CANCER IMMUNOLOGY

Interleukin-7 protects CD8$^+$ T cells from adenosine-mediated immunosuppression

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The nucleoside adenosine accumulates extracellularly in solid tumors and inhibits CD8$^+$ T cells by activating adenosine receptors. The cytokine interleukin-7 (IL-7), which is produced by various tissues and tumors, promotes the survival and maintenance of T cells. Adenosine and IL-7 signaling are being clinically targeted separately or in combination with other therapies for solid tumor indications. Here, we found that IL-7 signaling promoted the accumulation of tumor-associated CD8$^+$ T cells, in part, by preventing adenosine-mediated immunosuppression. Inhibition of the transcription factor FoxO1 downstream of IL-7 receptor signaling was important for protecting CD8$^+$ T cells from suppression by adenosine. These findings have implications for the development of new approaches for cancer immunotherapies that target the adenosine pathway.

INTRODUCTION

Signaling by the nucleoside adenosine is one of the immune checkpoint pathways in tumors. Unlike membrane-bound, receptor-ligand interactions, adenosine is a small molecule. Therefore, the presence of adenosine is not limited to the presence of a particular cell type but to the presence of events common to most tumors: hypoxia, inflammation, cellular stress, high metabolic activity, and cell death (*1*–*3*). Adenosine is a hallmark of the tumor microenvironment (TME) because these events occur in most tumors. Tumor-infiltrating suppressive cells and invasive tumors express adenosine-generating ectonucleotidases, such as CD38, CD39, and CD73, which break down adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) to adenosine to further increase adenosine concentrations and generate an immunosuppressive niche (*4*, *5*). Therefore, both adenosine receptors and adenosine-generating pathways are being targeted in clinical trials for many solid tumor indications (*5*). Extracellular adenosine can interact with four different adenosine receptor subtypes: A1, A2A, A2B, and A3 (*6*). Signaling through the adenosine A2A and A2B receptors inhibits immune cell activation and promotes tissue healing responses, such as angiogenesis and fibrosis (*1*).

Adenosine signaling strongly suppresses antigen-presenting cells and T cells. In T cells, adenosine A2A receptors (A2ARs) are the main adenosine receptors. Global or T cell–specific deletion of *adora2a* (the gene that encodes A2AR) increases the growth of syngeneic melanoma and bladder carcinoma tumors (*7*). Myeloid cell–specific deletion of *adora2a* in mice decreases tumor growth and dissemination to the lungs (*8*). Accordingly, adenosine gene signatures that predict the responsiveness of patients to A2AR blockade in the clinic consist mainly of myeloid cell–associated genes (*9*). Deletion of *adora2a* is associated with the reduced abundance of the interleukin-7 receptor (IL-7R) and reduced responsiveness to the cytokine IL-7, which is important for T cell development and maintenance. Therefore, in *adora2a*$^{-/-}$ mice, we observed a substantial reduction in the numbers of T cells in the thymus, lymphoid organs, and tumors (*7*, *10*). These findings suggest that interactions between adenosine signaling and IL-7 signaling may play important roles for T cell development in general or T cell maintenance in the TME (*7*, *10*).

The transcription factor FoxO1 plays an important role in naïve T cell maintenance by keeping naïve T cells quiescent and by promoting the expression of IL-7R (*11*). Sustained FoxO1 signaling can also stimulate proapoptotic pathways (*12*). One of the consequences of IL-7 signaling and antiapoptotic Akt signaling in general is inactivation of FoxO1 through phosphorylation of Ser^{256}, which prevents FoxO1 from entering the nucleus to perform transcriptional activities. This suggests that unrestrained FoxO1 activation can limit T cell survival by activating proapoptotic pathways (*13*). Similar to FoxO1, adenosine signaling through A2AR promotes T cell quiescence and maintenance by increasing IL-7R expression (*10*), suggesting a potential link between adenosine signaling and FoxO1 signaling through IL-7, which is yet to be identified.

In this study, in experiments with mice doubly deficient in A2AR and IL-7R, as well as with A2AR-deficient mice expressing IL-7R ectopically, we investigated the direct functional relationship between IL-7 signaling and A2AR signaling in the regulation of CD8$^+$ T cell responses in vivo and ex vivo. Our data suggest that IL-7 signaling protects CD8$^+$ T cells from adenosine-mediated suppression of their accumulation in tumors through inactivation of FoxO1. Because both IL-7 and adenosine signaling are being targeted in the clinic for cancer and because they are key molecules for the regulation of homeostatic pathways and immune responses, our data have implications for the treatment of cancer and other immunometabolic diseases.

