Adenosine A2A Receptors Intrinsically Regulate CD8+ T Cells in the Tumor Microenvironment

Caglar Cekic1,2 and Joel Linden1

Abstract

Adenosine A2A receptor (A2AR) blockade enhances innate and adaptive immune responses. However, mouse genetic studies have shown that A2AR deletion does not inhibit the growth of all tumor types. In the current study, we showed that growth rates for ectopic melanoma and bladder tumors are increased in Adora2a−/− mice within 2 weeks of tumor inoculation. A2AR deletion in the host reduced numbers of CD8+ T cells and effector–memory differentiation of all T cells. To examine intrinsic functions in T cells, we generated mice harboring a T-cell–specific deletion of A2AR. In this host strain, tumor-bearing mice displayed increased growth of ectopic melanomas, decreased numbers of tumor-associated T cells, reduced effector–memory differentiation, and reduced antiapoptotic IL7Rα (CD127) expression on antigen-experienced cells. Intratumoral pharmacologic blockade similarly reduced CD8+ T-cell density within tumors in wild-type hosts. We found that A2AR-proficient CD8+ T cells specific for melanoma cells displayed a relative survival advantage in tumors. Thus, abrogating A2AR signaling appeared to reduce IL7R expression, survival, and differentiation of T cells in the tumor microenvironment. One implication of these results is that the antitumor effects of A2AR blockade that can be mediated by activation of cytotoxic T cells may be overcome in some tumor microenvironments as a result of impaired T-cell maintenance and effector–memory differentiation. Thus, our findings imply that the efficacious application of A2AR inhibitors for cancer immunotherapy may require careful dose optimization to prevent activation-induced T-cell death in tumors.

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Introduction

Solid tumors produce high concentrations of adenosine in response to hypoxia, cell necrosis, and the rapid metabolism of extracellular adenine nucleotides by ecto-nucleotidases expressed on tumor cells, tumor cell exosomes, and T regulatory cells (1–4). Adenosine engages four adenosine receptor subtypes: A1, A2A, A2B, and A3. The adenosine A2A receptor (A2AR) is the predominant subtype found on T cells, and is induced upon cell activation (4, 5).

A2AR signaling inhibits innate and adaptive immune responses (5, 6). Global deletion of A2ARs facilitates activation of CD8+ T cells and enhances rejection of certain tumors that were genetically engineered to be highly sensitive to cytotoxic T-cell killing due to overexpression on tumor cells of MHC-I molecules (7). A2AR deletion enhances lymphoma killing and the effectiveness of an antilymphoma tumor vaccine (8). Hence, adenosine has been viewed as an inhibitor of T-cell–mediated tumor surveillance (9, 10), and blockade of lymphocyte A2ARs has been advocated to facilitate tumor immunotherapy. Curiously, global deletion of A2ARs did not affect the growth of B16F10 melanomas or MB49 bladder carcinomas that were not modified by genetic engineering (7, 11), despite the fact that these tumors produce immune cell activation (12). This might occur because adenosine levels are high in solid tumors, and A2AR signaling can inhibit activation-induced death of T cells and thus facilitate their survival (13). It is also possible that some of the effects of global A2AR deletion on tumor growth are due to disinhibition of tumor macrophages, dendritic cells (DC), or natural killer (NK) cells.

In contrast with the failure of global A2AR deletion to inhibit B16F10 growth, reduced adenosine production due to deletion of CD73, an ecto-enzyme that converts AMP to adenosine, was found to consistently enhance antitumor adaptive immune responses (6, 14–17). This could be in part due to the involvement of A2BRs in tumor suppression (11) or to differential effects on immune cell function caused by moderately reducing A2AR stimulation by deleting CD73 as opposed to eliminating A2AR signaling by deleting receptors.

In the current study, we evaluated the effects on B16F10 melanoma growth and tumor-associated T-cell survival of: (i) global A2AR deletion, (ii) LckCre-mediated T-cell selective

1Division of Inflammation Biology, La Jolla Institute for Allergy and Immunology, La Jolla, California. 2Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey.

