated kinase 1 (TAK1), IKKa and IKKβ. These data highlight some of the complex network interactions that act directly with cytoplasmic signaling molecules responsible for regulating NF-κB activity. Another example of links between PKC and NF-κB involves PKCδ and the protein kinase Syk [63]. Protein kinase C-δ (PKC-δ) is involved in mediating thrombin-induced protease-activated receptor-1 (PAR1) activation. PAR1 is a GPCR for thrombin and is irreversibly activated by proteolysis. Activation of PAR1 by thrombin is thought to induce PKC-δ–mediated activation of the protein tyrosine kinase Syk and the subsequent increase in transcriptional activity of NF-κB via tyrosine phosphorylation of RelA/p65.

PKC-mediated regulation is not only activated through ligand binding of GPCRs. In lymphocytes, antigen binding to the T-cell receptor and the B-cell receptor results in activation of NF-κB [64, 65]. PKCθ and PKCβ respectively are important kinases in signaling from the T-cell receptor and B-cell receptor respectively to CARMA1, the IKK complex, and ultimately NF-κB [66–70]. Activation of PKCθ and PKCβ occurs after presentation of antigen to the receptor, initiating a chain of intracellular tyrosine phosphorylation, resulting in activation of phospholipase C-γ (PLC-γ) (Fig. 2) [54]. Similar to PKC and CARMA3 activation by GPCRs, InsP3 and DAG are generated by PLC-γ, and DAG increases the activity of PKCθ and PKCβ. PKCθ and PKCβ
FIGURE 2 Regulation of NF-κB through CARMA.

then activate NF-κB through signal transduction involving CARMA1 [67–69]. CARMA1, like CARMA3, interacts with Bcl10 and MALT1. In an interesting cross-over, the PKCθ and PKCβ pathway converges with the TLR4, interleukin-1 receptor, and tumor-necrosis factor receptor1 (TNFR1) through signaling molecules TNFR-associated factor 2 (TRAF2) and TRAF6. TRAF2 and TRAF6 then in turn are involved in activation of TAK1 and the IKK complex, resulting in translocation of NF-κB to the nucleus [54]. The intracellular link to the innate immune response pathway and the induction of increased intracellular calcium influx by InsP3 results in coactivation of transcription factors AP-1 and NFAT, respectively. PKCθ deficient mice develop a thymus and mature peripheral T cells suggesting it is not critical in T-cell development [64, 71]. Peripheral mature T cells, however, are defective in TCR-mediated signaling, involving decreased activity of NF-κB, AP-1, and NFAT. In contrast, NF-κB is required for fetal development and normal fetal and adult lymphocyte development [72]. The selectivity of PKCθ in TCR activation makes it an attractive target for the development of new
drugs to treat T cell mediated inflammatory diseases [73]. Research suggests that activation of PKC\(\theta\) by T-cell receptor engagement may play a role in T cell–mediated diseases [42]. PKC\(\theta\) therefore is being targeted for drug discovery for T cell–mediated inflammatory diseases such as arthritis [74] and inflammatory bowel disease [75, 76].

Interestingly, PKC\(\theta\) has been implicated in progression of breast cancer mediated through the c-Rel member of the NF-\(\kappa\)B family [77]. Transgenic mice overexpressing c-Rel in mammary tissues develop mammary tumors [78], and high levels of nuclear c-Rel are found in the majority of human breast cancers [79–81]. In normal mammary epithelium, estrogen receptor-\(\alpha\) (ER-\(\alpha\)) expression is regulated by the transcription factor forkhead box O protein 3a (FOXO3a). ER-\(\alpha\) is a steroid receptor that upon associating with ligand can either bind directly to specific DNA sequences in promoter regions (estrogen receptor binding elements [EREs]) or directly interact with other transcription factors such as NF-\(\kappa\)B. ER-\(\alpha\) suppresses c-Rel activity. In breast cancer, a model has been proposed in which PKC\(\theta\) activates the kinase Akt, which subsequently inhibits FOXO3a, resulting in decreased expression of ER-\(\alpha\) and increased activity of c-Rel [77]. Therefore, it has been proposed that PKC\(\theta\) is directly involved on human breast cancer progression through increasing c-Rel activity and further extends the potential therapeutic utility by inhibiting PKC\(\theta\).