RESULTS

Adenosine signaling inhibits CD8$^+$ T cell activation in vitro

To test the effects of adenosine signaling on CD8$^+$ T cells in vitro, we used 5′-(*N*-ethylcarboxamido)adenosine (NECA), a cell-impermeable, stable adenosine analog. NECA significantly decreased the expansion of CD8$^+$ T cells after stimulation through the T cell receptor (TCR) complex with plate-bound anti-CD3 and anti-CD28 in vitro (fig. S1A). NECA also suppressed the activation-induced increase in CD25 cell surface expression (fig. S1B). However, NECA increased IL-7R (CD127 encoded by *il7r*) expression in activated CD8$^+$ T cells in a dose-dependent manner (fig. S1C).

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IL-7 signaling prevents adenosine signaling from inhibiting CD8$^+$ T cell accumulation in B16 melanoma tumors

To test whether IL-7R played an important role in the adenosine-dependent regulation of tumor-associated CD8$^+$ T cells, we crossed *adora2a*$^{-/-}$ mice with *il7r*$^{-/-}$ mice and tested these mice for tumor growth and infiltration of tumors by CD8$^+$ T cells and other immune cells in experiments in which *adora2a*$^{+/+}$*il7r*$^{-/-}$ littermates were used as controls. In *adora2*$^{-/-}$*il7r*$^{-/-}$ mice, tumor growth was significantly slower as compared with that in *adora2a*$^{+/+}$*il7r*$^{-/-}$ mice (Fig. 1A, right), which was associated with increased infiltration of the tumor by CD8$^+$ tumor-infiltrating lymphocytes (TILs) (Fig. 1B, right). However, tumors grew faster in *adora2a*$^{-/-}$ mice as compared with *adora2a*$^{+/+}$ littermates (Fig. 1A, left). In *adora2a*$^{-/-}$ mice, the proportion of CD8$^+$ TILs was less than that in *adora2a*$^{+/+}$ littermates (Fig. 1B, left), confirming our previous findings (*7*). The proportion of overall immune cells (CD45$^+$ cells) was also increased in tumors from *adora2a*$^{-/-}$*il7r*$^{-/-}$ mice as compared with that in tumors from *adora2a*$^{+/+}$*il7r*$^{-/-}$ mice (Fig. 1C, right). The proportions of tumor immune cells were comparable between *adora2a*$^{-/-}$ and *adora2a*$^{+/+}$ mice (Fig. 1C, left). The proportions of tumor-infiltrating immune cells (Fig. 1C) and the tumor density of various immune cell populations, including CD8$^+$ TILs, natural killer (NK) cells, and Ly6C$^+$ monocytes, were also increased in *adora2a*$^{-/-}$*il7r*$^{-/-}$ mice as compared with those in *adora2*$^{+/+}$*il7r*$^{-/-}$ littermates (Fig. 1D). Tumor infiltration by NK cells was also increased in *adora2a*$^{-/-}$ mice, whereas infiltration by monocytes was unchanged as compared with that in *adora2a*$^{+/+}$ littermates in our previous study (*7*), suggesting that the increase in NK cells was independent of IL-7 signaling in the context of A2AR deficiency, whereas changes in monocyte populations may be a secondary effect to the changes in CD8$^+$ T cell populations.

To test whether the effect of adenosine signaling on the accumulation of CD8$^+$ TILs was cell intrinsic, we reconstituted the bone marrow of irradiated wild-type mice (CD45.2$^+$) with a 1:1 mixture of bone marrow cells from *adora2a*$^{-/-}$ mice (CD45.2$^+$EGFP$^+$) and *adora2a*$^{+/+}$ (CD45.1$^+$) mice before inoculation with B16 melanoma tumors (fig. S2). The proportion of tumor-associated *adora2a*$^{-/-}$CD8$^+$ TILs was significantly reduced compared with that of *adora2*$^{+/+}$CD8$^+$ TILs, suggesting that A2AR signaling intrinsically mediates CD8$^+$ TIL accumulation in B16 melanoma tumors. Together, these results suggest that in the absence of IL-7Rs, A2AR signaling suppresses CD8$^+$ T cell responses and promotes tumor growth, whereas the presence of IL-7R can reverse this effect.