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Corresponding Author: Joel Linden, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037. Phone: 858-752-6603; Fax: 858-752-6985; E-mail: jlinden@liai.org
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deletion of floxed A2ARs, and (iii) adoptive cotransfer of T cells to tumor-bearing mice with and without A2ARs. The results indicate that T-cell–specific A2AR deletion does activate T cells, but can also lead to reduced numbers of tumor-associated T cells and an increase over time in the growth rate of large solid tumors. Hence, some degree of A2AR signaling is needed for maintenance and effector differentiation of tumor-associated T cells. Opposing effects of A2AR deletion to enhance T-cell activation but to reduce effector cell numbers in solid tumors provide an explanation for why global deletion of the A2AR causes inconsistent effects on tumor growth.

Materials and Methods

Cell lines, animals, and reagents

Animal experiments were approved by the ACUC of the La Jolla Institute (La Jolla, CA). B16F10 cells stably expressing luciferase were obtained from Caliper Life Sciences. MB49 bladder carcinoma cells were from Dr. Timothy Ratliff of Purdue University (West Lafayette, IN). MB49 Bladder carcinomas were characterized as indicated by Luo and colleagues (18) and further tested at the time of experimentation for adherence, freeze thaw viability, growth properties, and mouse MHCI expression, without further authentication. Ovalbumin-expressing B16F10 cells produced as described previously (19) were a gift of Dr. Stephen Schoenberger of the La Jolla Institute. Ovalbumin and luciferase-expressing B16F10 cells were obtained from Dr. Andreas Limmer of the University of Bonn (Bonn, Germany). Both ovalbumin-expressing melanoma cell lines were received within 6 months of experimentation and evaluated at the time of experimentation for morphology, adherence, freeze thaw viability, growth properties, and mouse MHCI expression before and after IFNγ treatment, cell surface expression of MHCI/Ova peptide complexes, and antigen-specific recognition of TRP2 or OVA peptides by respective transgenic T cells. B16F10 cells were cultured in RPMI-1640 medium containing 10% heat-inactivated FBS, 2 mmol/L l-glutamine, 1 mmol/L sodium pyruvate, 50 U/mL penicillin, 50 μg/mL streptomycin. Tumor cells were injected into mice after reaching 60% to 80% confluence. A2AR KO mice produced by Chen and colleagues (20) on a mixed genetic background were backcrossed onto C57BL/6. Six-week-old C57BL/6J, EGFP, and OT-I Rag−/− mice were purchased from Jackson Laboratories, crossed with Adora2afl/fl mice, and used for experiments after being acclimated for 2 to 6 weeks. LckCre+ mice (21) were obtained from Taconic (B6.Cg-Tg(Lck-cre)1Cwi N9) and used to create Adora2afl/fl-LckCre+ mice. Tail DNA from all mice was genotyped (Transnetyx, Inc.) to detect the presence of Cre recombine and to quantify by qPCR lckCre-mediated excision of floxed Adora2a DNA. Global versus lck-mediated Cre expression was found to increase the amount of excision by >20-fold in tail DNA. Hence, qPCR was used to exclude from experiments occasional mice with non-lymphoid deletion. As further evidence of lymphoid-selective deletion, we have shown previously by qPCR that thymocyte expression of A2AR mRNA in lckCre/Adora2afl/fl mice is only deleted after thymocytes activate lck (22). Yellow or aqua fluorescent reactive dyes were from Invitrogen.

Flow cytometry

Single-cell suspensions from indicated tissues were prepared by sequential pressing through 100 and 40 μm cell strainers. Dead cells were removed from tumor samples by Ficoll gradient centrifugation at 2,000 rpm (900 g) for 20 minutes at room temperature. After RBC lysis (Biolegend) of spleen samples, remaining cells were washed and resuspended in R10F, and counted in a Z2-Coulter particle counter (Beckman Coulter). Cells (3–5 × 106) were preincubated for 10 minutes in 100 μL FACS buffer with antibody to block Fc receptors. Each sample tube received 100 μL fluorescently labeled antibody cocktail and was incubated for 30 minutes at 4°C in the dark. Cells were analyzed using an LSRll equipped with 4 lasers or a LSR Fortessa equipped with 5 lasers and FACS Diva software (BD Biosciences). Live/dead fixable yellow, aqua, or blue (Invitrogen) were used to exclude dead cells before analysis. Flow-cytometric data were analyzed using Flowjo software (9.5.3 version, TreeStar Software Inc.).