Because of the large amount of evidence supporting the notion that PKCs may be involved in disease, significant efforts have been made to develop pharmacologically active PKC inhibitors. A number of PKC inhibitors have been and are in clinical trials, primarily for cancer and drug resistance [35]. It is not surprising to find PKC inhibitors have been shown to regulate NF-\(\kappa\)B–dependent transcription given the multiple intracellular connections, and inhibition is observed both in vitro and in vivo [53,82–86]. PKC inhibitors have also shown synergistic interactions with NF-\(\kappa\)B inhibitors in inducing apoptosis in tumor cells [87, 88]. PKC inhibitor PKC412 induced apoptosis in primary human multiple myeloma cells through Jun N-terminal kinases (JNKs), and, interestingly, upregulated NF-\(\kappa\)B activation [88]. NF-\(\kappa\)B inhibitors bortezomib and SN50 synergized with PKC 412 to enhance synergistically enhanced killing of multiple myeloma cells.

**cAMP/PKA AND NF-\(\kappa\)B IN INFLAMMATION AND CANCER**

In addition to GPCRs signaling through Gq and activating PLC\(\beta\) and PKC, an alternative signaling pathway can be induced by the G-protein \(\alpha\) subunit Gs, which associates with adenylyl cyclase (AC) and causes
elevated intracellular levels of cAMP. Similarly, an inhibitory signal can be initiated by the G-protein α subunit Gi, which inhibits AC resulting in a decrease in cAMP production. A number of cellular and physiologic activities and functions are regulated by the intracellular concentration of cAMP [38]. Cyclic AMP levels are increased by Gs binding and activating AC, which hydrolyzes ATP to produce pyrophosphate and cAMP [89]. Cyclic AMP levels are also regulated by AC binding Gi, which inhibits AC, resulting in a decrease in cAMP levels. Cyclic AMP levels are also controlled through degradation by phosphodiesterases (PDEs). There are 11 PDEs, each of which has several isoforms, which regulate hydrolysis and degradation of both cAMP and cyclic guanosine monophosphate (cGMP) [90]. PDEs can be grouped based on substrate specificity for cAMP and cGMP. Dual specific PDEs (PDE1, 2, 3, 10, and 11) target both cAMP and cGMP, whereas PDE4, 7, and 8 are cAMP specific and PDE5, 6 and 9 are cGMP specific. Selective PDE inhibitors that target hydrolysis of either cAMP or cGMP have been and continue to be developed for clinical use [91]. PDE4 is a major PDE found in immune and hematopoietic cells and is the target for inhibition to treat inflammatory diseases [92–95]. Inhibition of PDE4 with selective inhibitors in immune cells prevents degradation of cAMP and therefore accumulation and increased intracellular concentration. PDE5 inhibitors that selectively induce elevation of cGMP are used to treat erectile dysfunction and pulmonary hypertension [96].

It is clear that intracellular regulation of cAMP plays an important role in inflammation and in an immune response [37, 38]. Elevated cAMP activates two main signaling pathways through either exchange protein activated by cAMP (Epac) or protein kinase A (PKA) (Fig. 3). Epac binds cAMP, which then induces the activation of Rap, a GTPase member of the Ras family. Activated Rap has multiple biological functions, including induction of the MAP3K pathway [39]. Interestingly, Rap also activates other pathways including PLC, which converts PIP2 into InsP3 and DAG thereby increasing the activity of PKC [97]. As discussed earlier, PKC plays an important role in Gi-mediated signaling, and Rap therefore provides a link between the Gs to the Gi-coupled GPCR signaling pathway to NF-κB. Cyclic AMP also binds PKA, causing it to dissociate and release the catalytic subunit, which undergoes a conformational change and becomes active. The active catalytic subunit phosphorylates serines and threonines on target proteins [37] integrating the cAMP elevation with downstream signaling pathways, including NF-κB [98]. In an inflammatory response, cAMP and PKA can interact with NF-κB in different cell types including T cells and monocyte/macrophages. TLR4 activation with lipopolysaccharide (LPS)
activates NF-κB, resulting in expression of an array of proinflammatory mediators. One mechanism in which elevated cAMP inhibits NF-κB activation is through PKA, which phosphorylates the transcription factor cAMP-responsive element-binding protein (CREB). Phosphorylated CREB competes with NF-κB for a required cofactor CREB-binding protein (CBP) thereby limiting NF-κB activity [99]. In some cases, PKA induces cAMP-independent activation of NF-κB. LPS stimulation of macrophages results in phosphorylation and activation of PKA. The activated subunit of PKA then phosphorylates the NF-κB subunit p65, resulting in enhanced activity [100]. PKA also cooperates with NF-κB to inhibit LPS-induced cell death in macrophages. PKA activation by cAMP phosphorylates CREB, which cooperates with NF-κB to induce expression of the antiapoptotic protein plasminogen activator-2 (PAI-2), promoting macrophage survival [101].