Ectopic expression of *il7r* reverses the tumor-promoting effects of *adora2a* deletion

In *adora2a*$^{-/-}$ mice, thymic accumulation and peripheral maintenance of T cells were reduced, and this reduction was associated with decreased CD127 expression (*10*). Therefore, we next investigated the effects of ectopic expression of *il7r* in A2AR-deficient

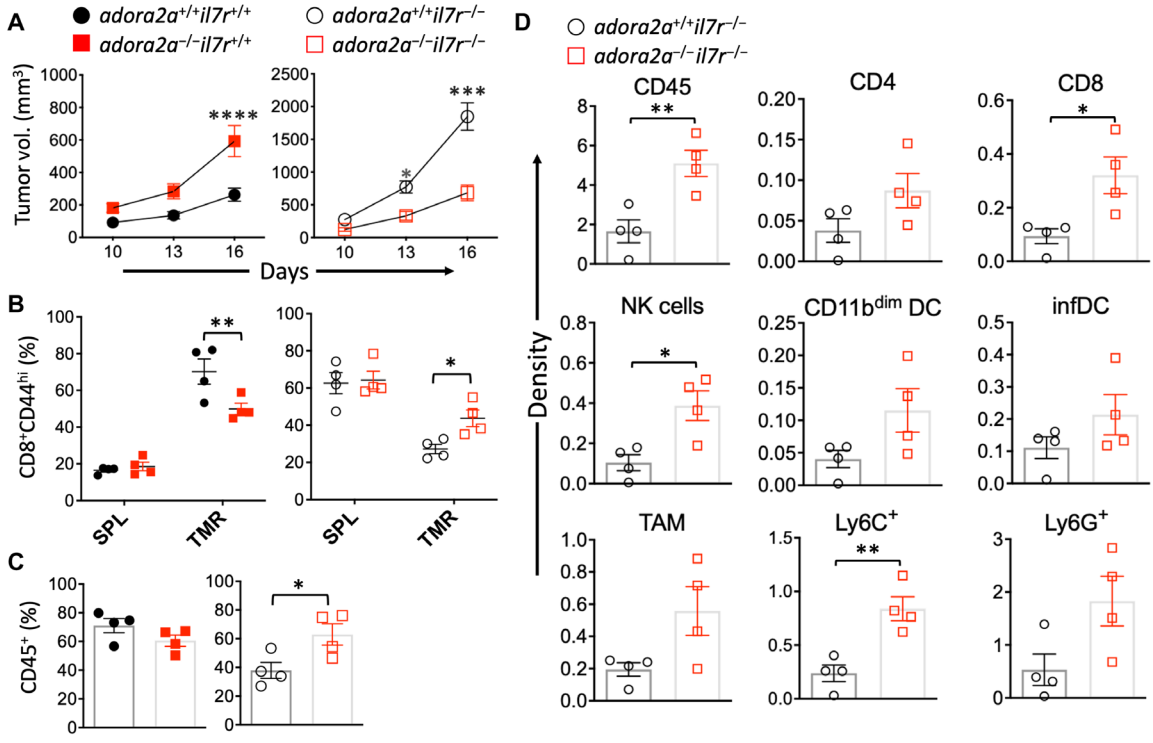

Fig. 1. IL-7R signaling protects T cells from A2AR-mediated suppression. (**A**) Analysis of in vivo tumor growth in *adora2a*$^{-/-}$*il7r*$^{+/+}$ versus adora2a$^{+/+}$*il7r*$^{+/+}$ littermates as controls (left) or adora2a$^{-/-}$*il7r*$^{-/-}$ versus adora2a$^{+/+}$*il7r*$^{-/-}$ littermates as controls (right) after the injection of 1×10^5 B16F10 melanoma cells. Data are pooled from two independent experiments with similar results; $N \geq 11$ mice. ***$P < 0.001$ and ****$P < 0.0001$ by two-way ANOVA and Sidak's multiple comparisons test. (**B**) Flow cytometry analysis of the proportions of CD8$^+$ TILs among total CD8$^+$ T cell populations in tumor tissues from the experiments shown in (A). Spleen tissues were used as a control. (**C** and **D**) Proportions of CD45$^+$ immune cell populations among live cells (C) and tumor density (numbers of cells/numbers of counting beads/tumor volume) of the indicated immune cell populations (D) in *adora2*$^{-/-}$*il7r*$^{-/-}$ versus *adora2a*$^{+/+}$*il7r*$^{-/-}$ mice. For (C) and (D), *$P < 0.05$ and **$P < 0.01$ by Student's *t* test. For (B) to (D), data are representative of one of two independent experiments, $n = 4$ mice per group. SPL: spleen; TMR: tumor; TAM: tumor-associated macrophages.