Establishment and in vivo imaging of solid tumors

B16F10 or MB49 cells (105) were injected into the right flanks of mice. B16F10 melanoma cells expressing luciferase were injected into Adora2afl/fl-LckCre+ and used for in vivo imaging. Tumor volumes were measured using digital calipers and calculated as height × width2/2. Luciferase activity was determined using an IVIS 200 Bioluminescence imager (Caliper Life Sciences) after intravenous injection of 1 mg p-Luciferin (Caliper Life Sciences) in 100 μL PBS to validate that tumor size differences were not due to infiltration of host cells. To measure tumor metastasis, 3 × 106 B16F10 melanoma cells expressing luciferase were injected intravenously into mouse tail veins and luciferase activity was measured in the lungs 1 and 2 weeks later. After measuring luciferase activity, lungs were removed, photographed, and weighted to validate that luciferase activity correlated with tumor mass.

Adoptive transfer and cotransfer of T cells

B16F10 cells (105) expressing ovalbumin (B16F10-OVA) were injected into mouse flanks and allowed to expand for 16 days. Mixtures of 3 × 106 OT-1 Rag−/− and 7 × 106 OT-1 Rag−/Adora2a−/− cells were injected intraperitoneally. Greater numbers of OT-1 Adora2a−/− cells were included in the mixture because A2AR deficiency substantially reduced their numbers. On days 3 or 5, tumors and spleens were harvested and stained for analysis by flow cytometry. For adaptive transfer experiments, 106 OT-1 Rag−/− or OT-1 Rag−/Adora2a−/− cells were injected intraperitoneally into the mice bearing B16F10-OVA tumors established for 2 weeks. Tumor growth was measured after T-cell transfer and on day 21. Mice were sacrificed and single-cell suspensions from tumors and spleen were analyzed for Annexin V staining, cell surface CD44 and CD127 expression, and cell number and density.
Results

Global deletion of Adora2a increases solid tumor growth and impairs CD8⁺ T-cell effector differentiation and accumulation in tumors