As discussed earlier, binding of the T-cell receptor to antigen results in activation of NF-κB [64, 65] via PKCθ CARMA1, Bcl10, MALT1, and the IKK complex. Localized cAMP levels are also elevated within TCR
containing lipid rafts after recognition of antigen. Localized elevated cAMP controls TCR responses by activating PKA associated with a closely associated complex of molecules involved in T-cell activation in the extracellular membrane termed lipid rafts [102]. PKA negatively regulates TCR responses by phosphorylating and activating C-terminal Src kinase (Csk) within T-cell lipid rafts [103]. Csk negatively regulates TCR signaling [103–105]. Interestingly, loss of PKA activity has been described in the autoimmune disease systemic lupus erythematosus (SLE), and T cells have a heightened response to antigen [106]. In addition to PKA/Csk negative regulation of TCR responses, a positive feedback mechanism exists to inhibit cAMP and PKA activation. TCR activation leads to recruitment of PDE4 to the lipid raft, which degrades cAMP thereby blocking PKA activation and allowing an uninhibited TCR response [104]. These mechanisms provide a means for cAMP to regulate NF-κB activity in T cells upstream of PKCθ signal transduction involving CARMA. In terms of network connectivity, the cAMP link to regulation of TCR activation provides one means for GPCR ligands to play a role in regulating TCR-mediated NF-κB activation. Within a local microenvironment, T cells are presented with antigen and costimulatory signals from antigen-presenting cells (APC) such as macrophages and dendritic cells but are also exposed to multiple ligands that signal through Gs and Gi coupled GPCRs. Other immune cells that are responsible for inducing and maintaining inflammation, such as macrophages, dendritic cells, and neutrophils, are also regulated by local ligands that bind GPCRs and elevate levels of cAMP. Multiple ligands, such as adenosine, prostaglandins, chemokines, and neurotransmitters, are produced locally at the site of inflammation and regulate cAMP through binding to Gs and Gi coupled GPCRs. Like for inflammatory diseases, a large body of data implicates both cAMP/PKA and NF-κB as playing an important role in cancer [107–111]. Both cAMP and NF-κB are potential drug targets for cancer, and inhibitors are under investigation for treating solid tumors as well as lymphoid and hematologic malignancies [109–114]. It is likely that given the complex nature of a tumor microenvironment, tumor cells will encounter ligands that activate NF-κB and at the same time encounter GPCR ligands that regulate cAMP, increasing the possibility of network interactions between the two pathways. Therefore, it is not surprising to find evidence of network interactions between cAMP and NF-κB in malignancies. A number of naturally occurring GPCR ligands that are frequently cited as being responsible for altering cAMP levels in tumor cells from various forms of cancer include chemokines, neurotransmitters (via the beta-2 adrenergic receptor [β2AR]), prostaglandin
GPCR Connectivity to NF-κB (PGE), and adenosine. Interestingly, these ligands are also known to regulate inflammation.