T cells by crossing *adora2a*$^{-/-}$ mice with *il7r*$^{-/-}$Lck*il7r* mice (mice expressing *il7r* ectopically under the proximal *lck* promoter) and compared their thymic and peripheral T cell populations with those of their *adora2a*$^{+/+}$*il7r*$^{-/-}$Lck*il7r* littermate controls (Fig. 2). Ectopic expression of *il7r* caused comparable cell surface expression of CD127 between *adora2a*$^{+/+}$*il7r*$^{-/-}$Lck*il7r* and *adora2a*$^{-/-}$*il7r*$^{-/-}$Lck*il7r* T cells from the thymus and spleen (Fig. 2, A and B, right). Ex vivo analysis indicated similar IL-7 responsiveness between *adora2a*$^{+/+}$*il7r*$^{-/-}$Lck*il7r* and *adora2a*$^{-/-}$*il7r*$^{-/-}$Lck*il7r* T cells (Fig. 2C, right). We observed decreased CD127 expression on thymic and peripheral T cells from *adora2a*$^{-/-}$ mice as compared with that on CD8$^+$ T cells from *adora2a*$^{+/+}$ mice (Fig. 2, A and B, left). Ex vivo, IL-7 responsiveness of CD8$^+$ T cells from *adora2a*$^{-/-}$ mice was reduced as compared with that of CD8$^+$ T cells from *adora2a*$^{-/-}$ mice (Fig. 2C, left). Proportions of peripheral T cells were similar between *adora2a*$^{-/-}$*il7r*$^{-/-}$Lck*il7r* and *adora2a*$^{-/-}$*il7r*$^{-/-}$Lck*il7r* mice, whereas proportions of peripheral T cells were reduced in *adora2a*$^{-/-}$ mice as compared with *adora2a*$^{+/+}$ mice (Fig. 2D). B16 melanoma growth, T cell infiltration, and overall immune cell infiltration (CD45$^+$ cells) in *adora2a*$^{-/-}$*il7r*$^{-/-}$Lck*il7r* mice were similar to those of *adora2a*$^{+/+}$*il7r*$^{-/-}$Lck*il7r* mice (Fig. 2, E and F, and fig. S3). A2AR-deficient mice ectopically expressing *il7r* (*adora2a*$^{-/-}$*il7r*$^{-/-}$Lck*il7r*) had similar numbers and proportions of CD4$^+$ and CD8$^+$ T cells in the thymus and in the periphery as compared with those of their *adora2a*$^{+/+}$*il7r*$^{-/-}$Lck*il7r* littermates (figs. S4 and S5). We observed decreased numbers of thymic and peripheral CD4$^+$ and CD8$^+$ T cells from *adora2a*$^{-/-}$ mice as compared with those of CD8$^+$ T cells from *adora2a*$^{+/+}$ mice (figs. S4 and S5), consistent with our previous observations (*10*). Note that neither A2AR deficiency nor ectopic expression of CD127 changed the proportions of T cells with specific TCR Vβ subunit subtypes, suggesting that these genetic modifications did not substantially influence T cell clonality (fig. S6). Expression of other cytokine receptors such as IL-4R, IL-15R, and IL-2R in thymocytes from *adora2a*$^{+/+}$ versus *adora2a*$^{-/-}$ mice or *adora2a*$^{+/+}$*il7r*$^{-/-}$Lck*il7r* versus *adora2a*$^{-/-}$*il7r*$^{-/-}$Lck*il7r* mice was comparable (fig. S7). Together, these results suggest that ectopic expression of CD127 rescues A2AR-deficient T cells and neutralizes the tumor-promoting effects of A2AR deletion.

Adenosine-mediated suppression of CD8$^+$ T cell expansion is reversed by FoxO1 inhibition, downstream of IL-7

To understand the molecular mechanism by which IL-7 rescued CD8$^+$ T cells from adenosine-mediated immunosuppression, we first replicated our in vivo observations in vitro. The adenosine analog NECA suppressed CD8$^+$ T cell expansion after activation in a dose-dependent manner, which was reversed by the addition of

