Chapter 14
The Hypoxia-adenosinergic Immunosuppression and Redirection of Immune Response in Tumor Microenvironment

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Abstract In this chapter, we will focus on physiological regulators of activated immune cells in cancerous tissue microenvironments. This consideration started when we were contemplating the molecular mechanism that would be responsible for the so-called Hellstrom Paradox. Indeed, it was not explained why cancer patients often have tumor-recognizing effector T cells without having tumor rejection. The latest great advances in identification of various immunological negative regulators of immune response still left room for tumor defense by physiological inhibitors of antitumor T and natural killer (NK) cells. We started by assuming that cancerous tissues could be misguidedly protected by the same mechanism, which saves lives by protecting vital tissues from collateral damage by overactive immune cells during the antipathogen immune response. In our search for a mechanism that protects tissues from collateral damage, we first focused on intracellular cyclic adenosine monophosphate (cAMP) which was long known to be immunosuppressive. It was important to identify which of the many Gs protein-coupled receptors is actually physiologically responsible for inhibition of immune response in tumor microenvironment. Levels of extracellular adenosine are high in inflamed and cancerous tissues corresponding to local hypoxia. A2A and A2B subtypes of adenosine receptor, which are coupled to cAMP-elevating Gs protein, are predominantly expressed in immune cells. Indeed, extracellular adenosine endogenously generated by degradation of adenosine triphosphate (ATP) could suppress immune response and immunoregulation by adenosine was notable in tumor microenvironment. Blockade of the hypoxia-adenosinergic immunosuppression may be a promising approach to eradicate cancer, especially when it is combined with adoptive immunotherapy or cancer vaccine.

Keywords Tumor microenvironment · Hypoxia · Adenosine · A2A adenosine receptor · A2B adenosine receptor · T cell · Regulatory T cell · Myeloid-derived suppressor cells · Adoptive immunotherapy · Cancer vaccine

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D. I. Gabrilovich, A. A. Hurwitz (eds.), *Tumor-Induced Immune Suppression,*
DOI 10.1007/978-1-4899-8056-4_14,

1 Hypoxia

1.1 Immunosuppression in Hypoxic Tissue Microenvironment

Tissue oxygen levels are not uniformly distributed in the same organ. Levels of oxygenation depend on diffusion of oxygen from blood vessels, distal area being more hypoxic [1], [2]. Pathological conditions in inflamed tissue and tumor are related to the formation of even less oxygenated microenvironments [2], [3]. Tissue inflammation inflicting damage to blood vessels reduces oxygen supply [3]. Combined with increased oxygen demand by accumulated inflammatory cells, inflamed tissue becomes deeply hypoxic [4].

Tumors are also often hypoxic for reasons different from those causing hypoxia in inflamed tissues. Oxygen demand is high in tumors because of aggressive proliferation of tumor cells. In addition, disorganized blood vessel formation in tumors is making blood flow sluggish, and therefore oxygen supply is low [5], [6]. Interface between tumor cells is tightly packed, preventing oxygen diffusion to the inside of tumor tissue [6], [7]. Hypoxia in tumors correlates with poor prognosis because hypoxic tumors are refractory to radiotherapy and chemotherapy [5]–[7]. Moreover, hypoxia is conductive to the establishment of tumor microenvironment, which is potentially suppressive to antitumor immune activities [8]–[10]. Hypoxia has been shown to suppress immune functions of T cells, natural killer (NK) cells, and antigen-presenting cells (APCs).

1.2 Suppression of T-cell Immunity Under Hypoxia

In vitro T-cell activation under hypoxia impairs proliferation of activated T cells and their effector functions such as cytotoxicity and cytokine production [1], [11]–[13]. Hypoxia blocks Ca^{2+} increase after stimulation of T-cell receptor (TCR) [14]. Whole body exposure of mice to hypoxic atmosphere inhibited T-cell activation in vivo [15]. In that study, the extent of T-cell activation correlated with the levels of oxygenation in the spleen. Indeed, degrees of T-cell activation were attenuated in poorly oxygenated environment as detected by covalent binding of nitroimidazole compound, Hypoxyprobe-1 [15].

Exposure to hypoxia induces cellular stress response to adapt energy deprivation. One of the most important events is stabilization of hypoxia-inducible factor-1α (HIF-1α), which upregulates glycolytic enzymes, angiogenesis, and erythropoiesis [16], [17]. In T cells, however, HIF-1α was reported to diminish TCR signaling [14]. Higher interferon gamma (IFN-γ) production and stronger cytotoxicity in T cells lacking HIF-1α suggest a negative regulatory role of HIF-1α [18], [19].

