Inhibition of T cell and natural killer cell function by adenosine and its contribution to immune evasion by tumor cells (Review)

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Abstract. The resistance of many human cancers to immune-based therapies, including adoptive immunotherapy and the administration of therapeutic cancer vaccines, has been attributed to tumor-associated immune suppression, due in part to immunosuppressive molecules located within the tumor microenvironment. Adenosine is a purine nucleoside found within the interstitial fluid of solid tumors at concentrations that are able to inhibit cell-mediated immune responses to tumor cells. It is well established that extracellular adenosine inhibits T lymphocyte activation and effector function, including T cell adhesion to tumor cells and cytotoxic activity, by signaling primarily through A_{2a} and A_3 adenosine receptors on the surface of T cells. Importantly, A_{2a} adenosine receptor-deficient mice exhibit enhanced anti-tumor immune responses by $CD8^+$ T cells, as well as a dramatic reduction in the growth of experimental tumors in comparison to wild-type controls. A_{2a} adenosine receptor signaling has also been implicated in adenosine-mediated inhibition of cytokine production and cytotoxic activity by activated natural killer (NK) cells, although the process of NK cell granule exocytosis is apparently suppressed via a distinct and as yet uncharacterized adenosine receptor. In this report, we review the evidence that adenosine is a potent inhibitor of cellular immune responses and may therefore be a major barrier to the successful immunotherapy of human carcinomas. The signaling pathways through which adenosine exerts its inhibitory effects on cell-mediated immune responses are also discussed. The accumulated evidence suggests that the effectiveness of immune-based therapies for solid tumors may be enhanced by selective antagonism of the adenosine receptor subtypes through which adenosine inhibits the anti-tumor activity of T lymphocytes and NK cells.

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1. Introduction: tumor evasion of immune-based therapies

Recent advances in our understanding of cell-mediated immune responses against tumor cells have spurred the development of immunotherapeutic strategies as a possible adjunct to, or even a replacement for, conventional surgical, radiotherapeutic and/or drug-based approaches to the treatment of advanced cancers. Dramatic results from preclinical research in animal models of cancer suggest that both cell-based and peptide-based cancer vaccines should evoke potent tumor-specific cellular immune responses, leading to complete tumor regression in cancer patients (1-4). Unfortunately, subsequent clinical trials have revealed that the efficacy of existing therapeutic cancer vaccines is at best limited and appears to be restricted to certain types of tumors (5-7). These less than optimal clinical outcomes echo the disappointing clinical results of earlier attempts at cancer immunotherapy using the adoptive transfer of activated natural killer (NK) cells (8). The refractory nature of many solid tumors to cellular immune responses has been attributed to a spectrum of tumor immune evasion mechanisms that include tumor-associated immune suppression, which has been well documented in both tumor-bearing animals and cancer patients (9-11). A great deal of attention has recently been focused on the role of regulatory T cells and myeloid suppressor cells as mediators of tumor-associated immune

suppression (12-14). However, tumor cells themselves can suppress tumor-specific cellular immune responses through the production of immunosuppressive cytokines that include transforming growth factor-ß (TGF-ß) and interleukin (IL)-10 (15,16), tumor necrosis factor (TNF) family ligands such as Fas ligand (17) and small inhibitory molecules like prostaglandin E_2 (18). The purine nucleoside adenosine is also present at immunosuppressive concentrations within the solid tumor microenvironment and may therefore be an important factor in immune evasion by tumor cells (19,20). In support of this, mutant mice that lack the A_{2a} adenosine receptor subtype through which adenosine mediates at least some of its immunosuppressive activity exhibit increased $CD8^+$ T cell-mediated anti-tumor immune responses and reduced growth of experimental tumors in comparison to wild-type mice (20). This review will focus on the potential impact of tumor-elaborated adenosine on the function of T lymphocytes and NK cells that mediate anti-tumor immune responses. Although the topic is beyond the scope of this review, adenosine also modulates the growth of tumor cells; however, controversy presently exists as to whether the predominant effect is one of growth inhibition or increased proliferation (21-23).