In prior studies, global deletion of A2ARs failed to slow the growth rate of B16F10 melanomas transplanted into syngeneic mice (7, 11). In the current study, we performed similar experiments in mice inoculated with B16F10 melanoma or MB49 bladder carcinomas and confirmed that A2AR deletion failed to decrease the rate of growth of either tumor; in fact, the growth rates of both tumors were significantly increased at days 14 to 18 after inoculation as the tumors became large (Fig. 1A). By preparing single-cell suspensions of tumors grown for 18 days after tumor inoculation, we next determined whether increased B16F10 growth was associated with reduced accumulation and/or impaired function of particular immune cell types within the tumor. Adora2a deletion significantly reduced the frequencies of CD8⁺ T cells (Fig. 1B) but not the frequencies of CD4⁺ T cells (Fig. 1B), myeloid cells, or CD11bdimCD11c⁺ cells (Fig. 1C). Adora2a deletion also caused a significant increase in frequencies of NK1.1⁺TCRβ⁻ cells (henceforth referred to as NK cells; Fig. 1C). Therefore, we calculated the density in tumors of NK and T cells by dividing the absolute numbers of these cells by tumor volume. Figure 1D shows that a large reduction in CD8⁺ T-cell density is associated with an increase in NK cell density in tumors. Local intratumoral injection of an irreversible A2AR blocker, 5-amino-7-[2-(4-fluorosulfonyl)phenylethyl]-2-(2-furyl)-pyrazolo[4,3-2]1,2,4-triazolo[1,5-c]pyrimidine (FSPTP) also reduced CD8 T-cell density but not CD4 T-cell or NK density within tumors, suggesting that local effects rather than global effects of Adora2a deletion are responsible for reduced T-cell numbers (Fig. 1E) and these effects are not dependent on elevated...
NK cell density. Additional experiments will be required to determine whether the increase in NK cell density in tumors caused by global A3R deletion is due to a cell intrinsic effect of A3R deletion on NK cells. Cell surface expression of CD44, KLRG1, and PD-1 was significantly lower in tumor-associated CD8+ T cells isolated from A3R-deficient mice as compared with tumor-associated CD8+ T cells from control animals (Fig. 2A). However, expression of CD25 tended to increase in A3R-deficient CD8+ T cells, suggesting that CD8+ T cells in tumors are activated but fail to become effector-memory cells in the absence of A3Rs (Fig. 2A, top). CD4+ T-cell effector differentiation (as measured by CD44 and KLRG1) was also significantly inhibited in the absence of Adora2a (Fig. 2A, bottom). After global A3R deletion,
CD80 expression on tumor-associated APCs increased, whereas CD86 expression and NK markers were unchanged (Fig. 2B and C). We also evaluated phenotypic markers in tumor-associated myeloid APCs such as MHCII, PD-1, and PD-L1, which regulate T-cell activation and CD39, which mediates tolerogenic activity of DCs by converting immunostimulatory ATP to ADP and AMP. A2AR deficiency did not cause significant changes among any of these markers except for a reduction in cell surface PD-L1 expression (Supplementary Fig. S1). PD-L1, although inhibitory for T-cell activation, can be upregulated by inflammatory signals. It is possible that a reduction in the production of inactivation CD39, which regulates T-cell activation and CD39, which mediates tolerogenic activity of DCs by converting immunostimulatory ATP to ADP and AMP, can be upregulated by inflammatory signals. It is possible that a reduction in the production of inflammatory cytokines due to reduced T-cell activation and activation contributes to reduced PD-L1 expression in A2AR-deficient mice. Overall, these results suggest that increased CD8T-cell infiltration and effector-memory differentiation in A2AR–/– mice are not due to APC inactivation. In fact, myeloid-selective deletion of A2AR decreases melanoma growth and increases the number of tumor-associated T cells and NK cells (23).

As in mice with B16F10 tumors, in mice with solid MB49 carcinomas A2AR deletion reduced CD8+ T-cell frequency and expression of the effector-memory marker CD44 (Fig. 3A and B). One possible explanation for the reduction in CD8+ T cells in the tumors of A2AR–/– mice is reduced expression of CXCR3, which is required for activated T cells to home to inflamed sites. We did not observe any reduction in CXCR3 expression after A2AR deletion (Fig. 3B).

Furthermore, local inhibition by intratumoral injection of the A2AR antagonist FSPTP, but not by selective A2BR blocker ATL-801, also significantly reduced the frequency of tumor-associated CD8+ T cells (Fig. 3C), suggesting that as in melanomas, A2AR signaling facilitates the accumulation CD8+ T cells within bladder tumors as well.

It is notable that A2AR blockade consistently increased the frequencies of tumor-associated NK cells (Figs. 1B and 3A and C). This observation agrees with findings by Beavis and colleagues (2) who found that blockade or global deletion of A2ARs reduced lung metastasis of CD73-expressing tumors by increasing NK cell activity and numbers, presumably by blocking A2AR-mediated effects of high adenosine in the tumor.