2 Adenosine

2.1 Formation of Extracellular Adenosine in Tumor

Tumors have been found to contain high levels of extracellular adenosine [20], [21], one of the potential immunosuppressive molecules. Enzymatic degradation of extracellular adenosine triphosphate (ATP) leads to an increase of extracellular adenosine. 5'-Ecto-nucleotidases are responsible for this metabolism: CD39 converting ATP to adenosine diphosphate (ADP) and to adenosine monophosphate (AMP) and CD73 catalyzing adenosine formation from AMP. CD73-deficient mice maintain extracellular adenosine concentration at low levels physiologically and even after the induction of inflammation, suggesting that conversion of adenine nucleotides accounts for a large part of extracellular adenosine formation [22]–[24]. Extracellular adenosine may be removed by further metabolism to inosine by adenosine deaminase and by cellular uptake through nucleoside transporters. Adenosine kinase in the intracellular compartment metabolizes adenosine to AMP, making room for further adenosine uptake. Inhibitors of adenosine deaminase, nucleoside transporters, and adenosine kinase increase extracellular adenosine levels, indicating significance of these mechanisms in the regulation of extracellular adenosine [25]–[28].

Increase of extracellular adenosine levels has been observed during inflammation [29]–[32]. By causing tissue injury, inflammation is able to increase extracellular content of adenine nucleotides and facilitate metabolism to produce adenosine. Cellular damage is considered to cause leakage of adenine nucleotides to extracellular space [4]. Increased release of adenine nucleotides was reported in activated neutrophils and irritant-treated keratinocytes [33], [34].

Subsequent to extracellular increase of adenine nucleotides, tissue hypoxia facilitates conversion to adenosine through upregulation of CD39 and CD73 levels [35], [36]. In parallel, hypoxia inhibits adenosine kinase [37]–[39]. Thus, tissue hypoxia is conductive to extracellular accumulation of adenosine [40] by increasing adenosine formation and by suppressing its removal. Intratumoral hypoxia caused by poor oxygen supply in spite of increasing demand of oxygen favors adenosine accumulation in the tumor. Various tumor cells expressing CD73 also contribute to the production of extracellular adenosine [41]–[44]. These findings correspond to the increase of adenosine levels in tumor tissue (Fig. 14.1).

2.2 Extracellular Adenosine as an Immunoregulatory Molecule

Adenosine is abundant in cells for its use in energy and nucleic acid metabolism. But its presence in the extracellular compartment results in distinctive effects on the cardiovascular system, neuronal cells, kidney, fat tissue, platelets, and leukocytes. In the mid-70s, incubation with adenosine was known to induce cyclic adenosine monophosphate (cAMP) in various cell types including T cells. The increase of cAMP

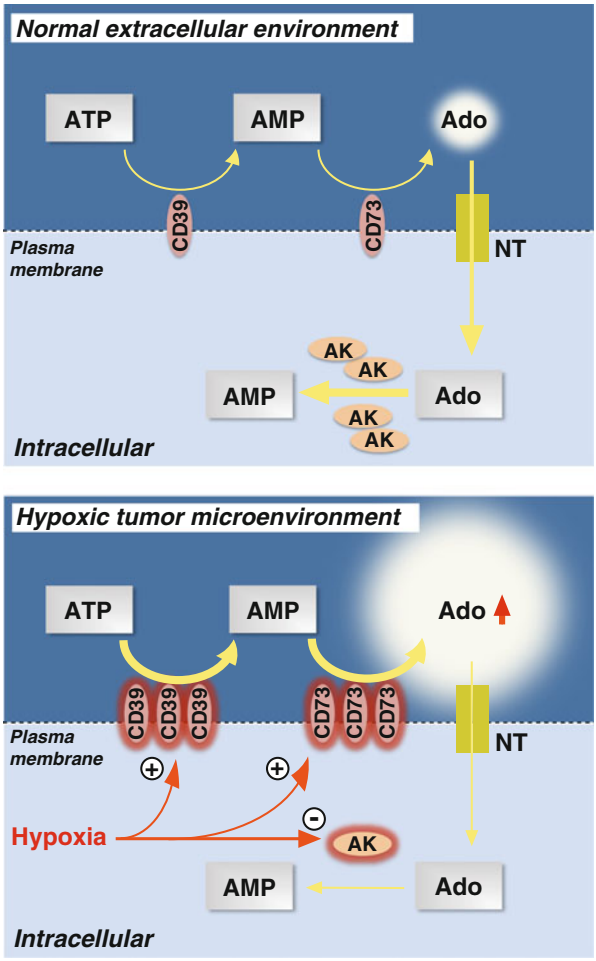

Fig. 14.1 The enhancement of extracellular adenosine generation in *hypoxic tumor microenvironment*. Adenine nucleosides (*ATP*, *ADP*, and *AMP*) in the extracellular compartment are catabolized to adenosine by the activities of *CD39* and *CD73* ecto-enzymes. In normoxic microenvironments (oxygen tension $> \sim 3\,\%$), the concentration of extracellular adenosine is kept low by, e.g., adenosine kinase and adenosine deaminase (not shown in this figure) and cellular uptake is regulated through nucleoside transporter (*NT*). However, hypoxia in tumor microenvironment can change the balance of extracellular adenosine formation and removal in favor of the accumulation of extracellular adenosine. Upregulation of *CD39* and *CD73* under hypoxia accelerates extracellular formation of adenosine. In addition, hypoxia down-regulates adenosine kinase (*AK*) and impairs removal of adenosine