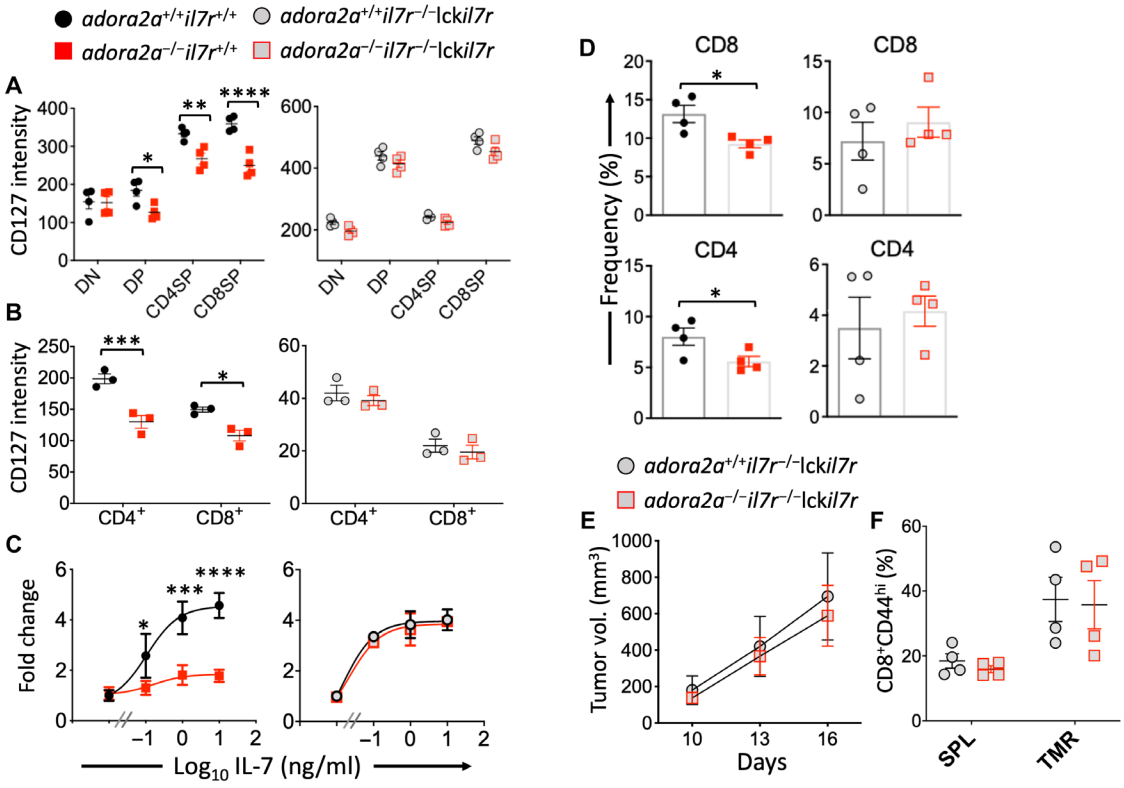

Fig. 2. Ectopic expression of *il7r* restores IL-7 responsiveness and tumor accumulation of A2AR-deficient T cells. (**A** to **D**) *adora2a*$^{-/-}$*il7r*$^{+/+}$ versus *adora2a*$^{+/+}$*il7r*$^{+/+}$ littermates as controls (left) or *adora2a*$^{-/-}$*il7r*$^{-/-}$lck*il7r* versus *adora2a*$^{+/+}$*il7r*$^{-/-}$lck*il7r* littermates as controls (right) were used to compare CD127 expression in thymocytes (A), CD127 expression in splenic T cells (B), IL-7 responsiveness of isolated CD8$^+$ T cells as calculated by the fold change in T cell numbers as compared to the average of vehicle-treated group (C), and proportions of CD4$^+$ and CD8$^+$ T cells from the spleen (D). For (A) to (D), $n \geq 3$ mice per group. For (A) to (C), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ by two-way ANOVA and post hoc Sidak's multiple comparisons test. For (D), *$P < 0.05$ by Student's *t* test. (**E**) Growth of B16F10 melanoma tumors. Data are pooled from two independent experiments, $n \geq 7$ mice per group. (**F**) Proportions of tumor- or spleen-associated CD8$^+$CD44$^+$ T cells from *adora2a*$^{-/-}$*il7r*$^{-/-}$lck*il7r* mice and *adora2a*$^{+/+}$*il7r*$^{-/-}$lck*il7r* littermates. Data in (E) and (F) were analyzed by two-way ANOVA and post hoc Sidak's multiple comparisons. CD127 intensity: geometric mean of CD127 expression by flow cytometry.

IL-7 (Fig. 3A). IL-7 signaling suppresses FoxO1 activities by decreasing its expression and by increasing its inactivation through the phosphorylation of Ser^{256} (*13*). FoxO1 activation can control expansion of T cells (*12*). Therefore, we investigated the involvement of FoxO1 signaling in the adenosine-mediated regulation of T cell activation. We stimulated $CD8^+$ T cells in the presence or absence of NECA with or without IL-7 and analyzed FoXO1 phosphorylation. The abundance of FoxO1 phosphorylated at Ser^{256} was decreased in NECA-treated cells compared to that in cells activated in the absence of NECA, suggesting that adenosine signaling may sustain FoxO1 activation after T cell stimulation (Fig. 3B). T cell activation led to increased FoXO1 phosphorylation. The addition of IL-7 did not further increase the FoxO1 phosphorylation after T cell activation; however, IL-7 reversed the inhibitory effects of NECA on FoxO1 phosphorylation (Fig. 3B), suggesting that IL-7 signaling prevented sustained FoXO1 activation in the presence of adenosine signaling. FoXO1 inhibition by the cell-permeable and selective FoxO1 inhibitor, AS1842856, together with NECA treatment not only completely reversed the suppression of T cell proliferation as compared with that of cells treated with NECA alone but also significantly increased T cell proliferation as compared with that of the positive control group (anti-CD3/anti-CD28). Inhibition of T cell proliferation by adenosine signaling was associated with increased caspase 3/7 activation (Fig. 3D). Adenosine receptor activation together with FoXO1 inhibition also caused a significant reduction in caspase activation as compared with that in the control cells (Fig. 3D).

