Cancer Immunology at the Crossroads: Experimental Immunotherapies

Cancer Immunology Research

Hostile, Hypoxia–A2-Adenosinergic Tumor Biology as the Next Barrier to Overcome for Tumor Immunologists

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Abstract

Hypoxia-driven, A2A adenosine receptor (A2AR)–mediated (hypoxia–A2-adenosinergic), T-cell–autonomous immunosuppression was first recognized as critical and nonredundant in protecting normal tissues from inflammatory damage and autoimmunity. However, this immunosuppressive mechanism can be highjacked by bacteria and tumors to provide misguided protection for pathogens and cancerous tissues. Inhibitors of the hypoxia–A2-adenosinergic pathway represent a conceptually novel type of immunologic coadjuvants that could be combined with cancer vaccines, adoptive cell transfer, and/or blockade of negative immunologic regulators to further prolong patient survival and to minimize treatment-related side effects. In support of this approach are preclinical studies and findings that some human cancers are resistant to chemotherapies and immunotherapies due to the tumor-generated extracellular adenosine and A2AR on antitumor T and natural killer (NK) cells. Among the coadjuvants are (i) antagonists of A2AR, (ii) extracellular adenosine-degrading drugs, (iii) inhibitors of adenosine generation by CD39/CD73 ectoenzymes, and (iv) inhibitors of hypoxia–HIF-1α signaling. Combining these coadjuvants with CTLA-4 and/or PD-1 blockade is expected to have additive or even synergistic effects of targeting two different antitumor protective mechanisms. It is expected that even after multicombinatorial blockade of negative immunologic regulators, the antitumor T and NK cells would still be vulnerable to inhibition by hypoxia and A2AR. Yet to be tested is the potential capacity of coadjuvants to minimize the side effects of CTLA-4 and/or PD-1 blockade by decreasing the dose of blocking antibodies or by eliminating the need for dual blockade. *Cancer Immunol Res; 2(7); 598–605. ©2014 AACR.*

Introduction

Recent advances in cancer vaccines, adoptive cell transfer, and blockade of negative immunologic regulators CTLA-4 and/or PD-1 are reflected in approvals by the FDA and represent a significant hope for many patients (1–7). However, there is still room for improvement in terms of further prolongation of patient survival and lessening of the immune-related adverse side effects (5, 6, 8–10). These goals may be accomplished only after careful and rigorous considerations and testing of other important and yet to be targeted immunosuppressive pathways. These immunosuppressive mechanisms may limit the clinical benefits of current cancer immunotherapies even after the depletion of all known negative immunologic regulators, such as CTLA-4/PD-1 or regulatory T cells (Treg).

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Hypoxia–A2-Adenosinergic Immunosuppression, Transcription, and Redirection of Antipathogen and Antitumor Immune-Cell Effector Functions

The concept of targeting the normally protective but sometimes usurped physiologic mechanisms, such as cellular metabolism and local tissue oxygen tension-dependent A2A and A2B adenosine receptor (A2AR/A2BR)–mediated immunosuppression in inflamed and cancerous tissues, is the basis of the therapeutic strategy and the subject of this Crossroads article (Fig. 1; refs. 11–18). This type of immunosuppression in the tumor microenvironment (TME) seems to be a misguided application of the evolutionarily critical and nonredundant negative feedback immunosuppressive mechanism that is otherwise lifesaving by protecting normal tissues from excessive collateral damage during the antipathogen immune response (13, 14, 18).

The identification of this indispensable immunoregulatory pathway may have provided one of the explanations for the paradoxical coexistence of tumors and antitumor immune cells in the same patient with cancer (19), which could be due to the A2AR-mediated inhibition of tumor-reactive T cells in the TME (12, 15).

It must be emphasized that hypoxia–A2-adenosinergic signaling is not only an immunosuppressive pathway that inhibits