in T cells led to speculation of receptor-mediated signaling, but at that time, the function of adenosine signaling was discussed in regard to energy production [45]–[47]. Effects of adenosine on T-cell function were reported in the 1980s, showing the inhibition of T-cell proliferation, interleukin-2 (IL-2) production, and B-cell helper function [48]–[50]. In parallel, the increase of cAMP was demonstrated to suppress IL-2 production, B-cell helper function, and cytotoxicity of T cells [51], [52]. These early studies implied that extracellular adenosine is inhibitory to T cells through the induction of cAMP.

Meanwhile, the presence of multiple adenosine receptor subtypes was speculated based on the different selectivity of synthetic adenosine derivatives [53], [54]. Since the first cloning of adenosine receptor in 1989, four different adenosine receptors have been identified to date. Among these, A2A and A2B adenosine receptors are cAMP-inducing receptors coupled to Gs protein, while A1 and A3 adenosine receptors are

coupled with Gi protein to reduce cAMP levels [55], [56]. Indeed, adenosine A2A receptor (A2AR) is the predominant subtype in T cells [57], [58].

Similar to the inhibitory effects on polymorphonuclear cells and macrophages, suppression of T-cell activation by A2AR agonist was shown in two papers published in 1997 [57], [59]. In these papers, treatment with A2AR agonist resulted in decreased T-cell proliferation, downregulation of activation marker CD25, and decreased cytotoxicity with a reduced level of Fas ligand expression.

2.3 Mechanism of A2AR-mediated T-cell Inhibition

Subsequent studies revealed more details of T-cell suppression due to A2AR signaling. A2AR stimulation at the time of T-cell activation significantly reduced proliferation of T cells and their effector functions including cytotoxicity and production of cytokines such as IL-2, IFN-γ, and TNF-α [60]–[62]. Both $CD4^+$ and $CD8^+$ T cells are susceptible to this mechanism [62]. A2AR is also expressed in human T cells, and A2AR agonist was shown to be suppressive to effector functions of human T cells such as cytokine production and cytotoxicity [63], [64]. Inhibition of T-cell activation correlates well with the interruption of TCR signaling by A2AR stimulation [61], [65], [66]. A2AR agonist diminished phosphorylation of ZAP70 after TCR stimulation together with downstream phosphorylation of ERK. Inhibition of Akt phosphorylation by A2AR agonist also suggests interruption of the phosphatidylinositol-3-kinase pathway. Since A2AR stimulation induces cAMP, protein kinase A-dependent phosphorylation of COOH-terminal Src kinase may inhibit Lck activation in the early stage of TCR signaling [67].

The helper function of $CD4^+$ T cells is important in activating both cellular immunity and humoral immunity depending on functional differentiation of $CD4^+$ T cells into T helper 1 (Th1) and Th2 cells. Although A2AR agonist can inhibit development of both Th1 and Th2 cells [68], large declines in IFN-γ and IL-2 production by exposure to A2AR agonist indicated a strong suppression of Th1-type cellular immune responses [60]–[62]. Inhibition of Th1 cell development is consistent with changes in cytokine production from APCs in which A2AR agonist diminishes IL-12, but augments IL-10 [69], [70].

A2AR stimulation not only blocks activation of T cells immediately, but also elicits sustained inhibition of T-cell activities by inducing activated T cells with impaired effector functions. As mentioned above, T-cell activation in the presence of A2AR agonist reduced IFN-γ production from activated cells. However, when these cells were restimulated after the removal of A2AR agonist, IFN-γ-producing activity was still less than normal activated T cells [61], [62]. The induction of such anergic T cells suggests that the T-cell inhibitory effect of adenosine may be persistent even after clearance of adenosine (Fig. 14.2a). This property of A2AR signaling may be relevant to the memory of exposure to extracellular adenosine, where persistent elevation of cAMP was observed after transient exposure to adenosine [57].