**Adenosine**

The role of extracellular adenosine interaction with adenosine receptors in regulating immune response and inflammatory cells including dendritic cells [115], T cells [116, 117], and neutrophils [118, 119] among others has been a subject of a large body of research. There are four adenosine receptors, A1 and A3, which are inhibitory GPCRs that inhibit AC activation through Gi, and the A2A and A2b, which are stimulatory receptors that elevate AC activity and cAMP by signaling through Gs. Depending on the context and cell type, all four receptors can engage other pathways including PKC and MAPK, therefore it is not surprising to find that adenosine has the ability to interact with and regulate NF-κB [120–122]. Adenosine has been shown to play a role in several diseases, and therefore a number of adenosine receptor agonists and antagonists have been developed for potential therapeutic application [123, 124]. Activation of the A2A adenosine receptor using a synthetic agonist has been shown to increase levels of cAMP and decrease expression of IL-2, TNF-α, and IFN-γ in activated T cells [125–127]. Mice deficient in the A2A adenosine receptor are hypersensitive to endotoxic shock, and have elevated levels of proinflammatory cytokines [128]. In addition, A2A-deficient mice have increased IκBα phosphorylation and degradation and increased levels of NF-κB DNA binding activity. Interestingly, both cAMP and adenosine have been shown to be potent inhibitors of antigen-induced NF-κB [129]. *In vitro* in macrophages, activation of the A2A receptor induces an anti-inflammatory response by reducing proinflammatory IL-12 expression [130], and expression of A2AR on leukocytes has been proposed as a mechanism for protection from ischemia-reperfusion–induced injury *in vivo* with selective A2AR agonists [131, 132]. However, the response to adenosine receptor ligation and interaction with the NF-κB pathway is gene and cell type specific. In macrophages, activation of the A2AR with a selective agonist synergizes with TLR4 signaling to induce NF-κB–dependent expression of vascular endothelial growth factor (VEGF) [133]. This data high-lights the need to understand signaling networks linking cAMP to NF-κB in physiologic responses in the correct cellular context, in that the structure of the network is likely dependent on the cell lineage and activation state, and the signals present from the immediate and systemic environment. The adenosine receptor A2B is another example of cell-specific responses. One function of the A2B
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receptor in macrophages is to inhibit expression of IL-1β and TNF-α, but has been shown in other cell types to induce a proinflammatory effect, increasing expression of IL-6 and the chemokines IL-8 and MCP-1 [134]. Mice deficient in adenosine deaminase have increased adenosine in lung tissue and develop severe airway inflammation and destruction [135, 136]. The inflammation, fibrosis, and elevated cytokine production can be alleviated using an A2B receptor antagonist [137]. These data suggest that in bronchial cells, adenosine induces inflammation through the A2B receptor, and blocking binding with a selective inhibitor may be an approach for treating respiratory diseases [138]. In contrast with the proinflammatory role in the lung, it has recently been shown that the A2B receptor plays an important role in preventing hypoxia-induced inflammation by inhibiting NF-κB activity. It has been proposed that accumulation of extracellular adenosine activates the A2B receptor resulting in suppression of NF-κB activity through deneddylation of Cul-1 [122]. Cul-1 belongs to the Cullin family of proteins, which target numerous cellular proteins for proteasomal degradation, and is involved in ubiquitination of IκBα. Deneddylated Cul-1 (removal of the Nedd8 polypeptide) is incapable of ubiquitination of IκB and therefore results in the inactivation of NF-κB. These results provide a direct link between adenosine receptor A2B activation and NF-κB inhibition through regulation of ubiquitination and proteasomal degradation. It has yet to be shown if cAMP plays a role in A2B receptor inhibition of ubiquitination and proteasomal degradation of IκB-α. However, cAMP and PKA mediated regulation of proteasomal degradation of other proteins involved in intracellular signaling have been reported [139–142]. In addition to A2A and A2B Gs-coupled receptors, the inhibitory Gi-coupled receptor A3 has also been suggested to play a role in regulating NF-κB during inflammation. In LPS-stimulated macrophages and microglial cells in vitro, A3 receptor activation by the A3 agonist N(6)-(3-iodobenzyl)-adenosine-5′-N-methyluronamide (IB-MECA) inhibited activation of NF-κB and TNF-α production [143, 144]. Similarly, IB-MECA inhibited NF-κB activation, inflammation, and bone destruction in vivo in rat models of arthritis [121, 145].