2. Adenosine production in the tumor microenvironment

The disordered growth of an expanding carcinoma often outstrips the development of a supportive vascular bed, which leads to a reduction in oxygen levels throughout much of the tumor mass (24,25). For example, the hypoxic fraction in squamous cell carcinomas of the cervix and head and neck can be as high as 20-32% (26). Adenosine is present at elevated levels in hypoxic tissues because of increased intracellular adenosine production and release from the cells. This is the result of oxygen deprivation and cellular ATP depletion (27) by activation of the 5'-nucleotidase pathway (28) and inhibition of adenosine kinase (29). Bidirectional equilibrative nucleoside transporters in the membrane are responsible for exporting intracellular adenosine to the extracellular compartment (30). As expected, hypoxia has been shown to stimulate adenosine production in cultures of 3LL Lewis lung carcinoma cells (31). Moreover, *in situ* microdialysis shows that extracellular concentrations of adenosine in mouse and human colorectal carcinomas are 10-20-fold higher than those measured in surrounding normal tissue (19). Elevated levels of extracellular adenosine in a solid tumor microenvironment have recently been confirmed by another laboratory (20). It is important to note that extracellular adenosine levels in solid tumors can be further supplemented or modified by ecto-enzymes that mediate adenosine production or degradation at the cell surface. Adenosine-producing ecto-enzymes that are expressed by both lymphocytes and cancer cells include NTPDase 1 (CD39) (32,33) and ecto-5'-nucleotidase (CD73) (34,35). Adenosine levels can be modulated through dipeptidyl peptidase IV/CD26 (36-38), which is the binding protein for adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4) (39). Importantly, adenosine down-regulates dipeptidyl peptidase IV/CD26 expression and subsequent ADA binding by colorectal carcinoma cells (37), which may result in a further increase in extracellular adenosine in certain solid tumors. An additional possible level of control results from the ability of ADA to bind A_{2b} adenosine receptors on lymphocytes (40) and A_1 adenosine receptors on non-lymphoid cells (41).

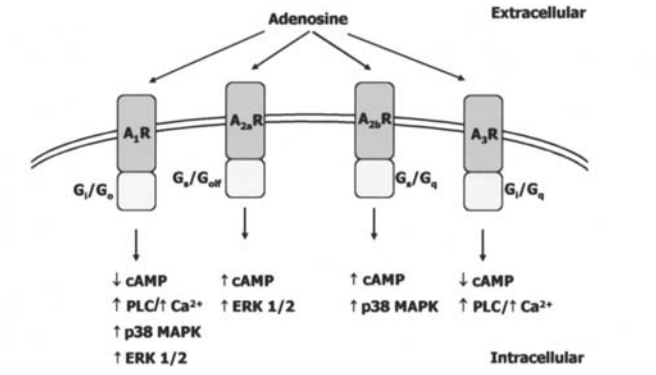

Figure 1. Second messenger pathways coupled to adenosine receptor subtypes. Extracellular adenosine can bind to 4 different G-protein-coupled receptor (R) structures that either stimulate ($A_{2a}R$ and $A_{2b}R$) or inhibit (A_1R and A_3R) adenylyl cyclase activity (42-47). Stimulation of A_1R and A_3R can also activate phospholipase C (PLC) with the consecutive release of Ca^{2+} from intracellular stores. In addition, all adenosine receptor subtypes couple to mitogen-activated protein kinase (MAPK) pathways, including extracellular signal-regulated kinase (ERK) 1/2 and p38 MAPK pathways (46,48).

3. Adenosine receptor subtypes and their expression by lymphocytes

Adenosine interacts with cell-surface adenosine receptors on T lymphocytes and NK cells that mediate cellular immune responses to tumor cells. There are currently 4 clearly defined adenosine receptor subtypes (A_1, A_{2a}, A_{2b} and A_3) that belong to the G-protein-coupled seven-transmembrane family of cell-surface receptors (42-44). These adenosine receptors have been extensively characterized on the basis of amino acid sequence, receptor affinity for selective ligands, and second messengers triggered downstream of receptor activation. A_1 and A_{2a} adenosine receptors (K_D for adenosine, ~10^{-8} to 10^{-7} M) exhibit higher relative affinities for adenosine than A_{2b} and A_3 adenosine receptors (K_D for adenosine, ~10^{-6} to 10^{-5} M) (43,45). As shown in Fig. 1, adenosine receptor subtypes are coupled to different combinations of G-protein family members: A_1 adenosine receptors to G_i/G_o, A_{2a} adenosine receptors to G_s/G_{olf}, A_{2b} adenosine receptors to G_s/G_q, and A_3 adenosine receptors to G_i/G_q (44,46,47). Stimulation of A_{2a} and A_{2b} adenosine receptors activates adenylyl cyclase, leading to elevated cellular cyclic AMP (cAMP) levels, whereas stimulation of A_1 and A_3 adenosine receptors inhibits adenylyl cyclase, resulting in a reduction in cellular cAMP levels. A_1 and A_3 adenosine receptor signaling can also activate phospholipase C and cause Ca^{2+} to be released from intracellular stores. In addition, all adenosine receptor subtypes have been shown to couple to mitogen-activated protein kinase (MAPK) pathways (46,48).