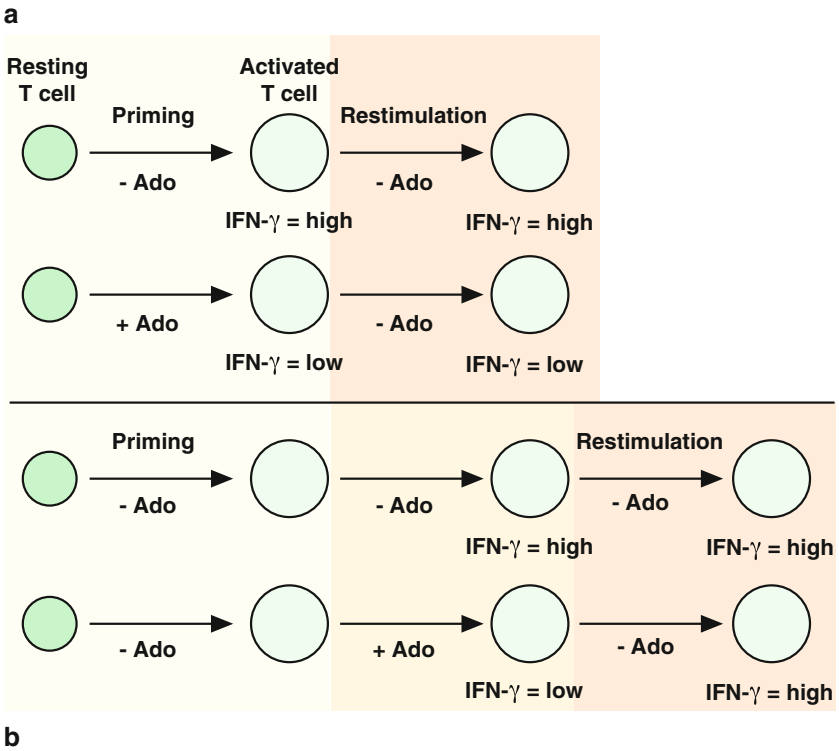

Fig. 14.2 Early stages of priming and activation of *resting T cells* are highly susceptible to the A2AR-mediated immunosuppression. **a** When *resting T* cells are stimulated in the presence of A2AR agonist, the *activated T cells* produce very low levels of *IFN-γ*. The impairment of IFN-γ-producing activity persists even after removal of A2AR agonist. **b** A2AR agonist can inhibit *IFN-γ* production from already *activated T cells*, but only in the very presence of A2AR agonist. After the removal of A2AR agonist, *IFN-γ* production from T cells returned to normal levels

Comparison between resting and activated T cells showed that already activated T cells are relatively resistant to A2AR-mediated inhibition (Fig. 14.2b). When activated T cells were restimulated, A2AR agonist still inhibited T-cell proliferation and IFN-γ production. After removal of the A2AR agonist, however, the effector function of these T cells came back to the same levels as in activated T cells that were never cultured with A2AR agonist [71], [72]. Therefore, although A2AR agonists can inhibit activities of the already activated T cells, the inhibitory effect did not persist after its removal. This result indicates that A2AR stimulation does not switch fully functional effector T cells to the anergic phenotype. The inhibition of activated effector cells by extracellular adenosine is highly territorial: only in extracellular adenosine-rich tissue microenvironment, but not in the neighboring adenosine-low microenvironment.

2.4 *Adenosine Promotes Immunosuppressive Activity of Regulatory T Cells*

Regulatory T cells (Treg) were initially identified as $CD4^+$ T cells constitutively expressing CD25 at high levels. Activation of Treg follows the normal scheme of T-cell activation, but activated Treg spontaneously inhibit activation of other effector T cells. Since the lack of immunoregulation by Treg causes severe autoimmune diseases, Treg are indispensable for the control of immune activation against self-antigens in peripheral tissues [73], [74].

It was suggested that Treg development and effector functions are under control of the hypoxia-adenosinergic pathway, and the model was proposed to potentially unify the diverse functions of Treg [75]. A large body of published data are consistent with the model where Treg development and their immunoregulatory activity are mediated by the interplay of the cAMP-elevating adenosine receptors, HIF-1α, and subsequent cAMP response element (CRE)- and hypoxia response element (HRE)-mediated transcription in Treg and effector cells. Accordingly, HRE- and CRE-driven activities of Treg may be required to achieve a maximal level of immune suppression.

As a subset of T cells, Treg express functional A2AR as well [76]. In contrast to negative effects on activities of most T cells, A2AR stimulation rather promotes immunoregulatory activity of Treg [76]. In isolated spleen cells, containing both effector T cells and Treg at physiological ratio, T-cell stimulation in the presence of A2AR agonist inhibited activation of effector T cells but increased Treg population. A2AR stimulation not only increased the number of Treg but also augmented the T-cell inhibitory activity of Treg. Corresponding to the enhanced immunoregulatory activity, A2AR agonist upregulated cytotoxic T-lymphocyte antigen 4 (CTLA-4) expression in these Treg. The importance of CTLA-4 in the immunosuppressive activity of Treg was demonstrated by systemic lymphoproliferation and autoimmune disease in mice with Treg-specific deletion of CTLA-4 [77].