Lymphoid-selective deletion of Adora2a reduces the number and differentiation to effector-memory cells of tumor-associated T cells and markedly increases the growth rate of large solid tumors

Because global A2AR deletion activates APCs (Fig. 2B), we hypothesized that A2AR signaling helps to maintain T-cell numbers in the solid tumor microenvironment in a T-cell intrinsic manner. To evaluate the effects of cell-intrinsic A2AR signaling on tumor growth and on T-cell populations from MB49 tumors injected with 100 µL, 1 µmol/L FSPTP, 1 µmol/L ATL-801 or vehicle every 3 days after tumor inoculation. Results are pooled from independent experiments with similar results (n ≥ 5; *P < 0.05; **P < 0.01; by two-way ANOVA and Bonferroni post hoc analyses.) A–C, all corresponding analyses were performed 3 weeks after tumor inoculation.
responses, we crossed mice with a floxed Adora2a gene with mice expressing Cre recombinase under control of the Lck promoter. Adora2a<sup>ff</sup>−LckCre<sup>+/−</sup> mice have normal numbers of thymic T-cell precursors in the absence (22) or presence (Supplementary Fig. S2) of solid tumors, suggesting that Adora2a<sup>ff</sup>−LckCre<sup>+/−</sup> mice have normal T-cell development compared with littermate controls. However, T-cell selective deletion of A2ARs markedly accelerated the growth rate of tumors after they reached a volume >500 mm<sup>3</sup> (Fig. 4A and B; see Supplementary Fig. S3 for results of individual experiments). These findings suggest that paradoxically, some degree of A<sub>2A</sub>R signaling in T cells is required to mount an optimal antitumor immune response in large solid tumors. A<sub>2A</sub>R signaling increases cAMP production (24). It has been suggested that a temporary increase in cAMP levels may be required for T-cell activation (25). However, A<sub>2A</sub>R deletion failed to affect the expression of CD69 in tumor-associated T cells (Supplementary Fig. S4). We reasoned that the absence of A<sub>2A</sub>R signaling in the tumor microenvironment might cause T cells to polarize toward a regulatory phenotype. Figure 4C shows that T-cell A<sub>2A</sub>R deletion does not enhance regulatory T-cell differentiation in the tumor or tumor draining lymph nodes. Therefore, we measured CD25 expression in antigen-experienced T-cell populations. The deletion of the A<sub>2A</sub>R from lymphocytes increased CD25 expression in tumor-associated/antigen-experienced CD4<sup>+</sup>Foxp3<sup>+</sup> T cells and did not affect CD8<sup>+</sup> T cells or lymph node CD4<sup>+</sup>Foxp3<sup>−</sup>CD8<sup>+</sup> T cells (Fig. 4D). These data suggest that deletion of A<sub>2A</sub>R signaling does not hamper T-cell activation in tumors.

We next considered the possibility that A<sub>2A</sub>R signaling sustains normal numbers of tumor-associated T cells. We found previously that A<sub>2A</sub>R signaling, by activating PKA, reduces the activity of the PI3K/Akt pathway (22). This suppresses TCR-mediated downregulation of antiapoptotic CD127, which is upregulated in long-lived effector–memory cells and required for their maintenance. LckCre-mediated deletion of A<sub>2A</sub>Rs significantly reduced CD127 expression in antigen-experienced T cells in the tumor (Fig. 5), and
significantly reduced the frequencies of tumor-associated T cells (Fig. 6A and B) but not NK cells (Fig. 6C). T-cell-selective A2A-R-deletion also significantly reduced the frequency of CD44+ effector-memory T cells in tumors, but not lymph nodes (Fig. 6D and E). Figure 6F shows a reduction in A2A-R−/− T-cell density in tumors. Hence, although A2A-R activation during TCR stimulation inhibits T-cell activation, the data suggest a role for adenosine in maintaining effector T cells within the tumor microenvironment. These opposing effects of A2A-R deletion to enhance T-cell activation but to reduce effector cell numbers provide an explanation for why global deletion of the A2A-R causes small or inconsistent effects on tumor growth.