Adenosine signaling through A2AR causes the accumulation of cyclic adenosine monophosphate (cAMP) to regulate immune responses (*6*). Accordingly, stimulation of $CD8^+$ T cells with an A2AR agonist caused the accumulation of cAMP, which was reversed by the addition of the A2AR antagonist 5-amino-7-[2-(4-fluorosulfonyl) phenylethyl]-2-(2-furyl)-pryazolo[4,3-ε]-1,2,4-triazolo[1,5-c] pyrimidine (FSPTP) (Fig. 3E). The addition of the stable cAMP analog, (*S*)-adenosine, cyclic 3′,5′-(hydrogenphosphorothioate), during anti-CD3/anti-CD28–dependent activation significantly reduced T cell proliferation (Fig. 3F). However, when the $CD8^+$ T cells were treated

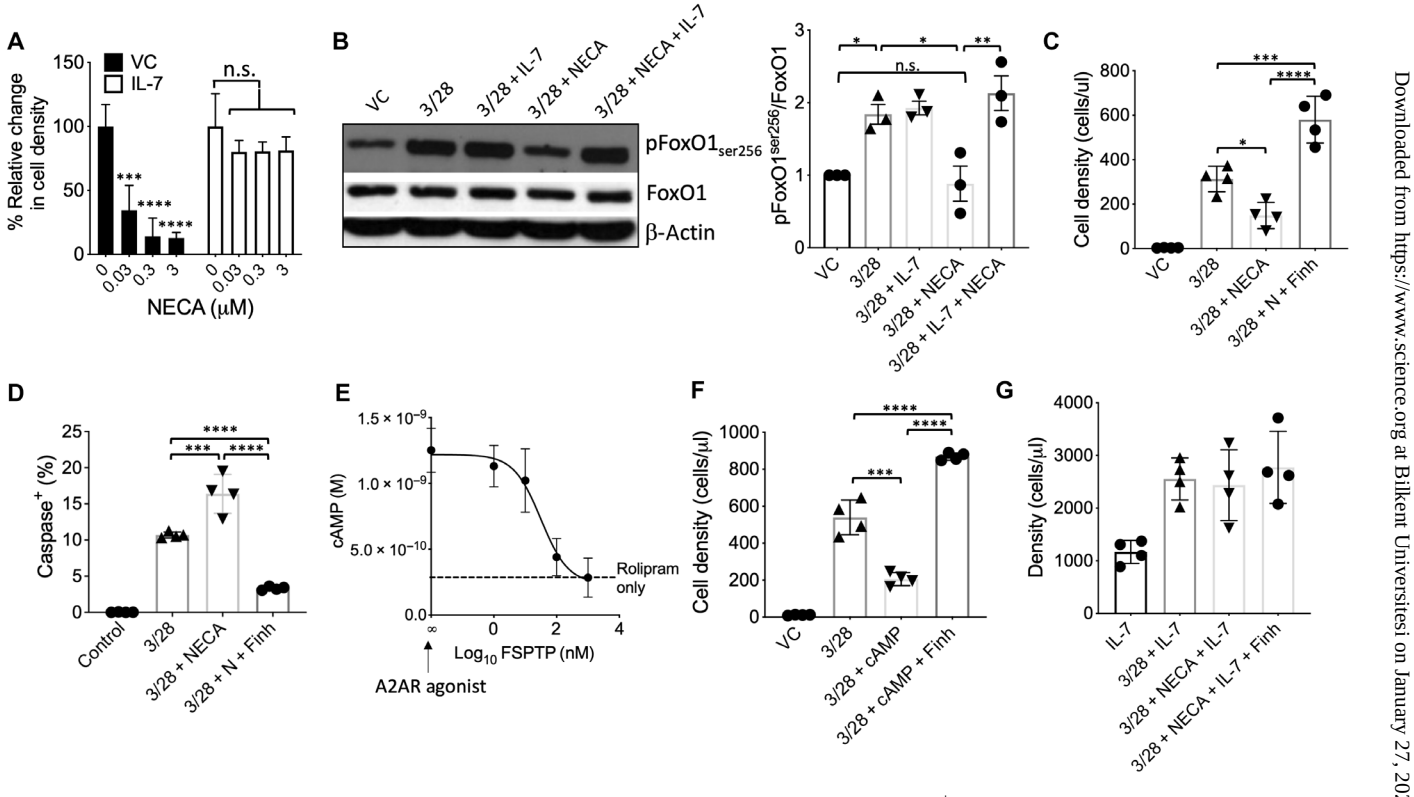

Fig. 3. IL-7 signaling prevents adenosine-mediated suppression of T cell accumulation in vitro. (**A**) Naïve $CD8^+$ T cells were isolated from the spleens of wild-type mice and stimulated with plate-bound anti-CD3/anti-CD28 (3/28). NECA (0.03 to 3 μM) with or without recombinant murine IL-1 (rmIL-7, 10 ng) were added to the culture medium before stimulation. Medium was replaced with fresh medium 48 hours after stimulation. T cell accumulation (numbers of cells/μl) was measured by flow cytometry 4 days after cell culture. DMSO was used as a vehicle. $n = 4$ replicates per group; $^{***}P < 0.001$ and $^{****}P < 0.0001$ by two-way ANOVA and Tukey's multiple comparisons test. n.s.: not significant. (**B**) Isolated naïve T cells were stimulated for 15 min with anti-CD3 and anti-CD28 in the presence or absence of 1 mM NECA and/or IL-17 (10 ng/ml). The cells were then lysed with RIPA buffer and analyzed by Western blotting with antibodies against for phosphorylated FoxO1 at Ser^{256}, total FoxO1, and β-actin as a loading control. Blots are representative of two independent experiments. (**C** and **D**) $CD8^+$ T cells were stimulated in the presence or absence of NECA alone or with the FoxO1 inhibitor (Finh) for 4 days with fresh medium added after 48 hours. (C) The numbers of cells were measured by flow cytometry. (D) Caspase 3/7 activation was measured by flow cytometry. (**E**) $CD8^+$ T cells were activated with plate-bound anti-CD3 and anti-CD28 for 24 hours and treated with A2AR agonist CGS 21680 (1 mM) in the absence or presence of various concentrations of the A2A antagonist FSPTP for 10 min. The concentration of cAMP was measured in the presence of 10 μM rolipram. (**F**) $CD8^+$ T cells were stimulated in the presence or absence of stable cAMP analog cAMP-Sp (cAMP) in the presence or absence of FoxO1 inhibitor. (**G**) $CD8^+$ T cells were stimulated with anti-CD3/anti-CD28 (3/28) and IL-7 in the presence or absence of NECA alone or with the FoxO1 inhibitor. Cell density was measured after 4 days of culture by flow cytometry. For (C), (D), (F), and (G), $n = 4$ replicates per group; $^*P < 0.05$, $^{***}P < 0.001$, and $^{****}P < 0.001$ by one-way ANOVA and post hoc Tukey's test. Results in all panels are representative of at least two experiments with similar results.