Adoptive transfer of Treg reduced ischemia-reperfusion injury in vivo, but pre-treatment of Treg with A2AR agonist before transfer augmented the efficacy of this treatment [78]. Moreover, A2AR-deficient Treg were less effective compared to wild-type Treg, suggesting the in vivo significance of A2AR signaling in regulating the immunosuppressive activity of Treg.

Treg may develop either during T-cell maturation in the thymus (natural Treg) or in the peripherals by functional differentiation of mature T cells (inducible Treg). Analysis of A2AR-dependent Treg expansion showed the involvement of natural Treg proliferation and induction of new Treg [76]. The promotion of inducible Treg has been speculated from an upregulation of FoxP3 mRNA in T-cell culture treated with A2AR agonist [61]. FoxP3 is a transcription factor involved in the regulation of immunosuppressive activity. It was further confirmed that A2AR agonist expanded transforming growth factor beta (TGF-β)-inducible Treg both in vitro and in vivo [79]. Besides A2AR, adenosine 2B receptor (A2BR) may be also involved in the increase of inducible Treg [80].

Upstream adenosine receptor signaling, hypoxia may be also involved in the regulation of Treg. Indeed, FoxP3 is inducible by hypoxia in T cells, and HIF-1α

mediates hypoxic induction of FoxP3 [81], [82]. However, subsequent studies provided evidence for complicated role of HIF-1α in the regulation of Treg. Initially, studies using mice with HIF-1α-deficient T cells demonstrated HIF-1α-mediated downregulation of Treg and reciprocal increase of Th17 [83], [84]. In contrast, more recent papers showed that hypoxia induces FoxP3 and increases Treg abundance [82]. In this setting, HIF-1α is necessary for optimal immunosuppressive activity of Treg. Nonetheless, local oxygen levels may be an important regulator of Treg. Hypoxia in tumors may be relevant to increase of Treg population in tumor microenvironment.

While adenosine can control the immunoregulatory activity of Treg, Treg may utilize adenosine in their mechanism of immunosuppression. Treg express CD39 and CD73, extracellular nucleotidases that catalyze degradation of ATP to adenosine and increase extracellular adenosine concentration [85]–[87]. The produced adenosine, in turn, interacts with A2AR and blocks activation of T cells. This mechanism may explain why A2AR-deficient effector T cells were resistant to immunoregulatory cells [88] and why CD73-deficient Treg were less effective in inhibiting ischemia-reperfusion injury [78]. Furthermore, adenosine produced from Treg may autonomously target Treg to enhance their activity.

Thus, A2AR-mediated signaling promotes immunoregulation by Treg both quantitatively and qualitatively. The outcome of this effect is consistent with the direct inhibition of effector T-cell activation by A2AR-mediated signaling. In addition to the direct inhibition of T-cell activation, A2AR agonist also provides longer lasting T-cell inhibition by at least two different mechanisms. When present at the time of T-cell priming, A2AR agonist induces longer lasting inhibition of antigen-specific T-cell response by developing anergic effector T cells. In addition, when enforced in the presence of A2AR agonist, Treg may provide long-lasting suppression of antigen-specific T-cell response ([76], [78]; Fig. 14.3).

2.5 Myeloid-derived Suppressor Cells

Together with Treg, myeloid-derived suppressor cells (MDSCs) represent major immunoregulatory cells contributing to immunosuppressive environment in tumors [89]. Adenosine promotes expansion of MDSCs in A2BR-dependent manner. Indeed, the number of tumor-infiltrated MDSCs is low in A2BR-deficient mice [90]. Hypoxia also promotes differentiation and function of MDSCs [91], suggesting significance of the hypoxia-adenosine pathway in regulating MDSCs in tumors.

2.6 Antigen-presenting Cells

Adenosine receptor stimulation of APCs inhibits T-cell stimulating activity. A2AR agonists inhibit IL-12 production but induce IL-10 from dendritic cells [69], [70]. This change in cytokine milieu is suppressive to the induction of Th1 cells and