Expression of different adenosine receptor subtypes on the surface of lymphocytes involved in tumor-specific

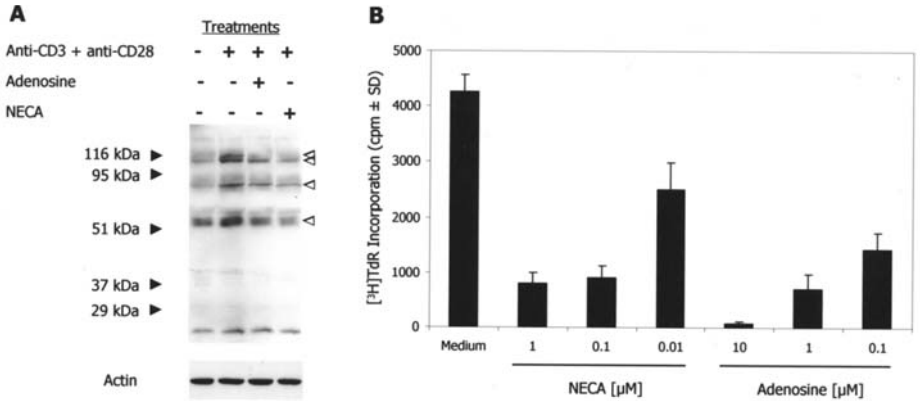

Figure 2. Inhibitory effect of adenosine on tyrosine phosphorylation and T cell proliferation. (A) Splenic T cells (purified using commercial T cell enrichment columns) from adult C57BL/6 mice were treated with medium alone, 10 μM 5'-*N*-ethylcarboxamidoadenosine (NECA), or 10 μM adenosine (plus 2.5 μM coformycin to inhibit endogenous ADA) for 15 min prior to stimulation with anti-CD3 and anti-CD28 monoclonal antibodies immobilized on 10 micron polystyrene beads (T cell to bead ratio of 2:1). After 15-min stimulation, T cells were pelleted and lysed. Ten microgram samples of cell lysate were run on a 12% SDS-polyacrylamide gel, blotted onto a nitrocellulose membrane, and probed with anti-phosphotyrosine monoclonal antibody followed by visualization using ECL reagents. The blot was then stripped and probed for actin to confirm equal loading of protein in each lane of the gel. (B) Splenic C57BL/6 T cells were added to wells of a 96-well microtitre plate, combined in a 2:1 ratio with 10 micron polystyrene beads coated with anti-CD3 and anti-CD28 monoclonal antibodies, and cultured in the absence (medium) or presence of the indicated concentrations of NECA or adenosine (plus 2.5 μM coformycin). After 42 h of culture, T cells were pulsed with 0.5 μCi tritiated thymidine ([^{3}H]TdR) and 6 h later well contents were harvested onto glass fibre mats. [^{3}H]TdR incorporation into the DNA of proliferating T cells was determined by liquid scintillation counting. Data are expressed as mean counts per minute (cpm) of quadruplicate cultures ± the standard deviation (SD).