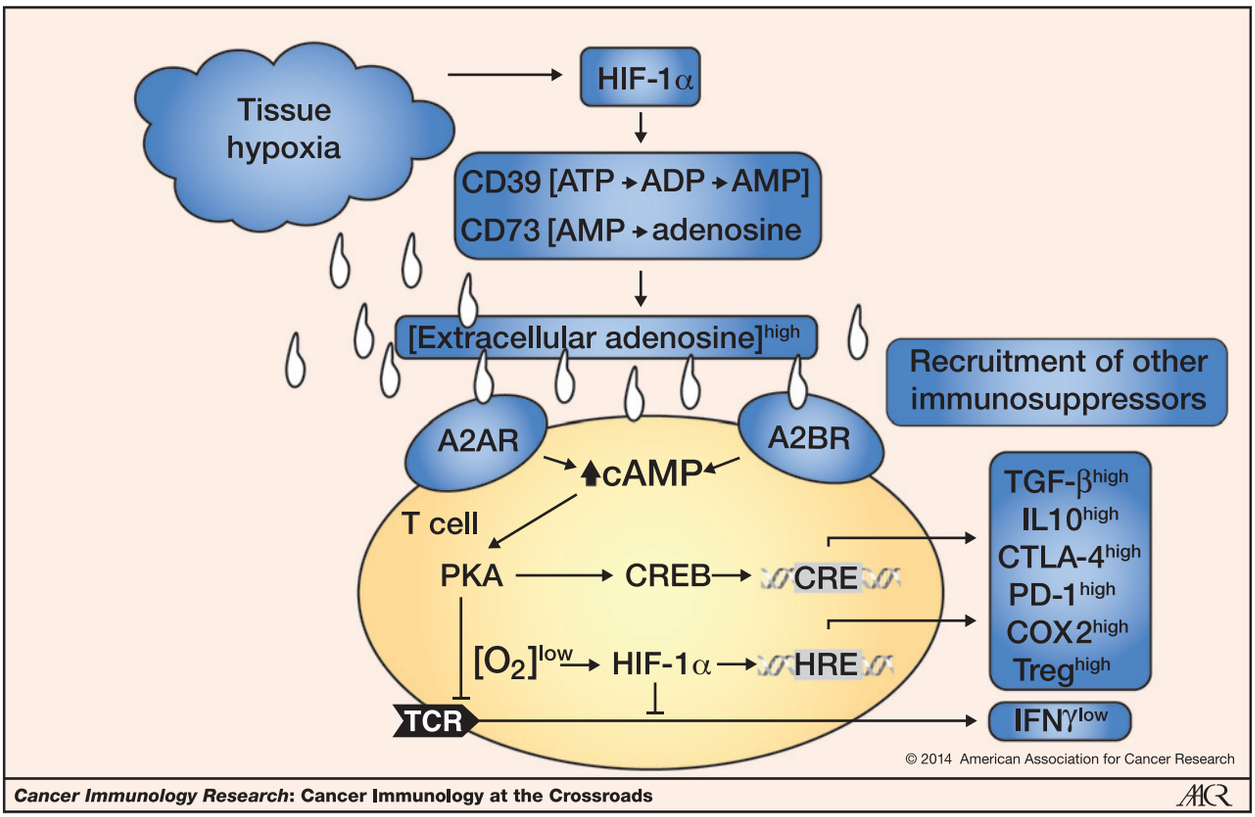

Figure 1. Schematic diagram of tissue hypoxia in inflamed and cancerous tissues. The hypoxia-driven stabilization of HIF-1α transcription factor leads to CD39/CD73 ectoenzyme–mediated generation of extracellular adenosine, which signals through the G_s-protein–coupled A2A and A2B adenosine receptors (A2AR and A2BR) and triggers the accumulation of intracellular cAMP. The increased expression of CD73 on the surface of Tregs (78) may generate extracellular adenosine that would further enhance their suppressor activities and add to the immunosuppressive effects of tumor-produced adenosine on $CD8^+$ T cells. The binding of cAMP to the regulatory subunit of cAMP-dependent PKA results in a cascade of phosphorylation events that inhibits the TCR-triggered signaling pathway and therefore inhibits the proinflammatory effects of T cells. After phosphorylation by PKA, CRE-binding protein CREB participates in the transcription of genes containing the CRE (79). In addition, HIF-1α participates in the transcription of genes containing HREs.

the T-cell receptor (TCR)–triggered production of proinflammatory cytokines, such as IFNγ (Fig. 1). This pathway may also redirect the immune response, as we discussed in detail in a model proposed previously (ref. 16 and Fig. 1). Briefly, the local tissue hypoxia and the A2AR signaling–mediated increase in intracellular cAMP and cAMP-dependent protein kinase inhibit the production of proinflammatory cytokines such as IFNγ in $CD8^+$ and $CD4^+$ T cells, while promoting transcription of genes that express the cAMP-response elements (CRE) or the hypoxia-response elements (HRE). This, in turn, may lead to the synthesis of immunosuppressive molecules and the development of Tregs (Fig. 1; ref. 16). Thus, the generation of anti-inflammatory mediators or the development or functions of immunosuppressive Tregs could be facilitated by hypoxia–A2-adenosinergic signaling. This may provide an explanation for the "infectious tolerance" of Tregs (16) in hypoxic and extracellular adenosine-rich inflamed tissues and in the TME (16, 20, 21).

The power and versatility of A2-adenosinergic immunosuppression is usurped by *Staphylococcus aureus* and other bacteria to suppress and escape host immune responses. These pathogens have acquired the ability to synthesize extracellular adenosine, leading to the inhibition of antibacterial effector functions of neutrophils through A2AR signaling (22).

Key Molecules of the Hypoxia–A2-Adenosinergic Signaling Pathway

Descriptions of the upstream and downstream stages of the hypoxia–A2-adenosinergic pathway in Figs. 1 and 2 and in this Crossroads article follow the history of the search for the molecular mechanism of physiologic immunosuppression, which started with the bet on the importance of intracellular cAMP. cAMP is a high-fidelity intracellular immunosuppressor that inhibits virtually all tested TCR-triggered effector functions of T cells through the activation of the cAMP-dependent protein kinase A (PKA), albeit with different efficacy (23–29).

Elevation of intracellular levels of cAMP in T cells, natural killer (NK) cells, or myeloid cells in hypoxic and extracellular adenosine-rich tissues is triggered by the binding of extracellular adenosine to G-protein–coupled, cAMP-elevating A2AR (high-affinity) and A2BR (low-affinity) adenosine receptors (30, 31). Our focus on studying A2AR in T cell–mediated