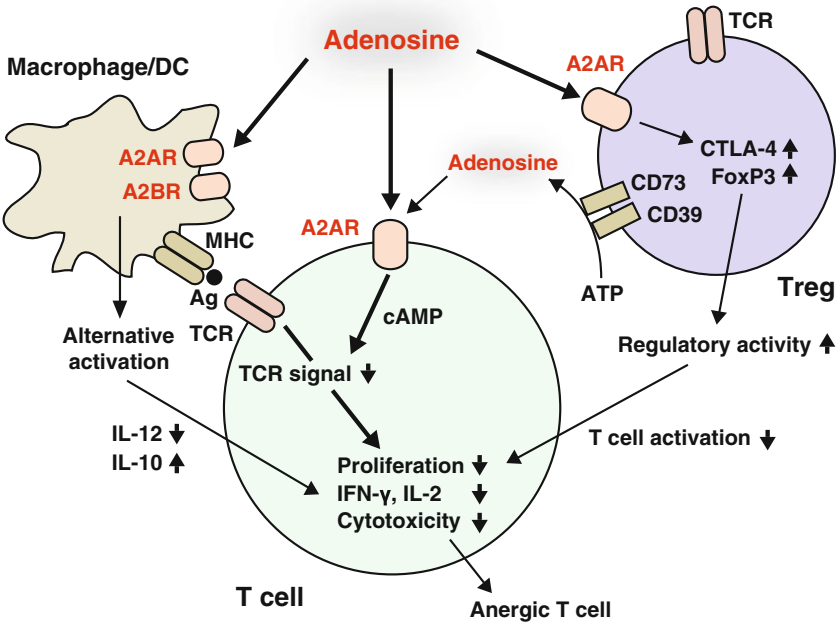

Fig. 14.3 Regulation of T cells' effector functions by extracellular adenosine. Signals from *A2AR* on *T cell*'s surface directly inhibit the TCR-mediated activation. As a result, *A2AR* stimulation diminishes various T-cell functions including proliferation, cytokine production, and cytotoxicity. The impairment of effector functions in activated T cells can persist even after removal of agonist, suggesting the development of anergic T cells. Adenosine also indirectly influences T-cell activation by inducing alternative activation of antigen (*Ag*)-presenting cells. Macrophages and dendritic cells (*DC*s) activated in the presence of adenosine produce less *IL-12* and more *IL-10*, changing cytokine milieu for functional differentiation of T cells. Moreover, A2AR stimulation promotes Treg expansion and their immunosuppressive function. Thus, adenosine signaling suppresses T-cell activation both directly and indirectly. Therefore, T-cell inhibitory effect of A2AR/A2BR-mediated immunosuppressive signaling is both immediate (i.e., by directly inhibiting the T-cell activation signal) and long-lasting (anergic T cells and Treg)

therefore inhibitory to cellular immune responses. While A2AR stimulation suppresses activation of APCs to proinflammatory phenotype, adenosine induces alternative activation of APCs via A2BR [69], [70], [92], [93]. Alternative activation induces arginase, indoleamine-2,3-dioxygenase (IDO), TGF-β, and COX-2 in APCs, and such APCs inhibit optimal activation of T cells [94]. A2AR agonist also induces VEGF from macrophages [95], [96], suggesting adenosine switches APCs to tolerogenic and angiogenic phenotype.

Dendritic cells exposed to hypoxia express lesser levels of major histocompatibility complex (MHC) and co-stimulatory molecules [97], [98]. Hypoxia inhibits phagocytosis by dendritic cells, decreasing capture of antigen [99]. Therefore, those dendritic cells under hypoxia have impaired T-cell stimulatory capacity as APCs.

2.7 NK Cells

Adenosine suppresses NK cell activities. A2AR agonists are suppressive to IFN-γ production and cytotoxicity of lymphokine-activated killer (LAK) cells and NK cells from mice and humans [100]–[102].

Hypoxia also suppresses activity of NK cells [103]. Closely relevant to the inhibition of NK cell-dependent cytotoxicity, hypoxia downregulates NKG2D ligands including MHC class I chain-related molecules on tumor cells [104], [105]. Since NKG2D is an activating receptor of NK cells, hypoxic tumor cells are induced to be resistant to NK cell-dependent cytotoxicity. These observations suggest biological significance of oxygen tension in the regulation of antitumor immune responses.

3 Endogenous Adenosine as a Physiological Regulator of Immune Response

3.1 A2AR

A2AR stimulation suppresses immune responses through Gs protein-mediated cAMP increase. However, there are many other cAMP-elevating receptors on the surface of immune cells that can transduce immunosuppressive signals when activated pharmacologically. A brief inventory of such molecules includes prostaglandin E_2, adrenaline, histamine, and small peptides such as vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP). Adenosine has been regarded as just one of such anti-inflammatory small molecules, but in recent years, recognition of adenosine became prominent because of its nonredundancy as an endogenously produced immunoregulator [106]–[108].

One of the most important features of A2AR is its critical role in physiological regulation of immune responses. Acute hepatitis induction in A2AR-deficient mice resulted in remarkable exaggeration of liver damage and proinflammatory cytokine levels [109], [110]. The result demonstrated that (1) endogenously produced adenosine can control the intensity of immune response through A2AR and (2) A2AR signaling is critical to stop inflammation because other immunoregulatory mechanisms could not compensate for the lack of A2AR-mediated immunosuppression.