immune responses, as well as the relative affinities of these adenosine receptor subtypes for adenosine, dictate the effect that extracellular adenosine will have on lymphocyte function. Mouse T lymphocytes express mRNA transcripts coding for A_{2a}, A_{2b} and A_3 adenosine receptors (49-51), with A_{2a} adenosine receptor expression being the most abundant (50). In contrast, little if any A_1 adenosine receptor-encoding mRNA is present in mouse T cells (49-51), although a low level of A_1 adenosine receptor expression has been reported in the mouse thymus (50). A_{2a}, A_{2b} and A_3 adenosine receptors are also expressed by human T lymphocytes (52-54); A_1 adenosine receptor expression has not yet been examined on human T cells. It is noteworthy that adenosine receptor subtype expression by T lymphocytes can be modulated by T cell receptor signaling. In the mouse system, A_{2a} adenosine receptor mRNA expression becomes elevated in CD4$^+$ T cells following their activation (55). Similarly, activated human T lymphocytes exhibit elevated A_{2a}, as well as A_{2b} and A_3 adenosine receptor expression (52-54). Activated tumor-specific T cells that migrate into the tumor microenvironment may therefore have increased sensitivity to adenosine-mediated immune suppression. Less is known about adenosine subtype receptor expression by NK cells, although differential responses to adenosine subtype receptor-selective agonists suggest the presence of A_1, A_{2a} and, possibly, A_{2b} adenosine receptors on murine NK cells (31,56). The A_3 adenosine receptor may also be expressed on mouse NK cells since oral administration of an A_3 adenosine receptor-selective agonist leads to enhanced NK cell activity (57); however, confirmation at the mRNA or protein level is required because increased IL-12 production by dendritic cells in response to the A_3 adenosine receptor-selective agonist could account for the observed increase in NK cell activity. Adenosine receptor subtype expression by human NK cells has not yet been investigated.

4. Adenosine inhibition of T lymphocyte function

CD4$^+$ and CD8$^+$ T lymphocytes that recognize major histocompatibility class (MHC) II- and I-restricted tumor-associated antigens, respectively, have been identified in the circulation of cancer patients, as well as in the tumor microenvironment (58-60). CD4$^+$ Th1 cells are an important source of the type 1 cytokines that drive anti-tumor immune responses whereas CD8$^+$ cytotoxic T lymphocytes (CTL) eliminate tumor cells via granule-dependent and -independent cytotoxicity (61,62). On the other hand, the inhibitory activity of CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells has been implicated in tumor evasion of immune responses (12,14). Interestingly, one mechanism by which CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells have been suggested to suppress T cell responses is through extracellular adenosine production catalyzed sequentially by CD39 and CD73 co-expressed on the surface of regulatory T cells (63). Tumor-associated extracellular adenosine is predicted to affect cytokine production and cytotoxic effector function by tumor-infiltrating T lymphocytes because CD4$^+$ and CD8$^+$ T cells express each of the adenosine receptor subtypes except A_1 (see previous section). Indeed, adenosine potently inhibits a wide range of T lymphocyte responses to antigenic stimulation, including cellular proliferation (49,64), synthesis of IL-2 and proinflammatory cytokines such as interferon γ and TNF α (55,65,66), up-regulation of CD25 (IL-2 receptor α chain) (64,65), expression of cytotoxic effector molecules such as perforin and Fas ligand (49,67), CTL adhesion to tumor target cells (68,69) and granule exocytosis by CTL (67). In fact, adenosine inhibits some of the earliest steps in T cell activation associated with signal transduction through the T cell receptor and costimulatory CD28 molecules. As shown in Fig. 2A, low micromolar concentrations of adenosine or 5'-*N*-ethylcarboxamidoadenosine (NECA), a non-selective adenosine receptor agonist, inhibited T cell receptor/CD3-

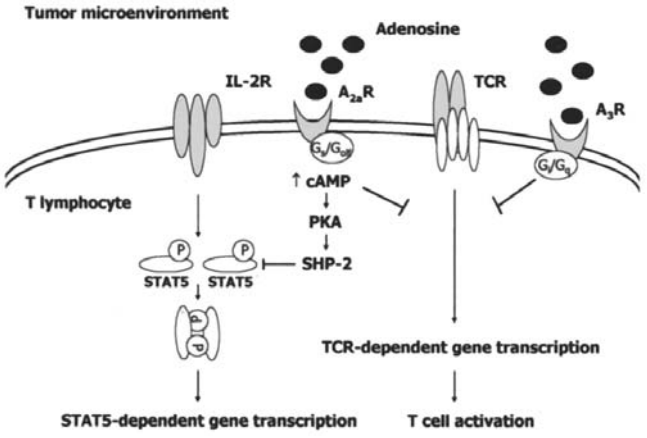

Figure 3. Inhibitory effect of adenosine on T cell signaling pathways. T cell proliferation and differentiation is initiated by T cell receptor (TCR) signaling and the subsequent transcription of genes involved in T cell activation, such as those coding for c-myc, interferon γ, IL-2, and CD25 (71). Clonal expansion of T cells and the expression of effector molecules such as perforin involve IL-2 receptor (IL-2R) signaling and the subsequent tyrosine phosphorylation and activation of the transcription factor STAT5 (72,73). TCR-dependent gene transcription is suppressed following adenosine stimulation of A_{2a} and A_3 adenosine receptor (R) subtypes (49,55,64,66,74). Adenosine also interferes with IL-2-driven T cell expansion (51,74). $A_{2a}R$ (and $A_{2b}R$) stimulation induces the cAMP- and protein kinase A (PKA)-dependent tyrosine phosphatase SHP-2, which interferes with IL-2R signaling by dephosphorylating and inactivating STAT5 (51).