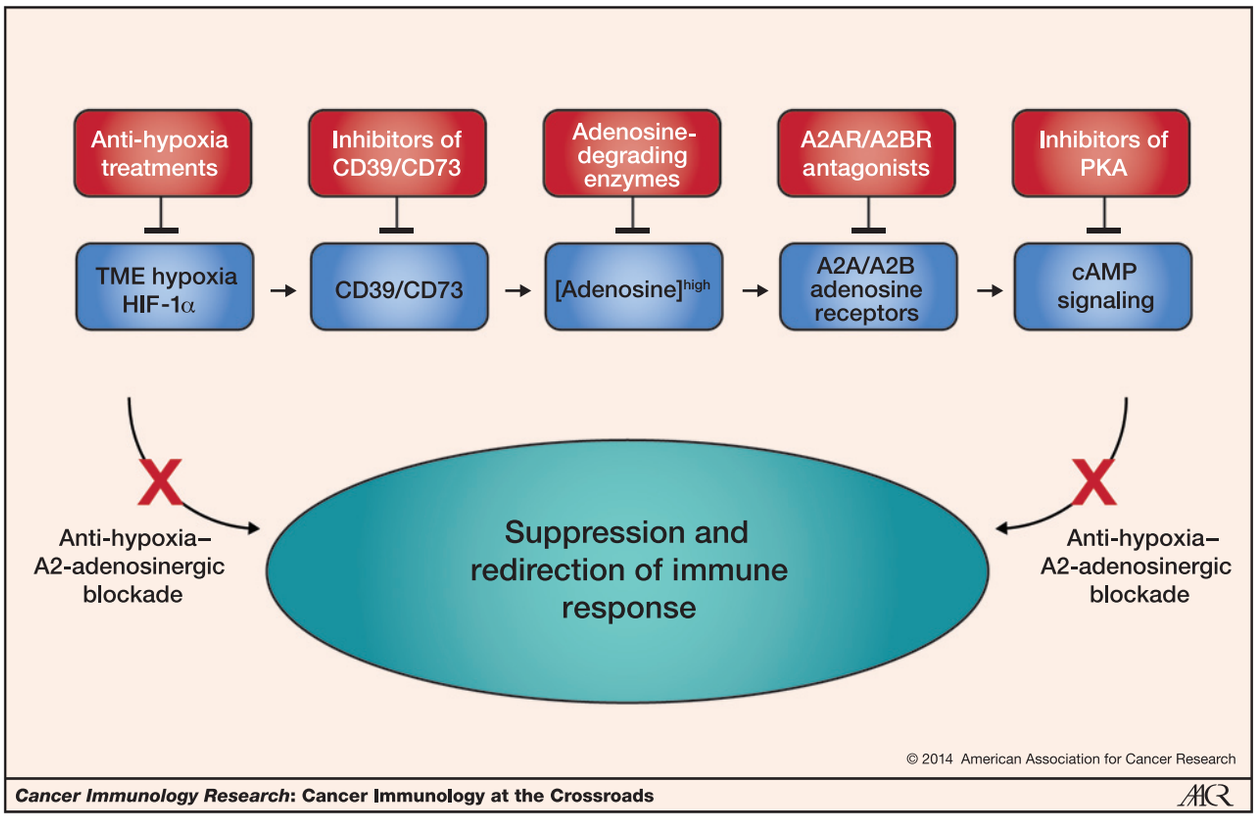

Figure 2. Anti-hypoxia–A2-adenosinergic coadjuvant-mediated inhibition of the hypoxia–A2-adenosinergic pathway. The anti-hypoxia–A2-adenosinergic drugs shown were developed for other therapeutic purposes. Inhibitors of the hypoxia→HIF-1α early stage, including inhibitors of HIF-1α, can weaken hypoxia and promote destabilization and degradation of HIF-1α in antitumor T and NK cells. Inhibitors of CD39 ecto-ATPase/ADPase and CD73 5′-nucleotidase may prevent the accumulation of extracellular adenosine in the TME, thereby decreasing the intensity of immunosuppressive signaling through A2AR or A2BR. Commercially available drugs such as stabilized adenosine deaminase may be tested to degrade extracellular adenosine. Alternatively, enzymes such as adenosine kinase may be tested for the ability to re-phosphorylate adenosine to generate AMP, thus decreasing the levels of extracellular adenosine. Antagonists of A2A/A2B adenosine receptors compete with the tissue-produced adenosine for binding to the adenosine receptor. The antagonist-bound adenosine receptors are not activated and do not increase intracellular cAMP levels. Inhibitors of cAMP-dependent PKA could decrease cAMP signaling, but the associated side effects may outweigh potential benefits.

immunity was based on the original observations that murine T cells preferentially express A2AR (32–34), which is likely to be activated in the lower ranges (~50 nmol/L) of extracellular adenosine in inflamed and cancerous tissues *in vivo*. Human antitumor T cells also express both A2AR and A2BR (Sitkovsky et al.; unpublished data).

Importantly, T cells do not have "spare" A2AR (i.e., there is no receptor reserve; ref. 35), suggesting that the number of A2AR molecules per T cell is the limiting factor in determining the maximal cAMP response of T lymphocytes to adenosine and the extent of the inhibition of T cells by adenosine–A2AR–cAMP signaling. This important property provides yet another mechanism to fine-tune the intensity of immunosuppressive effects of extracellular adenosine. It was also shown that T cells have a "memory" of signaling through A2AR (36). Therefore, T cells experience the A2AR-triggered suppression long after exposure to the short-lived *in vivo* extracellular adenosine has ceased.

Extracellular adenosine is generated by at least two known mechanisms, from intracellular ATP or from extracellular ATP due to activities of extracellular adenosine-generating tandem ectoenzymes CD39 (ecto-ATPase/ADPase) and CD73 (ecto-5′-nucleotidase), which was recently reviewed in ref. (17). The CD39 and CD73 ectoenzymes were shown to be important in limiting the inflammatory damage (20, 37–40) by generating extracellular adenosine and thereby enabling the downstream adenosine → A2AR-mediated signaling (11).

Hypoxia → hypoxia-inducible factor-1α (HIF-1α)–mediated events have been implicated in upstream events that trigger anti-inflammatory CD39/CD73 → $[adenosine]^{High}$ → A2AR/A2BR signaling. The first evidence of the inhibitory role of hypoxia–HIF-1α in T cells was obtained in HIF-$1\alpha^{-/-}$Rag$2^{-/-}$ chimeric mice, which are characterized by HIF-1α deficiency only in cells of the adaptive immune system (41). Results from these studies revealed that HIF-1α not only regulates lymphocyte development and functions, but it also protects against autoimmunity and inflammatory tissue damage. The immunosuppressive role of HIF-1α in T cells was confirmed using mice with targeted deletion of the *HIF-1α* gene in T cells. The genetic "knockdown" of HIF-1α in T cells prevented the

inhibition of T cells in hypoxic inflamed tissues, increased antibacterial response, and improved survival in mice (42). These observations also established that HIF-1α effects are T-cell autonomous.