with both the cAMP analog and the FoXO1 inhibitor, they showed increased proliferation compared with that of cells stimulated with anti-CD3/anti-CD28 alone (Fig. 3F). The addition of the FoXO1 inhibitor did not further promote T cell proliferation in the presence of IL-7 alone or with NECA (Fig. 3G). Together, these results suggest that FoxO1 inactivation downstream of IL-7R signaling may be one of the main pathways that promotes T cell expansion in the presence of immunosuppressive adenosine.

Expression of *adora2a* positively correlates with *il7r* expression and is associated with increased survival among patients with melanoma

Our analysis using The Cancer Genome Atlas (TCGA) database showed that *adora2a* rather than *adora2b* positively correlated with *il7r* expression in human melanoma samples (Fig. 4A). Increased expression of *il7r* was associated with the increased survival of patients with melanoma (Fig. 4B). Similar to *il7r*, increased expression of *adora2a* was associated with prolonged survival in patients with melanoma (Fig. 4C), whereas expression of *adora2b* was associated with poorer survival (Fig. 4D), indicating an overlap between clinical outcomes and our findings.

DISCUSSION

The evidence presented here suggests that IL-7 signaling regulates T cell responses to extracellular adenosine. We found that in the absence of IL-7R expression, signaling by the adenosine receptor A2AR was protumoral, limiting the infiltration of tumors by $CD8^+$ T cells, NK cells, and monocytes. However, in the presence of IL-7R signaling, deletion of A2AR promoted tumor growth and limited T cell accumulation in the tumor. Previously, we observed that above-threshold TCR signaling due to A2AR deficiency causes a reduction in the cell surface abundance of CD127 and reduces T cell development and maintenance (*10*). Here, we showed that ectopic expression of IL-7R reversed the impaired T cell development and peripheral maintenance due to A2AR deficiency. Ectopic expression of IL-7R also reversed the protumoral effects of A2AR deficiency. In vitro, adenosine signaling suppressed T cell activation but sustained CD127 expression. Adenosine-mediated suppression of T cell proliferation was prevented by the addition of IL-7 or by inhibition of a downstream target of IL-7, FoxO1. In patients with human melanoma, the expression of *adora2a* and *il7r* was correlated and associated with increased survival.