and CD28-induced phosphorylation of tyrosine residues on intracellular mouse T cell proteins with molecular weights corresponding to the protein tyrosine kinases $p56^{lck}$ (56 kDa) and ZAP-70 (70 kDa), which are essential components of the T cell receptor signal transduction pathway (70). DNA synthesis by T cells was also suppressed in parallel with reduced tyrosine phosphorylation of intracellular mouse T cell proteins in the presence of adenosine or NECA (Fig. 2B).

Studies performed in the mouse system using adenosine receptor subtype-selective agonists and antagonists indicate that adenosine inhibits T cell activation and effector function by signaling primarily through A_{2a} and A_3 adenosine receptors (Fig. 3); however, different aspects of T cell activation and effector function appear to be affected by A_{2a} and A_3 adenosine receptor stimulation (49,51,55,64,67,68). For example, A_{2a} adenosine receptor signaling suppresses CD25 and cytotoxic effector molecule expression (64,67), whereas A_3 adenosine receptor signaling inhibits T cell proliferation in response to T cell receptor stimulation (49), as well as the adhesion of activated T cells to syngeneic adenocarcinoma cells (68). Adenosine also acts through the A_{2a} adenosine receptor to prevent IL-2 and TNF α secretion by mouse type 1 and type 2 $CD8^+$ CTL, while maintaining interferon γ production at levels similar to control cells (74). By inhibiting IL-2 production, tumor-associated adenosine prevents the clonal expansion of activated tumor antigen-specific T cells, while inhibition of the secretion of TNF α, which is a major proinflammatory cytokine, results in reduced protective inflammation at the tumor site. Importantly, antagonism of the A_{2a} adenosine receptor or siRNA-mediated down-regulation of A_{2a} adenosine receptor expression enhances the ability of $CD8^+$ T cells to retard tumor growth (20). It is also important to note that A_{2a} adenosine receptor stimulation has different effects on $CD8^+$ and $CD4^+$ T cells since, unlike the effect on $CD8^+$ T cells (74), signaling through this receptor blocks interferon γ secretion by murine $CD4^+$ T cells (55).

The inhibitory effect of A_{2a} adenosine receptor signaling on some aspects of T cell function is caused by adenylyl cyclase-mediated accumulation of cAMP (55,64,67). A recent study suggests that activation of protein kinase A type I via the A_{2a} adenosine receptor is also involved in adenosine-mediated inhibition of cytokine production and cytotoxicity by T cells (66). A_{2a}, as well as A_{2b}, adenosine receptor signaling also activates the protein tyrosine phosphatase SHP-2, which results in the dephosphorylation of IL-2 receptor-associated STAT5 and impaired signal transduction through the high affinity IL-2 receptor of T cells (51). Thus, elevated extracellular adenosine in the tumor microenvironment has the potential to inhibit both IL-2 production and utilization by tumor-infiltrating T lymphocytes (51,65). In contrast to the well described mechanisms by which A_{2a} adenosine receptor signaling blocks T cell activation and effector function, little is known about the mechanism of A_3 adenosine receptor-mediated T cell inhibition. Moreover, while the importance of A_{2a} adenosine receptor signaling in adenosine-mediated suppression of T cell responses has been confirmed using A_{2a} adenosine receptor-deficient mice (50), similar confirmatory studies have not yet been performed with A_3 adenosine receptor-deficient mice.