The immunosuppressive role of HIF-1α in T cells was also indirectly supported by studies of the effects of pharmacologic weakening of tissue hypoxia and of hypoxia → HIF-1α signaling by systemic oxygenation (43) that weakens hypoxia and destabilizes HIF-1α. The observations of increased immune response and exacerbation of collateral inflammatory damage in these experiments extended the earlier genetic evidence. HIF-1α was shown to induce the expression of CD73, a membrane-bound glycoprotein that generates the immunosuppressive adenosine following binding of HIF-1α to HRE sites in the *CD73* gene promoter. Thus, the inhibition of *HIF-1α* expression by antisense oligonucleotides led to the inhibition of hypoxia-inducible CD73 expression (44). Another immunosuppressive molecule, A2BR, was also shown to be regulated transcriptionally by HIF-1α (45).

Hypoxia–A2-Adenosinergic Protection of Tumors from Antitumor Immune Cells

Previous demonstrations supported the view that inflammatory damage-associated interruption in local blood supply and the ensuing tissue hypoxia may lead to the accumulation of extracellular adenosine and recruitment of anti-inflammatory A2AR on T cells in the adjacent normal tissues (11). This led these authors to a straightforward assumption that hypoxic and extracellular adenosine-rich cancerous tissues may have highjacked this A2AR-based mechanism to inhibit the incoming antitumor T cells by elevating the levels of their immunosuppressive intracellular cAMP (12, 15).

Indeed, many solid TMEs are hypoxic (46), and tissue hypoxia is conducive to the generation of extracellular adenosine (47). Tumors were shown to contain extracellular adenosine (48), although the intracellular cAMP-elevating A2AR or A2BR were explicitly excluded as possible candidates to mediate the immunosuppression by extracellular adenosine in tumors (48–50).

The key test to confirm or disprove the potential roles of extracellular adenosine and A2AR as mediators of immunosuppression in tumors was the comparison of antitumor immune response in A2AR gene-deficient mice with that of their wild-type (WT) littermates. On the basis of insights about the role of adenosine → A2AR in immunosuppression in inflamed tissues, it was expected that A2AR gene-deficient mice would have much stronger and longer-lasting antitumor immunity. This hypothesis was validated by findings that the genetic deletion of A2AR resulted in much stronger antitumor immunity and rejection of established tumors and prolonged survival of A2AR-deficient mice compared with those of control tumor-bearing A2AR-expressing mice (12, 15).

The ability to recapitulate the antitumor effects of genetic deficiency in A2AR by pharmacologic maneuvers pointed to the feasibility and promise of the novel therapeutic approach of using small molecules to unleash the antitumor T cells from hypoxia–A2-adenosinergic inhibition (15).

Anti-hypoxia–A2-Adenosinergic Coadjuvants Enable the Antitumor Capacity of Current Cancer Immunotherapies

The pharmacologic inhibition of A2AR-mediated immunosuppressive signaling in T cells with synthetic and natural A2AR antagonists, or the pretreatment of tumor-reactive T cells with A2AR siRNA before adoptive transfer, led to much stronger antitumor effects of the transferred T cells or of the elicited endogenous antitumor immunity (12). This included reduced neovascularization of tumors, stronger rejection of lung metastases, and stronger inhibition of tumor growth. Similar increases in antitumor effects of transferred T cells are expected with the negatively selected $A2AR^{low}$ antitumor T cells that are more resistant to inhibition by adenosine (51).

The demonstrated ability of A2AR antagonists to interrupt the hypoxia → CD39/CD73 → $[adenosine]^{High}$ → A2A-adenosinergic signaling at the last stage and to unleash the antitumor effects (Fig. 2) provided additional pharmacologic confirmation *in vivo* of the role of A2AR in antitumor immunity. These data also provided a proof-of-principle (12) for the therapeutic use of novel immunologic coadjuvants to block physiologic negative regulators of antitumor immunity (Fig. 2; refs. 11, 12). Figure 2 shows different types of therapeutically feasible treatments that target the individual upstream and downstream stages of the hypoxia → CD39/CD73 → $[adenosine]^{High}$ → A2AR/A2BR → cAMP signaling to mitigate immunosuppression in the TME.

Inhibitors of cAMP-dependent PKA

PKA inhibitors were considered first as a potential approach to prevent the inhibition of antitumor T cells (23–29). However, these efforts were abandoned because such inhibitors likely have unacceptable side effects due to the crucial roles of the cAMP binding site and PKA in many fundamental biologic processes.

A2A adenosine receptor antagonists

In contrast with targeting PKA, a much more fruitful approach has been to block the intracellular cAMP-elevating high-affinity A2AR (30, 31) using synthetic or natural antagonists of these receptors. The biologic effects of antagonists of A2AR or A2BR are due to their competition with the tissue-generated, endogenous extracellular adenosine for binding to the same site on A2AR or A2BR. However, the receptor-bound antagonists do not trigger the accumulation of intracellular cAMP.

Thus, the binding of the antagonists to these adenosine receptors prevents the inhibition of T cells by adenosine. In addition, by blocking A2AR, it is expected that A2AR antagonists may also shorten the "memory" of exposure of T cells to immunosuppressive signaling through A2AR (36). Effects of A2AR antagonists are facilitated by the lack of spare A2AR (i.e., no "receptor reserve" in T cells; ref. 35), thereby allowing antagonists to further minimize the immunosuppressive effects of extracellular adenosine. The focus on high-affinity A2AR was because of its pattern of expression

(30–34) and because A2AR will likely be activated even at relatively modest increases in the levels of extracellular tissue adenosine.