A2A-R signaling prolongs the maintenance of T cells in tumor-bearing hosts

Global A2A-R deletion significantly reduces the development and peripheral maintenance of naive T cells (22). Although Lck-mediated Adora2afl/fl deletion did not affect thymic progression of T cells, it did cause a decrease in the number of naive T cells in the periphery. This decrease in the naive T-cell population may contribute to reducing numbers of T cells in tumors after global or LckCre-mediated deletion of Adora2af/f. Also, reduced naive T-cell numbers in mice lacking T-cell A2A-Rs could be a consequence of high tumor burden rather than to an intrinsic effect of A2A-R signaling. To evaluate in vivo competition and phenotypic differentiation of antigen-specific T cells lacking or expressing A2A-Rs in the same tumor microenvironment, we performed adoptive cotransfer experiments. When cotransferred into the same host-bearing B16F10-OVA tumors, the proportion of Adora2afl/fl OT-I T cells was markedly decreased in the tumor relative to Adora2ax/y OT-I T cells (Fig. 7A). A2A-R deletion also reduced cell surface expression of PD-1 (Fig. 7B), whereas CD25 expression was increased (Fig. 7C), a phenotype similar to what was observed after global deletion of the A2A-R (Fig. 2A). Figure 7D shows that A2A-R deletion caused a significant decrease in CD127 expression in both spleen and tumor-associated OT-I T cells.

To directly test the effects of Adora2a deletion on T-cell survival and effector-memory differentiation, we adoptively transferred Adora2afl/fl and Adora2ax/y OT-I T cells 2 weeks after establishment of B16F10-OVA tumors in congenic hosts. One week after adoptive transfer, we prepared single-cell suspensions from tumor tissue by ficoll gradient and measured cell surface staining of Annexin V as an apoptosis marker, CD44 as marker for effector-memory differentiation, and CD127 as mediator of T-cell survival. Tumor-associated but not splenic Adora2afl/fl OT-I T cells expressed significantly more Annexin V than Adora2ax/y OT-I T cells and this was associated with decreased expression of CD44 and CD127 within tumors (Supplementary Fig. S5). Transfer of either Adora2afl/fl or Adora2ax/y OT-I cells induced a transient decrease in tumor growth, suggesting that Adora2afl/fl cells are initially functional, but immunostimulatory effects of Adora2a deletion appear to be counteracted by reduced survival/effector-memory differentiation (Supplementary Fig. S5). Overall, these data show that in the tumor environment, A2A-R-deficient T cells have a survival disadvantage as compared with A2A-R-proficient T cells.

Discussion

Adenosine accumulates to high levels in solid tumors (1–4). A2A-Rs on antigen-presenting cells and T cells, and A2B receptors on antigen-presenting immune cells are primarily responsible for immunosuppression by adenosine (26, 27). Global deletion or intratumoral blockade of A2A-Rs delays the growth of lung and bladder carcinoma and breast cancers, consistent with the immunosuppressive roles of these receptors (11). Curiously, global A2A-R deletion failed to slow the growth of bladder carcinomas and B16BL6 melanomas (7, 11), but was found to enhance the rejection of CL8-I cells that were genetically engineered to be highly immunogenic
by transfection with H-2K^b (7). These findings, and the results of the current study suggest that A2AR blockade, as a strategy to treat cancer, is more complex than previously thought (9, 10). Although T cells are acutely activated by A2AR deletion, long-term T-cell–mediated solid tumor rejection is compromised, likely as a result of impaired maintenance of T cells and reduced effector–memory differentiation in the tumor. It is important to point out, however, that the...
effect of $A_2A$R deletion on T-cell functions is not necessarily indicative of the effects of $A_2A$R blocking drugs that lessen, but do not eliminate $A_2A$R signaling. In this respect, a more clinically relevant reversible $A_2A$R antagonist rather than an irreversible antagonist may have different properties.

Ohta and colleagues previously showed that silencing of $A_2A$ and $A_2B$ adenosine receptors by siRNA in adoptively transferred, tumor-specific T cells significantly reduced lung metastasis of H2-Kb-expressing RMA T-cell lymphoma cells and improved the survival of tumor-bearing mice (7). The current study shows that Adora2a-deficient OT-I T cells do not slow the growth of solid B16F10 cells expressing ovalbumin, which can be recognized by OT-I T cells. The data suggest that: (i) antitumor effects of Adora2a deletion vary among different types of tumors; (ii) $A_2B$R signaling in tumor-associated T cells may contribute to adenosome suppression of T-cell activity; (iii) solid versus metastatic tumor growth may be differentially in the expression of T-cell activity; (iv) highly immunogenic tumors may preferentially elicit antitumor effects of Adora2a deletion; and (v) siRNA silencing of Adora2a expression or pharmacologic inhibition may result in $A_2A$R residual signaling and contribute to tumor killing by sustaining T cells in the tumor microenvironment.