Like for inflammatory diseases, adenosine and adenosine receptors have been shown to play a role in cancer and are under investigation to evaluate potential therapeutic applications for treating solid tumors as well as lymphoid and hematologic malignancies [146–148]. Although adenosine has been shown to inhibit TNF-α–induced NF-κB activation in cell lines [149], there is limited evidence to suggest network interactions occur between adenosine receptors and NF-κB
in cancer. The strongest body of data relates to the Gi-coupled adenosine receptor A3 [150]. A3 receptor expression is elevated in primary colon and breast tumors compared with that of normal tissue, and elevated expression correlated with increased levels of NF-κB [151]. The adenosine A3 receptor agonist IB-MECA decreases expression of NF-κB and inhibits the growth of a human prostate carcinoma cell line [152]. In vivo, IB-MECA has been shown to inhibit human and mouse colon carcinoma cell line growth and metastasis [153]. The A3 receptor is linked to G-protein α subunit Gi, which inhibits AC, resulting in decreased production of cAMP and decreased activation of PKA [148]. Tumor lesions derived from human colon carcinoma xenografts treated with IB-MECA have decreased expression of protein kinase A (PKA) and an increase in glycogen synthase kinase-3 beta (GSK-3 beta) and decreased expression and DNA-binding capacity of NF-κB [154]. A major concern in cancer therapy is the selection and expansion of drug-resistant tumors. Anticancer cytotoxic (chemotherapeutic) agents activate NF-κB, thereby inducing expression of antiapoptotic proteins [9]. Activation of the adenosine A3 receptor with agonist IB-MECA reduces activation of NF-κB, suggesting a potential mechanism for sensitizing tumors to chemotherapy. 5-Fluorouracil (5-FU) is a commonly used drug in therapeutic regimens for the treatment of several malignancies. Activation of the A3 adenosine receptor in human colon carcinoma cells enhances 5-FU cytotoxicity in vitro. IB-MECA sensitized human colon xenograft tumors to 5-FU–induced inhibition of growth and showed downregulation of NF-κB activity in vivo [155]. Overall, there is clear evidence to support a network relationship between adenosine receptors and NF-κB in inflammation and cancer.

Prostaglandins

Prostaglandins are lipids that have potent bioactivity through binding and activating specific GPCRs and play a major role in both inflammation and cancer. Prostaglandins are produced in response to external stimuli that upregulates the enzyme cyclooxygenase-2 (COX-2), which converts arachidonic acid to intermediate prostaglandins. Interestingly, COX-2 expression is regulated by a number of transcription factors including CREB, AP1, NFAT, and NF-κB [156, 157]. COX-2 plays a role in regulating a number of cellular processes through prostaglandins and in disease contributes to inflammation and pain. COX-2 inhibitors have been developed for treating inflammation, pain, and for several forms of cancer [158–163]. Once converted to intermediate prostaglandins by COX-2, subsequent enzymatic steps
result in the production of thromboxane (TX) A2, prostacyclin (PGI2), and an important regulatory molecule in inflammation and cancer, prostaglandin E2 (PGE2).

Like adenosine, PGE2 is a ligand for four GPCRs (EP1, EP2, EP3, and EP4) and induces different responses depending on the cell type and situation. The EP1 signaling response is not well characterized but has been shown to increase intracellular Ca^{2+}. Both EP2 and EP4 are coupled to Gs and increase intracellular cAMP, and EP3 is coupled to Gi and inhibits AC [164]. PGE2 has variable effects on regulation of expression depending on the gene, blurring the line between it being categorized as a proinflammatory or anti-inflammatory molecule. PGE2-induced elevation of cAMP inhibits expression of a number of inflammatory mediators such as cytokines IL-1, IL-2, IFN-γ, TNF-α, IL-12, chemokines CCL3, CCL4, CXCL1, and cell surface adhesion molecule ICAM-1 and costimulatory molecules B7-1 and B7-2 [165–171]. A model has been proposed as a feedback mechanism in arthritis to control inflammation, where IL-1 or TNF-α actives NF-κB via ERK in synovial fibroblasts, increasing production of PGE2, which in an autocrine fashion binds its receptor resulting in blocking ERK activation and inducing upregulation of IκBα which inhibits nuclear translocation of NF-κB [172]. PGE2 also induces upregulation of key inflammatory molecules CXCL1, IL-23, and IL-6, highlighting the complexity of signaling responses [165, 173, 174]. IL-6 is a good example of complex regulation by multiple pathways. The IL-6 promoter has binding sites for multiple transcription factors including AP-1, ets, C/EBPB, glucocorticoids, and NF-κB [175]. IL-6 expression can be elevated by PGE2 (and elevated cAMP) and TLR4. In myeloid cells (macrophages, monocytes, dendritic cells), cytokine production induced by stimulation of TLR4 induces NF-κB activation and expression of proinflammatory mediators including cytokines, chemokines, and proteases [176]. Eliminating the NF-κB binding site from the IL-6 promoter eliminates expression induced by LPS, whereas elimination of individual transcription factor binding sites has little effect on PGE2-induced expression. EP4 was identified as the receptor responsible for PGE2 inhibition of NF-κB and inhibition of chemokine expression induced by LPS. Recently, EP4 receptor-associated protein (EPRAP) has been identified as an intracellular molecule responsible for PGE2-mediated inhibition by blocking NF-κB precursor p105 from being phosphorylated, ubiquitinated, and processed to p50, which forms an active dimer with p65 [26].