Even the short-lived "first-generation" synthetic A2AR antagonists have demonstrated an increase in antitumor immunity *in vivo*, suggesting their use as coadjuvants in cancer immunotherapies (12). Subsequent extensive and well-controlled studies revealed the potent antitumor effects of longer-lived A2AR antagonists (52, 53) and provided a strong rationale for clinical trials of existing cancer immunotherapies in combination with the currently available synthetic A2AR antagonists (53).

The use of synthetic A2AR antagonists in combination with cancer immunotherapies was the much desirable outcome of fundamental studies of antipathogen immune responses and autoimmunity (11–18). That alone would be sufficient to justify the large-scale research and development of this class of synthetic drugs. Fortuitously, these drugs have been developed by neurobiologists because of the role of A2AR in the central nervous system and their original promise in slowing the progression of Parkinson disease.

Several synthetic A2AR antagonists have been shown to be safe in phase II and III clinical trials of Parkinson disease (30, 54, 55). One such A2AR antagonist, KW6002 (istradefyline), is approved for the treatment of patients with Parkinson disease in Japan. These drugs can be easily re-purposed and tested in combination with existing cancer immunotherapies. In contrast with the use of A2AR antagonists, considerations of the clinical utility of antagonists of A2BR are premature due to insufficient preclinical data, low affinity to adenosine, and potential cardiovascular side effects.

Extracellular adenosine-generating or -degrading enzymes

Approaches to target CD39 and CD73 ectoenzymes, which function in tandem to generate extracellular adenosine, have been developed in innovative research by Robson's research group in studies of CD39 (20, 56, 57), by Smyth and Stagg's research group (58–61), and by Zhang's research group (52, 62) in studies of CD73. Drugs such as adenosine deaminase (ADAGEN; Enzon) that degrade the accumulated extracellular adenosine in the TME (63), and drugs that inhibit the CD39/CD73 ectoenzyme-generated accumulation of extracellular adenosine in the TME, may provide yet another tool to inhibit the CD39/CD73 → A2A/A2B axis. Future studies may reveal relative advantages and disadvantages of using anti–CD39- or anti–CD73-blocking monoclonal antibody compared with small-molecule inhibitors to decrease the intratumoral levels of extracellular adenosine.

Inhibitors of TME hypoxia–HIF-1α

Drugs that inhibit hypoxia–HIF-1α signaling are in high demand due to the well-established understanding of the protumor effects of hypoxia (46) and HIF-1α (64). Promising inhibitors of HIF-1α, including digoxin and acriflavine (65, 66), were shown to decrease lung metastasis in an orthotopic breast cancer model. Other HIF-1α inhibitors such as sirtuin-7 and ganetespib, a new therapeutic candidate targeting triple-negative breast cancer cells, were also found to have antitumor activities (67, 68), and are candidates for testing as anti-hypoxia–A2-adenosinetgic immunologic coadjuvants.

Hypoxia–A2-Adenosinergic Immunosuppression in Human Cancers

The original observations of the critical role of hypoxia–A2-adenosinergic immunosuppression in tumor protection (12, 15) have been confirmed in extensive and well-controlled studies by several groups in different models of tumor rejection. These studies have looked at the effects of A2AR genetic deletion in mice, A2AR antagonists and/or genetic deletion or pharmacologic inhibition of upstream stages of adenosine generation by the CD39/CD73 ectoenzymes (52, 53, 60, 62, 69–71).

The most significant are clinical implications of the recent extensive analysis of gene-expression data from more than 6,000 samples of triple-negative breast cancers. These studies provided evidence for the correlation between (i) high levels of expression of extracellular adenosine-generating ectoenzyme CD73 on human triple-negative and chemotherapy-resistant breast cancers, (ii) the inhibition of antitumor T cells and NK cells by A2AR and A2BR, and (iii) the poor prognosis of patients with such tumors (61).

Anti-hypoxia–A2-Adenosinergic Coadjuvants May Also Block Other Immunosuppressive Pathways

The hypoxia–A2-adenosinergic pathway not only may be the oldest in evolution but also the most influential in recruiting other immunosuppressive pathways. Indeed, it is challenging to come up with older biochemical entities/parameters than the lack of oxygen (anoxia, hypoxia) or adenosine, as discussed in an earlier review (16). It was proposed and confirmed that A2AR and A2BR, CRE and HRE-mediated transcription, and HIF-1α have key roles in governing the functions of Tregs and effector cells (Fig. 1; ref. 16).

Thus, blocking hypoxia–A2-adnosinergic signaling should block at least partially many other immunosuppressive mechanisms. Indeed, it is already established that the hypoxia–A2-adenosinergic pathway also recruits other immunosuppressive molecules such as cyclooxygenase-2 and eicosanoid mediators (72–74). This pathway is also implicated in the development and functions of Tregs (16, 20, 75). Interestingly, both CD73-mediated generation of extracellular adenosine and A2AR were required for the suppressive effects of Tregs through a PD-1-dependent mechanism in the kidney ischemia–reperfusion injury model (76). It was also shown that the activation of A2AR recruited the negative immunologic regulators PD-1 and CTLA-4 on T cells (77).

Conclusions and Expectations

Hypoxia–A2-adenosinergic immunosuppression negates the antitumor effects of tumor-reactive T cells and NK cells. Published data and insights from yet to be published studies have identified this pathway as an important remaining barrier to more effective tumor rejection. Immunosuppressive

adenosine → A2AR/A2BR–mediated signaling can be weakened by anti-hypoxia–A2-adenosinergic coadjuvants, thereby further enhancing the antitumor potential of current cancer immunotherapies.