Adenosine-dependent immunoregulation may represent the tissue's negative feedback response to overwhelming inflammation [106], [110]. Tissue damage inflicted by proinflammatory activities triggers an accumulation of extracellular adenosine. Indeed, an increase of adenosine levels was observed during inflammation [29]–[32]. Tissue hypoxia and nucleotidase activities of CD39 and CD73 are responsible, at least in part, for the increase in extracellular adenosine [22]–[24]. The increased adenosine transmits a signal to immune cells through A2AR to stop proinflammatory activities and prevent further tissue damage. Interruption of this sequence, e.g., A2AR-deficiency and A2AR antagonism, means loss of a brake on inflammation.

Exaggerated inflammation in A2AR-deficient mice was demonstrated in various tissues and in various causes of inflammation [32], [111]–[114], suggesting that the adenosine–A2AR system is a universal mechanism in the vital body to prevent excessive tissue damage.

This discovery offered a solution to a clinically important issue that, as opposed to recruitment of anti-inflammatory effects by targeting A2AR with agonists, it is possible to enhance inflammation by blocking the action of endogenous adenosine by A2AR antagonist. Intake of A2AR antagonists may be detrimental to inflammatory disorders; however, we may take advantage of this mechanism in the treatment of cancer.

3.2 A2BR

Another Gs protein-coupled adenosine receptor is adenosine A2B receptor (A2BR). Affinity of adenosine to A2BR is lower than A2AR; however, local adenosine levels in hypoxic tissue can be high enough to stimulate A2BR [115], [116]. A2BR is expressed on macrophages, dendritic cells, endothelial cells, epithelial cells, mast cells, and fibroblasts, and it has distinctive effects on inflammatory responses [115], [116]. A2BR agonist was shown to block inflammatory tissue injury in experimental models. Exacerbation of colitis, lung inflammation, and ischemia-reperfusion injury in A2BR-deficient mice suggests pathophysiological significance of endogenous adenosine signaling through A2BR [117]–[121]. Since A2BR stimulation changes the functions of macrophages and dendritic cells as APCs, T cells may receive indirect immunoregulatory effects from A2BR [92], [93], [115], [116]. Thus, increase of extracellular adenosine triggers anti-inflammatory negative feedback responses via A2AR and A2BR. The adenosine–A2AR/A2BR pathway may be vital as an immunoregulatory mechanism in tumor.

4 Cancer

Tremendous efforts by tumor immunologists have significantly advanced the understanding of tumor-associated antigens and improved induction of effector T cells recognizing tumor cells as foreign [122], [123]. It also became clear that the immunosuppressive environment in tumors is a potential problem in tumor eradication by immune cells. Tumors often have infiltration of T cells that can be reactive against the tumor cells, but the tumor-infiltrated T cells are inactive in attacking the tumor in vivo. In mice manipulated to express the same antigen in both normal and tumor tissues, the same effector T cells were disabled only in tumor [124]–[126]. Such studies provide a direct evidence for the existence of potentially immunosuppressive tumor microenvironment. Tumors may employ various mechanisms to evade immune response, e.g., Treg, MDSCs, anti-inflammatory cytokines, and IDO [9], [127].

Advances in T-cell technology developed methods of inducing antitumor effector T cells. However, efficacy of these antitumor T cells may be limited if they are sensitive to immunosuppression in tumor microenvironment. Disengagement of antitumor effectors from immunosuppressive mechanism in tumor will significantly improve the outcome of tumor immunotherapy.

Hypoxia, which is frequently observed in tumors, may play a role in the establishment of immunosuppressive environment. Hypoxia is conductive to the increase of extracellular adenosine levels, and indeed high levels of extracellular adenosine were observed in tumors [20], [21]. Various effects of hypoxia in vivo and in vitro are mediated by the interaction of extracellular adenosine with A2AR [32], [40], [128]–[132]. There is a similarity between tumor-infiltrated T cells and T cells activated in the presence of adenosine in terms of preferential suppression of effector functions [62]. Thus, adenosine may represent one of the potentially immunosuppressive mechanisms in tumors. This concept was established in a tumor inoculation study in which A2AR-deficient mice, but not wild-type mice, demonstrated regression in growing tumors [21]. Improvement of T cell-mediated tumor eradication upon inactivation of A2AR suggests nonredundance of the adenosine–A2AR pathway in the immunosuppressive tumor microenvironment. Tumors protect themselves utilizing the body's common rule: You shall not take vengeance when you see adenosine.

The enhanced tumor regression in A2AR-deficient mice suggested that A2AR-antagonists might be useful to break immunosuppression in tumors and improve tumor immunotherapy. Indeed, A2AR antagonists such as caffeine, ZM241385, and SCH58261 blocked tumor growth by promoting antitumor immune responses [21], [42], [133]. Significant reduction of intratumoral blood vessels in A2AR antagonist-treated mice suggests that the treatment not only enhances antitumor immune response but also blocks adenosine-induced angiogenesis in tumors [21]. The countermeasure to immunosuppression in tumors in conjunction with successful induction of antitumor effector T cells may significantly improve the outcome of tumor immunotherapy.