T cell receptor-driven activation of $CD4^+$ T helper cells requires the participation of antigen-presenting cells (APCs) such as dendritic cells, macrophages, and B lymphocytes, which present antigenic peptides to $CD4^+$ T cells in the context of MHC class II molecules and provide an essential source of costimulation for T cells (75,76). It is likely that tumor-associated adenosine indirectly prevents the activation of tumor-specific $CD4^+$ T cells by interfering with APC function. Mature human dendritic cells express A_{2a} adenosine receptors through which adenosine suppresses IL-12 production (77). Adenosine also inhibits IL-12 synthesis by mouse macrophages via A_{2a} adenosine receptor-dependent and -independent mechanisms (78). Since IL-12 plays a critical role in the development of Th1 cells (79), diminished IL-12 synthesis by dendritic cells and macrophages in the presence of extracellular adenosine is predicted to interfere with the induction of Th1-dependent cell-mediated immune responses to tumor cells. Another means by which adenosine might interfere with the induction of a strong Th1-directed anti-tumor immune response is by enhancing dendritic cell secretion of anti-inflammatory IL-10, which has a negative effect on Th1 cell development (80). Furthermore, the adenosine analogue NECA inhibits the expression of CCR5, MIP-3ß/CCL19, and MDR-1 by mature human dendritic cells and retards the migration of mouse dendritic cells to draining lymph nodes (81). Tumor-associated adenosine might therefore prevent dendritic cells from promoting T cell-mediated immune responses. As shown in Fig. 4A, expression of costimulatory molecules by APCs is also

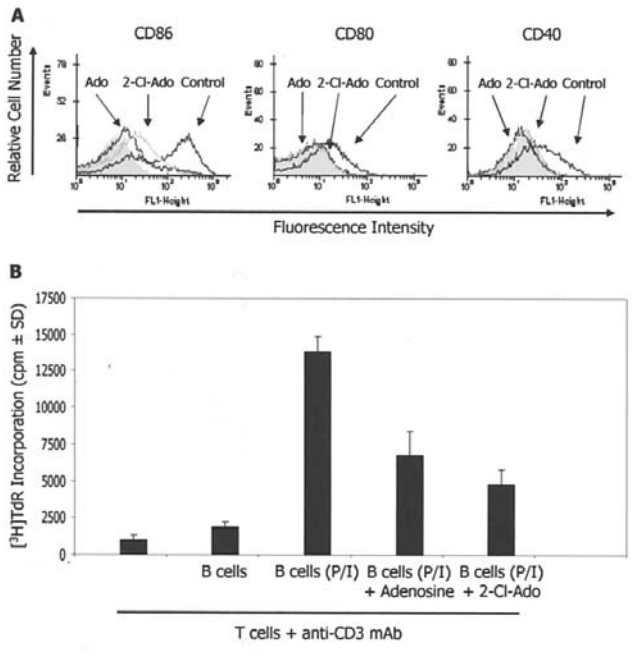

Figure 4. Adenosine diminishes the costimulatory capacity of B cells. Splenic B cells (enriched by sequential antibody depletion of T cells and macrophages) from adult C57BL/6 mice were stimulated with 10 ng/ml phorbol 12-myristate 13-acetate (P) and 100 ng/ml ionomycin (I) for 24 h in the absence (control) or presence of 10 μM 2-chloro-adenosine (2-Cl-Ado) or 10 μM adenosine (Ado; plus 2.5 μM coformycin to inhibit endogenous ADA). B cells were then harvested and washed extensively prior to use in experiments. (A) B lymphocytes were stained for CD86, CD80, and CD40 expression using fluorochrome-labeled monoclonal antibodies and analysed by flow cytometry. Shaded peaks indicate background staining by isotype control antibodies. (B) B cells were inactivated by treatment with 25 μg/ml mitomycin C (20 min at 37˚C) and combined in a 1:3 ratio with syngeneic T cells (purified using commercial T cell enrichment columns) and anti-CD3 monoclonal antibody (1/20 dilution of hybridoma culture supernatant) in wells of a 96-well microtitre plate. After 42 h of culture, wells were pulsed with 0.5 μCi tritiated thymidine ([^{3}H]TdR) and 6 h later well contents were harvested onto glass fibre mats. [^{3}H]TdR incorporation into the DNA of proliferating T cells was determined by liquid scintillation counting. Data are expressed as mean counts per minute (cpm) of quadruplicate cultures ± the standard deviation (SD).