As depicted in Figs. 1 and 2 and reviewed here, the inhibition of hypoxia–A2-adenosinergic immunosuppression should improve the antitumor immunity of tumor-reactive T cells that have been induced by other immunotherapeutic protocols, including the mono- or dual immunotherapies with CTLA-4 or PD-1 blockade. This hypothesis was supported by recent observations of stronger antitumor effects of CTLA-4 or PD-1 blockade when combined with reducing levels of extracellular adenosine and inhibiting the adenosine → A2AR and A2BR signaling (53, 60). It was shown that inhibition of the accumulation of extracellular adenosine by anti-CD73 monoclonal antibody did indeed enhance the antitumor activity of dual CTLA-4 and PD-1 blockade in models of transplanted and chemically induced mouse tumors (60). It would be interesting to test whether the efficacy of A2AR antagonists could be further increased by lowering the concentration of extracellular adenosine in the TME by drugs that either inhibit the accumulation or induce the degradation of extracellular adenosine.

It must be emphasized that treatments with cancer vaccine–induced tumor-reactive T cells, adoptively transferred tumor-reactive T cells, or inhibitors of CTLA-4/PD-1, are highly complementary with anti-hypoxia–A2-adenosinergic coadjuvant treatments. Indeed, inhibition of all known immunologic negative regulators along with the depletion of Tregs will still leave T cells vulnerable to multifaceted and powerful immunosuppression by tumor hypoxia and A2AR that can be weakened by anti-hypoxia–A2-adenosinergic coadjuvants.

Finally, inhibitors of the hypoxia–A2-adenosinergic pathway may have additional favorable anti-immunosuppressive effects by decreasing the intensity of many other immunosuppressive mechanisms such as Tregs, CTLA-4, TGF-β, cyclooxygenase-2, and eicosanoid-mediator immunosuppression. This, in turn, may allow for treatment with lower therapeutic levels of the checkpoint inhibitors of CTLA-4 or PD-1, thereby decreasing the treatment and immune-related side effects. Taken together, the available data strongly justify targeting the hypoxia → adenosine →A2AR/A2BR pathway to prevent the inhibition of antitumor T cells and NK cells in the TME.

Disclosure of Potential Conflicts of Interest

M. Sitkovsky is the founder and president of Redoxtherapies, Inc., has ownership interest (including patents) in a U.S. patent, and is a consultant/advisory board member for NewVac. A. Ohta has ownership interest in a patent. No potential conflicts of interest were disclosed by the other authors.

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References

1. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer 2012;12:237–51.
2. Barrett DM, Singh N, Porter DL, Grupp SA, June CH. Chimeric antigen receptor therapy for cancer. Annu Rev Med 2014;65:333–47.
3. Ramos CA, Narala N, Vyas GM, Leen AM, Gerdemann U, Sturgis EM, et al. Human papillomavirus type 16 E6/E7-specific cytotoxic T lymphocytes for adoptive immunotherapy of HPV-associated malignancies. J Immunother 2013;36:66–76.
4. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 2012;12:269–81.
5. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711–23.
6. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443–54.
7. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.
8. Bakacs T, Mehrishi JN, Moss RW. Ipilimumab (Yervoy) and the TGN1412 catastrophe. Immunobiology 2012;217:583–9.
9. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013;369:122–33.
10. Curran MA, Callahan MK, Subudhi SK, Allison JP. Response to "Ipilimumab (Yervoy) and the TGN1412 catastrophe." Immunobiology 2012;217:590–2.
11. Ohta A, Sitkovsky M. Role of G-protein–coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature 2001;414:916–20.
12. Ohta A, Gorelik E, Prasad SJ, Ronchese F, Likashev D, Wong MK, et al. A2A adenosine receptor protects tumors from antitumor T cells. Proc Natl Acad Sci U S A 2006;103:13132–7.
13. Sitkovsky MV, Lukashev D, Apasov S, Kojima H, Koshiba M, Caldwell C, et al. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. Annu Rev Immunol 2004;22:657–82.
14. Sitkovsky M, Lukashev D. Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors. Nat Rev Immunol 2005;5:712–21.
15. Sitkovsky MV, Kjaergaard J, Lukashev D, Ohta A. Hypoxia-adenosinergic immunosuppression: tumor protection by T regulatory cells and cancerous tissue hypoxia. Clin Cancer Res 2008;14:5947–52.
16. Sitkovsky MV. T regulatory cells: hypoxia-adenosinergic suppression and re-direction of the immune response. Trends Immunol 2009;30:102–8.
17. Eltzschig HK, Sitkovsky MV, Robson SC. Purinergic signaling during inflammation. N Engl J Med 2013;368:1260.
18. Sitkovsky MV, Ohta A. The 'danger' sensors that STOP the immune response: the A2 adenosine receptors? Trends Immunol 2005;26:299–304.
19. Hellstrom I, Hellstrom KE, Pierce GE, Yang JP. Cellular and humoral immunity to different types of human neoplasms. Nature 1968;220:1352–4.
20. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 2007;204:1257–65.
21. Ohta A, Kini R, Subramanian M, Madasu M, Sitkovsky M. The development and immunosuppressive functions of CD4(−) CD25(−) FoxP3