In addition to A2AR, A2BR also participates in the protective mechanism of tumor against immune response. Retardation of tumor growth was observed in A2BR-deficient mice [134]. Treatment with A2BR antagonist is also inhibitory to tumor growth in wild-type mice, but not in T cell-deficient mice [135]. Enhanced T-cell infiltration into the tumor by A2BR antagonist suggests that extracellular adenosine in tumor discourages antitumor immune response through both A2AR and A2BR.

The critical role of adenosine-dependent immunosuppression in tumors was also demonstrated by the promotion of antitumor immunity in the absence of CD73 [136], [137]. The lack of CD73, ecto-nucleotidase, sharply decreases extracellular adenosine formation and promotes proinflammatory responses [22]–[24]. Neutralization of CD73 by the injection of antibody inhibited tumor growth and promoted antitumor immune response [42], [138]. CD73 expression on tumor cells plays an important role in immunosuppression and tumor metastasis. Indeed, tumor cells lacking CD73 are susceptible to antitumor immunity [42], [43]. Not only CD73 expression on tumor cells but also CD73 expression on normal cells plays a significant role. Inoculated tumors grow slower in CD73-deficient mice because of stronger antitumor

T-cell response [139]. Moreover, CD73 deficiency resulted in the inhibition of carcinogenesis thanks to T cell- and NK cell-dependent immune response [140]. These studies suggest that, besides blockade of adenosine signaling by adenosine receptor antagonists, prevention of extracellular adenosine formation by targeting CD73 may be a promising countermeasure to immunosuppressive tumor microenvironment.

5 Natural A2AR Antagonists: Caffeine and Theophylline

Caffeine and theophylline are representative nature-derived methylxanthines, and they are the most widely consumed A2AR antagonists in the form of beverage, food, and medication. It is known that the psychostimulatory effect of caffeine is attributable to antagonism of the adenosine–A2AR interaction [141], [142]. Indeed, caffeine exacerbated inflammatory tissue damage in experimental acute hepatitis by blocking A2AR [133], [143]. While caffeine and theophylline block A2AR-mediated cAMP increase, a high concentration of these compounds actually increase cAMP levels by inhibiting cAMP phosphodiesterase. Therefore, while low doses of caffeine exacerbate inflammatory tissue damage, caffeine can be anti-inflammatory at high doses [133], [143]. Normal caffeine consumption in humans raises caffeine concentration enough to antagonize A2AR [141], [144], [145]. Since anti-inflammatory high dose may not be reproduced by normal caffeine consumption in humans, the immune-enhancing effect will be clinically more relevant.

In tumor immunotherapy, proinflammatory action of natural adenosine receptor antagonists may be beneficial in promoting antitumor immune response. Co-treatment with caffeine significantly improved tumor eradication by endogenously developed and adoptively transferred antitumor T cells [21]. The enhancement of antitumor activity by caffeine may be relevant to some epidemiological studies that have suggested inverse association between cancer incidence and coffee consumption. The statistics suggest that coffee consumption dose-dependently decreased incidence of breast, liver, colon, lung, skin, and endometrial cancer [146]–[154].

6 Conclusion

Hypoxia in tumors may be implicated to the establishment of immunosuppressive environment. Hypoxia inhibits diverse antitumor immune responses at least in part by upregulation of extracellular adenosine. Adenosine stops antitumor immune response through A2AR and A2BR on immune effector cells. This direct action of adenosine can immediately suppress immune responses in tumor microenvironment. Adenosine evokes longer lasting immunoregulation, which persists in immune cells even after the disappearance of adenosine. Cell activation in the presence of adenosine induces anergic T cells and alternative activation of APCs.

Furthermore, adenosine promotes cellular immunosuppressive activities. Adenosine promotes expansion of Treg and their immunoregulatory activity. MDSCs were

also shown to increase in response to adenosine and hypoxia. The increase of professional immunoregulatory cells may be an important component of tumor microenvironment, which is harsh to immune effectors. Thus, the hypoxia-adenosine pathway involves direct inhibition of antitumor effector cells and long-term effect by developing tumor microenvironment favoring immunosuppression.

Treatment with adenosine receptor antagonist and CD73 inhibitor may be a promising approach to improve antitumor immunity. Since this treatment is compensatory to the current approach that focuses on the numerical increase of antitumor T cells, it will be more efficacious when combined with cancer vaccines and adoptive immunotherapy [21], [123], [155]. In cancer adoptive immunotherapy, downregulation of A2AR on the antitumor T cells is expected to promote their efficacy in vivo. In addition to A2AR antagonist treatment after cell transfer, transfer of cells that were created to be insensitive to adenosine may also be worth exploring [21], [72].

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