diminished in the presence of adenosine. Mouse B lymphocytes that were activated with phorbol ester and ionophore in the presence of adenosine or its stable analogue 2-chloroadenosine showed reduced expression of CD80 and CD86 (ligands for CD28 on T cells), as well as CD40 (ligand for CD40 ligand on T cells). Moreover, diminished expression of costimulatory molecules by B cells activated in the presence of adenosine or 2-chloroadenosine correlated with a reduced capacity to costimulate T cell activation through the T cell receptor/CD3 complex (Fig. 4B). This negative effect of adenosine on costimulatory molecule expression by B cells is most likely mediated through A_{2a} adenosine receptors since B cells exhibit strong A_{2a} adenosine receptor expression but little or no expression of other adenosine receptor subtypes (50). Adenosine may have a similar inhibitory effect on the expression of these or other costimulatory molecules by dendritic cells and macrophages. In addition to interfering with T cell costimulation at the level of the APC, adenosine inhibits T cell receptor- and IL-2-dependent up-regulation of costimulatory CD2 and CD28 by mouse T cells (65), which may further impair T cell activation in the solid tumor microenvironment.

5. Adenosine inhibition of natural killer cell function

NK cells are able to secrete proinflammatory cytokines, lyse certain MHC class I-deficient cancer cells and, upon stimulation by cytokines that include IL-2, IL-12, and IL-15, become lymphokine-activated killer (LAK) cells that exhibit enhanced cytolytic activity against a wider spectrum of tumor cells (82). However, as with T cell-stimulating cancer vaccines, overall clinical responses to NK cell-based immunotherapies (e.g., adoptive transfer of LAK cells) have to date been disappointing (8). Tumor-associated adenosine may, at least in part, account for the minimal impact that NK cell-based immunotherapy has had on human cancer since adenosine and adenosine analogues are potent inhibitors of NK cell function (56,83,84), as well as LAK cell function (31,85). Exposure to A_2 adenosine receptor agonists suppresses the cytotoxic activity of mouse NK cells whereas A_1 adenosine receptor agonists have a stimulatory effect (56). Given that intracellular cAMP levels modulate the killing activity of NK cells (86), the differential effect of A_1 and A_2 adenosine receptor ligation on NK cell-mediated cytotoxicity are most likely due to an A_1 adenosine receptor-induced decrease in intracellular cAMP, resulting in enhanced cytolytic activity, and an A_2 adenosine receptor-induced increase in intracellular cAMP, leading to diminished cytotoxic activity. Adenosine and its stable analogue 2-chloro-adenosine also act through A_{2a} adenosine receptors to inhibit the killing of 3LL Lewis lung carcinoma cells by mouse LAK cells (31). Parallel studies using LAK cells generated from A_1 and A_3 adenosine receptor-deficient mice have ruled out any involvement of these adenosine receptor subtypes in the adenosine-mediated inhibition of LAK cell function. Recently, cAMP-dependent activation of protein kinase A type I has been implicated in adenosine-mediated inhibition of proinflammatory cytokine production and cytotoxic activity by mouse LAK cells (85). Interestingly, oral administration of the A_3 receptor agonist 2-chloro-N^6-(3-iodobenzyl)-adenosine-5-*N*-methyl-uronamide (Cl-IB-MECA) has been suggested to enhance the cytotoxic activity of mouse NK cells, as well as cause increased serum IL-12 and reduced *in vivo* growth of B16-F10 melanoma cells (57). However, since IL-12 is a potent stimulator of NK cell activity (87), it is equally likely that the apparent potentiating effect of Cl-IB-MECA on NK cell-mediated cytotoxicity is in fact an indirect result of increased IL-12 synthesis in response to the A_3 adenosine receptor agonist. In any case, adenosine has an overall inhibitory effect on the cytotoxic function of NK and LAK cells (31,56,83,85). The accumulation of intracellular cAMP is also associated with the inhibitory effect of adenosine on IL-2-induced cellular proliferation and TNF α production in human NK cell cultures; however, in this instance adenosine failed to inhibit cytotoxic activity, most likely due to a counteracting effect by the high concentration of cytotoxicity-promoting IL-2 that was added to the NK cell cultures (84). In addition, mouse NK cells exhibit defective granule exocytosis in the presence of adenosine, although in this case the inhibitory effect is