(–) regulatory T cells are under influence of the adenosine-A2A adenosine receptor pathway. Front Immunol 2012;3:190.
22. Thammavongsa V, Kern JW, Missiakas DM, Schneewind O. Staphylococcus aureus synthesizes adenosine to escape host immune responses. J Exp Med 2009;206:2417–27.
23. Takayama H, Sitkovsky MV. Antigen receptor–regulated exocytosis in cytotoxic T lymphocytes. J Exp Med 1987;166:725–43.
24. Trenn G, Takayama H, Sitkovsky MV. Antigen-receptor regulated exocytosis of cytolytic granules may not be required for target cell lysis by cytotoxic T lymphocytes. Nature 1987;330:72–4.
25. Sitkovsky MV. Mechanistic, functional and immunopharmacological implications of biochemical studies of antigen receptor-triggered cytolytic T-lymphocyte activation. Immunol Rev 1988;103:127–60.
26. Sitkovsky MV, Trenn G, Takayama H. Cyclic AMP-dependent protein kinase as a part of the possible down-regulating pathway in the antigen receptor-regulated cytotoxic T lymphocyte conjugate formation and granule exocytosis. Ann N Y Acad Sci 1988;532:350–8.
27. Takayama H, Sitkovsky MV. Potential use of antagonists of cAMP-dependent protein kinase to block inhibition and modulate T-cell receptor–triggered activation of cytotoxic T-lymphocytes. J Pharm Sci 1988;78:8–10.
28. Takayama H, Trenn G, Sitkovsky MV. Locus of inhibitory action of cAMP-dependent protein kinase in the antigen-receptor triggered cytotoxic T-lymphocyte activation pathway. J Biol Chem 1988;263: 2330–6.
29. Bjorgo E, Moltu K, Tasken K. Phosphodiesterases as targets for modulating T-cell responses. Handb Exp Pharmacol 2011:345–63.
30. Fredholm BB, AP IJ, Jacobson KA, Linden J, Muller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update. Pharmacol Rev 2011;63:1–34.
31. Chen JF, Eltzschig HK, Fredholm BB. Adenosine receptors as drug targets—what are the challenges? Nat Rev Drug Discov 2013;12: 265–86.
32. Huang S, Koshiba M, Apasov S, Sitkovsky M. Role of A2a adenosine receptor-mediated signaling in inhibition of T cell activation and expansion. Blood 1997;90:1600–10.
33. Apasov SG, Koshiba M, Chused TM, Sitkovsky MV. Effects of extracellular ATP and adenosine on different thymocyte subsets: possible role of ATP-gated channels and Gprotein-coupled purinergic receptors. J Immunol 1997;158:5095–105.
34. Koshiba M, Rosin DL, Hayashi N, Linden J, Sitkovsky MV. Patterns of A2A extracellular adenosine receptor expression in different functional subsets of human peripheral T cells. Flow cytometry studies with anti-A2A receptor monoclonal antibodies. Mol Pharmacol 1999;55:614–24.
35. Armstrong JM, Chen JF, Schwarzschild MA, Apasov S, Smith PT, Caldwell C, et al. Gene dose effect reveals no Gs-coupled A2A adenosine receptor reserve in murine T-lymphocytes: studies of cells from A2A-receptor-gene-deficient mice. Biochem J 2001;354:123–30.
36. Koshiba M, Kojima H, Huang S, Apasov S, Sitkovsky MV. Memory of extracellular adenosine/A2a purinergic receptor-mediated signalling in murine T cells. J Biol Chem 1997;272:25881–9.
37. Eckle T, Krahn T, Grenz A, Kohler D, Mittelbronn M, Ledent C, et al. Cardioprotection by ecto-5′-nucleotidase (CD73) and A2B adenosine receptors. Circulation 2007;115:1581–90.
38. Eckle T, Fullbier L, Wehrmann M, Khoury J, Mittelbronn M, Ibla J, et al. Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury. J Immunol 2007;178:8127–37.
39. Colgan SP, Eltzschig HK, Eckle T, Thompson LF. Physiological roles for ecto-5′-nucleotidase (CD73). Purinergic Signal 2006;2:351–60.
40. Antonioli L, Pacher P, Vizi ES, Hasko G. CD39 and CD73 in immunity and inflammation. Trends Mol Med 2013;19:355–67.
41. Kojima H, Gu H, Nomura S, Caldwell CC, Kobata T, Carmeliet P, et al. Abnormal B lymphocyte development and autoimmunity in hypoxia-inducible factor 1alpha-deficient chimeric mice. Proc Natl Acad Sci U S A 2002;99:2170–4.
42. Thiel M, Caldwell CC, Kreth S, Kuboki S, Chen P, Smith P, et al. Targeted deletion of HIF-1alpha gene in T cells prevents their inhibition in hypoxic inflamed tissues and improves septic mice survival. PLoS ONE 2007;2:e853.
43. Thiel M, Chouker A, Ohta A, Jackson E, Caldwell C, Smith P, et al. Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury. PLoS Biol 2005;3:e174.
44. Synnestvedt K, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, et al. Ecto-5′-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. J Clin Invest 2002;110:993–1002.
45. Hart ML, Grenz A, Gorzolla IC, Schittenhelm J, Dalton JH, Eltzschig HK. Hypoxia-inducible factor-1alpha-dependent protection from intestinal ischemia/reperfusion injury involves ecto-5′-nucleotidase (CD73) and the A2B adenosine receptor. J Immunol 2011;186:4367–74.
46. Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nat Rev Cancer 2008;8:425–37.
47. Sitkovsky MV. Damage control by hypoxia-inhibited AK. Blood 2008;111:5424–5.
48. Blay J, White TD, Hoskin DW. The extracellular fluid of solid carcinomas contains immunosuppressive concentrations of adenosine. Cancer Res 1997;57:2602–5.
49. Hoskin DW, Butler JJ, Drapeau D, Haeryfar SM, Blay J. Adenosine acts through an A3 receptor to prevent the induction of murine anti-CD3–activated killer T cells. Int J Cancer 2002;99:386–95.
50. Williams BA, Manzer A, Blay J, Hoskin DW. Adenosine acts through a novel extracellular receptor to inhibit granule exocytosis by natural killer cells. Biochem Biophys Res Commun 1997;231:264–9.
51. Ohta A, Kjaergaard J, Sharma S, Mohsin M, Goel N, Madasu M, et al. *In vitro* induction of T cells that are resistant to A2 adenosine receptor-mediated immunosuppression. Br J Pharmacol 2009;156:297–306.
52. Jin D, Fan J, Wang L, Thompson LF, Liu A, Daniel BJ, et al. CD73 on tumor cells impairs antitumor T-cell responses: a novel mechanism of tumor-induced immune suppression. Cancer Res 2010; 70:2245–55.
53. Beavis PA, Divisekera U, Paget C, Chow MT, John LB, Devaud C, et al. Blockade of A2A receptors potently suppresses the metastasis of $CD73^{+}$ tumors. Proc Natl Acad Sci U S A 2013;110:14711–6.
54. Pinna A. Novel investigational adenosine A2A receptor antagonists for Parkinson's disease. Expert Opin Investig Drugs 2009;18:1619–31.
55. Jacobson KA. Introduction to adenosine receptors as therapeutic targets. Handb Exp Pharmacol 2009:1–24.
56. Feng L, Sun X, Csizmadia E, Han L, Bian S, Murakami T, et al. Vascular CD39/ENTPD1 directly promotes tumor cell growth by scavenging extracellular adenosine triphosphate. Neoplasia 2011;13:206–16.
57. Kunzli BM, Bernlochner MI, Rath S, Kaser S, Csizmadia E, Enjyoji K, et al. Impact of CD39 and purinergic signalling on the growth and metastasis of colorectal cancer. Purinergic Signal 2011;7:231–41.
58. Stagg J, Divisekera U, Duret H, Sparwasser T, Teng MW, Darcy PK, et al. CD73-deficient mice have increased antitumor immunity and are resistant to experimental metastasis. Cancer Res 2011;71:2892–900.
59. Stagg J, Beavis PA, Divisekera U, Liu MC, Moller A, Darcy PK, et al. CD73-deficient mice are resistant to carcinogenesis. Cancer Res 2012;72:2190–6.
60. Allard B, Pommey S, Smyth MJ, Stagg J. Targeting CD73 enhances the antitumor activity of anti-PD-1 and anti-CTLA-4 mAbs. Clin Cancer Res 2013;19:5626–35.
61. Loi S, Pommey S, Haibe-Kains B, Beavis PA, Darcy PK, Smyth MJ, et al. CD73 promotes anthracycline resistance and poor prognosis in triple negative breast cancer. Proc Natl Acad Sci U S A 2013;110: 11091–6.
62. Zhang B. CD73: a novel target for cancer immunotherapy. Cancer Res 2010;70:6407–11.
63. Hershfield MS, Buckley RH, Greenberg ML, Melton AL, Schiff R, Hatem C, et al. Treatment of adenosine deaminase deficiency with polyethylene glycol-modified adenosine deaminase. N Engl J Med 1987;316: 589–96.
64. Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell 2012;148:399–408.
65. Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, et al. Digoxin and other cardiac glycosides inhibit HIF-1alpha synthesis and block tumor growth. Proc Natl Acad Sci U S A 2008;105:19579–86.