T cells go through an expansion phase after activation but many fail to survive either due to excess activation of inhibitory signals, or due to the absence of costimulation by certain cytokines or homing signals in the tumor (9). Interestingly, the absence of T-cell intrinsic inhibitory $A_2A$R signaling reduces numbers of tumor-associated T cells after 2 to 3 weeks of solid tumor expansion. Our recent findings indicate that $A_2A$R signaling can prevent TCR-induced downregulation of antiapoptotic CD127 (22). $A_2A$R deficiency significantly reduced the development and peripheral maintenance of naive T cells. This decrease in the naive T-cell population may contribute to reduced numbers of T cells in tumors after Lck-mediated deletion of Adora2a (9). However, the irreversible $A_2A$R antagonist, FSPTP, injected directly into solid tumors, also reduced tumor-associated T-cell numbers in wild-type recipients. Also, the ratio of antigen-specific $A_2A$R-deficient/$A_2A$R-proficient T cells decreased within the same tumor after adoptive cotransfer, suggesting a T-cell survival defect due to either deletion or irreversible blockade of $A_2A$R signaling in the tumor environment.

We show that CD127 expression by effector-memory cells in tumors is significantly reduced in T cells lacking $A_2A$Rs, whereas CD25 expression is largely intact or even increased. This supports the notion that although $A_2A$R signaling acutely inhibits initial T-cell activation, $A_2A$R-dependent control of CD127 expression may be necessary for the maintenance of T cells after they differentiate into long-lived effector-memory cells. Consistent with this idea, we noted a significant impairment in the ex vivo survival of $A_2A$R+/− T cells in response to IL7 (22). The pattern of tumor T-cell responses...
caused by deletion of the A2A R is very similar to the effect of IL2 in tumor immunotherapy: although IL2 increases the activation and early expansion of T cells, it causes increased activation-induced death (28). Therefore, it is possible that CD127 deficiency and increased IL2 signaling due to increased CD25 expression impairs T-cell survival in the tumor microenvironment.

A2A R signaling may sustain tumor-associated T cells by inhibiting the PI3K/Akt pathway. As with A2A R agonists, PI3K is also inhibited by rapamycin, which has been shown to increase numbers of long-lived effector–memory T cells in virally infected mice (29). Expansion of tumor-associated effector–memory T cells might account for the observations that despite their immunosuppressive properties, adenosine (this study) and rapamycin (30) have antiapoptotic effects on T cells in some tumors.

The A2A R is upregulated in multiple immune cell types upon activation (5, 31, 32). Blockade or deletion of A2A Rs in NK cells (2) or myeloid cells (23) significantly inhibits tumor growth and metastasis and is associated with transactivation of cytotoxic lymphocytes. Consistent with these findings, the current study suggests that to avoid apoptosis of tumor-associated T cells, myeloid or NK cell targeted therapies should be considered as preferable targets for A2A R deletion/blockade as a strategy for tumor immunotherapy. Alternatively, to optimize the beneficial effects of T-cell activation by A2A R blockade for solid tumor killing, it may be necessary to find complementary strategies to enhance T-cell survival and effector–memory differentiation to counteract activation-induced cell death. These concepts apply not only to the potential use of adenosine receptor blockade or deletion to treat cancer, but also more broadly to the general use of T-cell activators.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: C. Cekic, J. Linden

Development of methodology: C. Cekic

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Cekic, J. Linden

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Cekic, J. Linden

Writing, review, and/or revision of the manuscript: C. Cekic, J. Linden

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Cekic

Study supervision: J. Linden

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