In contrast with the clear role of PGE2 in regulating NF-κB in inflammatory cells, there is little evidence for an equivalent role in regulating cancer cells. PGE2 is implicated in tumorigenesis in several forms of
cancer, and COX-2 inhibitors are used clinically for cancer therapy [161, 177–179]. NF-κB regulates apoptosis and cell proliferation and a number of other cellular functions important in tumor development and progression, and inhibitors are being developed for cancer therapy [180, 181]. As mentioned above, PGE2 regulates cytokines, chemokines, and NF-κB during inflammatory responses, and a large amount of data has been generated recently supporting a role of inflammation and NF-κB in the development and progression of cancer [11, 12, 182]. Based on this, it is tempting to speculate that PGE2 would also regulate NF-κB activity in tumors, but to date a strong body of evidence is not available to definitively make that association.

**Beta Adrenergic Receptors**

For decades, a connection between the nervous and immune systems has been studied [183]. The key regulatory connection between systems involves the interaction of neurotransmitters with functional neurotransmitter receptors on immune cells [184]. The primary neurotransmitter that is produced by the sympathetic nervous system and is involved in regulating immune function is norepinephrine (NE), and the receptors are the adrenergic GPCR receptors. Macrophages and monocytes express alpha and beta adrenergic receptors (αAR, βAR), and lymphocytes express beta2 adrenergic receptors (β2AR) [185, 186]. For several years, it has been known that β-AR regulate NF-κB [187]. βAR agonists such as isoproterenol inhibit LPS-induced cytokine production, potentially through increasing protein levels of IκBα. Increased expression of IκBα by isoproterenol was shown to be dependent on NF-κB (the IκBα promoter has an NF-κB binding site) and was PKA dependent [188]. Recently, it has been suggested that IκBα inhibitory function can also be upregulated by decreased interaction with β-arrestin-2 after βAR activation [189]. An additional independent mechanism for regulating NF-κB has been proposed for regulating inflammatory gene expression in bronchial cells. βAR activation causes chromatin remodeling in the promoter region of the chemokine eotaxin, preventing NF-κB binding to DNA and decreasing expression [190]. Interestingly, eotaxin is a chemokine involved in airway inflammation, and it has been shown that clinical use of β2AR agonists in asthma enhances the effects of glucocorticoids in preventing exacerbations [191, 192]. A large body of research over decades has focused on the interaction of epinephrine and other β2AR agonists in control of lymphocytes [185, 186]. Norepinephrine regulates CD4+ T cells and B cells in vitro [185, 193]. Interestingly, it has recently been found that
human CD4⁺CD25⁺ regulatory T cells (Treg), which are suppressors of immune response, express significant amounts of catecholamines, including norepinephrine [194]. Given the role of GPCRs, cAMP, and PKA in regulating NF-κB in T-cell responses, it is not surprising to find evidence of NF-κB regulation by βAR. Similar to what has been observed in LPS-stimulated macrophages, βAR agonists increase IκBα protein levels and inhibit NF-κB in PMA/Ionomycin activated human CD3⁺ T-cells [195]. Human T cells polarized to differentiate into Th2 cells in vitro respond to both βAR agonists and PGE2 by activating PKA, suppressing p38 MAPK and NF-κB, resulting in suppression of IFN-γ, IL-2, and IL-13 production [31]. A large body of evidence collected over decades supports the role of βAR as an immune modulator, in part through regulating NF-κB.