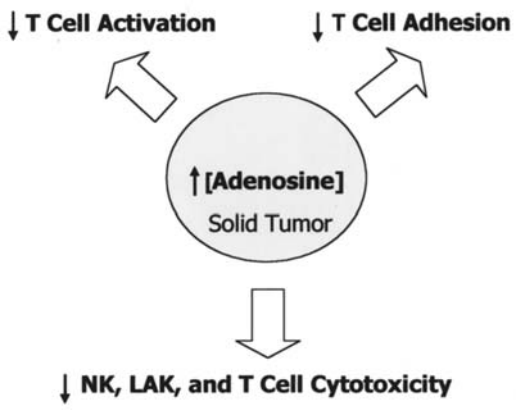

Figure 5. Schematic diagram illustrating the impact of tumor-associated adenosine on cell-mediated anti-tumor immune responses. Solid tumors contain immunosuppressive concentrations of adenosine (19,20) that inhibit the activation, differentiation, and clonal expansion of tumor-specific T cells (20,66), CTL binding to syngeneic tumor cells (68,69), and tumor cell destruction by NK cells (56,83), LAK cells (31) and CTL (66).

mediated through a distinct and as yet uncharacterized cell-surface adenosine receptor (83). Collectively, these findings indicate that extracellular adenosine has the potential to be an important inhibitor of tumor cell destruction by NK and LAK cells within the microenvironment of solid tumors.

6. Impact of lymphocyte-associated adenosine deaminase on adenosine receptor stimulation

The severe state of B cell and T cell immunodeficiency that occurs as a result of a genetic deficiency of ADA underscores the essential role played by ADA in the proper development and function of the immune system (88). For example, thymocytes from ADA-deficient mice exhibit a profound defect in T cell receptor signaling due to reduced phosphorylation of the CD3 ζ chain (89). ADA is normally anchored to the cell surface by CD26/dipeptidyl peptidase IV (39), which is up-regulated on activated $CD4^+$ and $CD8^+$ T cells (90,91). NK cells also show CD26 expression, which is increased in response to IL-2, IL-12, or IL-15 (92). Ecto-ADA can also bind to A_1 and A_{2b} adenosine receptors, the latter of which are present on T lymphocytes (49-51). However, ADA-A_{2b} adenosine receptor complexes are not likely to be a major source of T cell-associated ecto-ADA because A_{2b} adenosine receptor expression by T lymphocytes is low in comparison to CD26. Rather, the principal role of ADA that binds to A_{2b} adenosine receptors is believed to involve the modulation of receptor signaling (40). ADA that is complexed with CD26 on T cells renders them resistant to the inhibitory effect of adenosine on cellular proliferation and IL-2 synthesis (93). Since the amount of ADA that can bind to lymphocytes increases with increasing CD26 expression (90,94), activated T cells and NK cells that infiltrate the microenvironment of solid tumors would be predicted to have an increased capacity to deaminate adenosine, thereby ameliorating the inhibitory effect of tumor-associated adenosine. However, this does not appear to be the case as there is ample evidence that ADA activity associated with peripheral lymphocytes from patients with solid tumors is significantly lower than healthy controls (95-97). Furthermore, it is possible that the high levels of adenosine in the tumor microenvironment (19,20) may down-regulate lymphocyte-associated CD26 and therefore levels of ADA in that context, as has been shown for epithelial cells (37,38). It is therefore most likely that immune effector cells in tumor-bearing individuals have accentuated sensitivity to the inhibitory effects of adenosine within the solid tumor microenvironment.

7. Conclusions

We were the first to hypothesize that elevated levels of adenosine in solid tumors might result in impaired killing of tumor cells by immune effector cells (98). Indeed, it is now apparent that the immunosuppressive activity of tumor-elaborated adenosine may constitute a significant impediment to the success of immunotherapeutic strategies that seek to elicit curative cell-mediated anti-tumor immune responses either by the stimulation of tumor-specific T cell responses or adoptive transfer of tumor-reactive killer cells such as LAK cells. As depicted in Fig. 5, this problem is further complicated by the ability of adenosine to negatively affect cellular anti-tumor immune responses at multiple levels, including the activation, development, and clonal expansion of tumor-specific T cells with helper and cytotoxic effector function (20,66), the adhesion of CTL to syngeneic carcinoma cells (68,69), and tumor cell killing by NK cells (56,83), LAK cells (31) and CTL (66). However, the identification of adenosine receptor subtypes and/or signal transduction pathways through which adenosine exerts its inhibitory effects on cell-mediated anti-tumor immune responses may allow for the development of focused pharmacologic strategies to reduce or ablate the impact of adenosine-mediated immune suppression in cancer patients, thereby increasing the effectiveness of therapeutic cancer vaccines and other immune-based cancer therapies.

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