66. Wong CC, Zhang H, Gilkes DM, Chen J, Wei H, Chaturvedi P, et al. Inhibitors of hypoxia-inducible factor 1 block breast cancer metastatic niche formation and lung metastasis. J Mol Med 2012;90:803–15.
67. Hubbi ME, Hu H, Kshitiz, Gilkes DM, Semenza GL. Sirtuin-7 inhibits the activity of hypoxia-inducible factors. J Biol Chem 2013;288:20768–75.
68. Xiang L, Gilkes DM, Chaturvedi P, Luo W, Hu H, Takano N, et al. Ganetespib blocks HIF-1 activity and inhibits tumor growth, vascularization, stem cell maintenance, invasion, and metastasis in orthotopic mouse models of triple-negative breast cancer. J Mol Med 2014; 92:151–64.
69. Allard B, Turcotte M, Spring K, Pommey S, Royal I, Stagg J. Anti-CD73 therapy impairs tumor angiogenesis. Int J Cancer 2014; 134:1466–73.
70. Zarek PE, Huang CT, Lutz ER, Kowalski J, Horton MR, Linden J, et al. A2A receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. Blood 2008;111:251–9.
71. Waickman AT, Alme A, Senaldi L, Zarek PE, Horton M, Powell JD. Enhancement of tumor immunotherapy by deletion of the A2A adenosine receptor. Cancer Immunol Immunother 2012;61: 917–26.
72. Pouliot M, Fiset ME, Masse M, Naccache PH, Borgeat P. Adenosine up-regulates cyclooxygenase-2 in human granulocytes: impact on the balance of eicosanoid generation. J Immunol 2002;169:5279–86.
73. Cadieux JS, Leclerc P, St-Onge M, Dussault AA, Laflamme C, Picard S, et al. Potentiation of neutrophil cyclooxygenase-2 by adenosine: an early anti-inflammatory signal. J Cell Sci 2005; 118:1437–47.
74. McColl SR, St-Onge M, Dussault AA, Laflamme C, Bouchard L, Boulanger J, et al. Immunomodulatory impact of the A2A adenosine receptor on the profile of chemokines produced by neutrophils. FASEB J 2006;20:187–9.
75. Sitkovsky M, Lukashev D, Deaglio S, Dwyer K, Robson SC, Ohta A. Adenosine A2A receptor antagonists: blockade of adenosinergic effects and T regulatory cells. Br J Pharmacol 2008;153(Suppl 1): S457–64.
76. Li L, Huang L, Ye H, Song SP, Bajwa A, Lee SJ, et al. Dendritic cells tolerized with adenosine A2AR agonist attenuate acute kidney injury. J Clin Invest 2012;122:3931–42.
77. Sevigny CP, Li L, Awad AS, Huang L, McDuffie M, Linden J, et al. Activation of adenosine 2A receptors attenuates allograft rejection and alloantigen recognition. J Immunol 2007;178:4240–9.
78. Kobie JJ, Shah P, Yang L, Rebhahn A, Fowell DJ, Mosmann TR. T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5′-adenosine monophosphate to adenosine. J Immunol 2006;177:6780–6.
79. Wen AY, Sakamoto KM, Miller LS. The role of the transcription factor CREB in immune function. J Immunol 2010;185:6413–9.