Evidence also suggests βAR are involved in tumor development and growth [196, 197]. In contrast with the role in inflammation, however, βAR induce tumor growth, and antagonists are considered as therapeutic tools. Norepinephrine is active in promoting growth and survival of various types of cancer cells. Some examples of norepinephrine activities include protection of prostate cancer cells from apoptosis by inhibiting an antiapoptotic protein, upregulation of vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-2, and MMP-9 in nasopharyngeal cancer cells, stimulation of esophageal squamous-cell carcinoma cell-proliferation by transactivation of the COX-2 pathway, and increase of invasive properties of ovarian cancer cells through regulation of STAT3 [198–201]. Because of this wide array of protumorigenic activities, there may be potential for using βAR antagonists for cancer therapy.

Inhibitors of α1AR are effective in inducing apoptosis in prostate tumor cells, as well as inhibiting migration and invasion ability. However, evidence suggests induction of apoptosis may be independent of α1AR through upregulation of TGF-β and IκBα expression [202]. α1AR antagonists also inhibit proliferation and induce apoptosis in breast cancer cells. The suggested mechanism in this case is thought to be through reduction in expression of the phosphorylated EGFR and inhibition of several key transcription factors including NF-κB [203]. Interestingly, EGF stimulates esophageal cancer cells to proliferate, but also induces expression of epinephrine, elevated cAMP levels, and activated PKA [204]. These EGF-induced responses are blocked by α1AR antagonists, suggesting an autocrine mechanism. Recently, it has been shown that epinephrine inhibits prostate cancer cell apoptosis by PKA-dependent phosphorylation and inactivation of the proapoptotic protein BAD [198]. Beta AR are implicated in cancer development and progression.
and regulate cancer cell proliferation, MMP expression, and apoptosis. Similarly as mentioned previously, NF-κB regulates apoptosis and cell proliferation and a number of other cellular functions important in tumor development and progression, and inhibitors are being developed for cancer therapy [180, 181]. βARs regulate cytokines, chemokines, and NF-κB during inflammatory responses, and inflammation and NF-κB are important in the development and progression of cancer [11, 12, 182]. Based on this, it is tempting to speculate that βAR would also regulate NF-κB activity in tumors, but to date a strong body of evidence has yet to be generated to definitively make that association.

**CONCLUSION**

NF-κB is a key regulatory factor that has been implicated as playing a major role in the development and progression of inflammatory disease and cancer and therefore has been a major target for drug development. Multiple signaling pathways regulate NF-κB. The most well characterized are those that regulate innate and adaptive immune response (TNFR/IL-1R/TLR, TCR and BCR), among others. In addition to these activators of NF-κB, several other ligands and signals are present within an inflammatory site or tumor, a number of which signal through GPCRs. The GPCR family consists of approximately 800 members that regulate several cellular activities including proliferation, differentiation, and chemotaxis. Both Gq and Gs coupled GPCRs are involved in inflammation and cancer. Intracellular signaling from Gq and Gs coupled GPCRs are mediated through PKC and cAMP/PKA, respectively. A strong body of evidence indicates that NF-κB is regulated by PKC in lymphocytes and macrophages during an inflammatory response. In addition, it is clear that PKC regulates NF-κB through multiple intracellular mechanisms in cancer cells. Gs-coupled GPCRs and cAMP/PKA clearly play a role in inflammation and cancer. Whereas there are clear links between cAMP/PKA and NF-κB in inflammation, similar connections in cancer are not as obvious. PGE, adenosine, and βAR agonists are ligands that activate the cAMP/PKA pathway and regulate inflammation and NF-κB through multiple mechanisms. In cancer, the connection between PGE, adenosine, and βAR agonists and NF-κB is less clear. This is surprising because both cAMP/PKA and NF-κB regulate a number of the same cellular functions in tumor cells. Adenosine, PGE, and βAR regulate inflammation-induced NF-κB activation. Inflammation and NF-κB induce tumorigenesis and metastasis in some forms of cancer. It is therefore tempting to speculate that regulators of the PKC or the cAMP/PKA pathway are involved in inflammation-induced
tumorigenesis. Both GPCRs and NF-κB are targets for drug discovery. Continued research aimed at unraveling the network interactions between GPCR signaling and NF-kB may lead to better understanding of gene regulation and the development of better drugs and drug combinations for treatment of inflammatory diseases and cancer.

REFERENCES


GPCR Connectivity to NF-κB


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