In preclinical settings, IL-7 suppresses tumor growth in several tumor models, including murine melanoma, by increasing T cell infiltration and persistence (*14*, *15*). IL-7 increases $CD8^+$ T cell infiltration in tumors by decreasing T cell exhaustion and increasing cell renewal capacity. IL-7 also antagonizes the immunosuppression by myeloid-derived suppressor cells (MDSCs) and regulatory T (T_{reg}) cells without changing their proliferation (*16*, *17*). T_{regs} and MDSCs produce ectonucleotidases that catalyze the dephosphorylation of ATP to adenosine (*4*, *18*). Adenosine generation by these cells is viewed as one of the main mechanisms for the suppression of $CD8^+$ T cells. Our data suggest that one of the mechanisms by which IL-7 can be effective is to maintain activated $CD8^+$ T cells despite the presence of immunosuppressive extracellular adenosine. In this scenario, adenosine signaling would also support the IL-7 responsiveness of $CD8^+$ T cells by sustaining CD127 expression to obtain this combinatorial effect.

IL-7 is important for the development and peripheral homeostasis of T cells (*19*). Our previous observations suggested that excessive TCR signaling due to A2AR deficiency causes decreased IL-7R expression and impaired T cell development and homeostasis (*1*, *8*). In this study, we provided further evidence that ectopic expression of the IL-7R can reverse this phenotype. Deletion of A2AR or ectopic expression of IL-7R in the presence or absence of A2AR did not change the proportion of T cells with particular Vβ chain expression, suggesting that this interaction may mostly influence T cell maintenance rather than T cell clonality. However, further research is required to determine whether A2AR deficiency or inhibition could change the proportion of thymic T cell clones and, therefore, the T cell subsets that recognize tumor neoantigens.

Tumor growth was slower in A2AR/CD127 co-deficient mice than in CD127-deficient mice, whereas in A2AR-deficient mice, tumor growth was faster than that in A2AR-proficient mice. Note that ectopic expression of CD127 in A2AR-deficient mice did not cause slower tumor growth as compared with that in littermate controls ($il7r^{-/-}$Lck*il7r*), suggesting that A2AR signaling not only controls CD127 expression but also influences other aspects of T cell responses to cause slower tumor growth in wild-type mice as compared with A2AR-deficient mice in the presence of IL-7R signaling. These results, together with the fact that there are only a few T cells in CD127-deficient mice, also emphasize the potential importance of adenosine-mediated suppression of innate immune

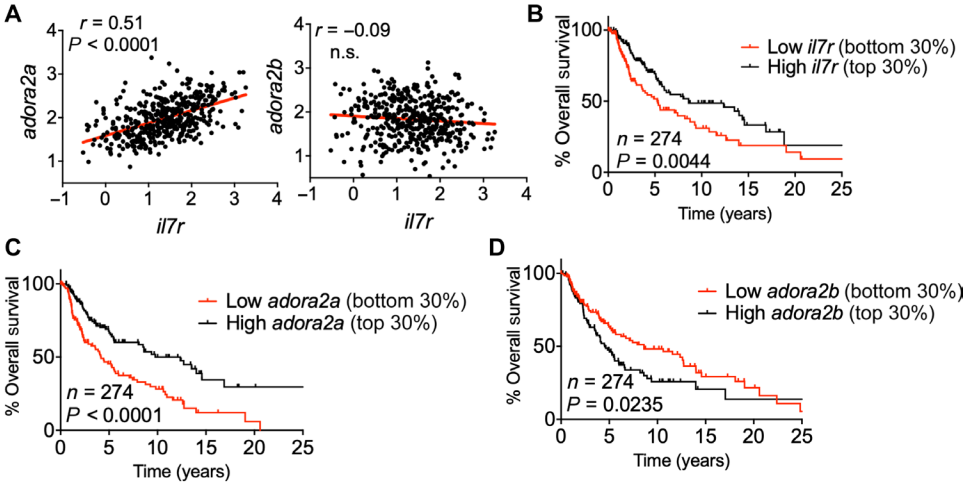

Fig. 4. Expression of CD127 and A2AR is positively correlated and associated with prolonged survival in patients with human melanoma. (**A** to **D**) Gene expression and survival data for the *il7r*, *adora2a*, and a*dora2b* genes among patients with melanoma were downloaded from the TCGA database using the OncoLnc tool. (A) Correlation between *il7r* and *adora2a* or *adora2b* expression was calculated and graphed by simple linear regression. (B to D) The survival of high versus low *il7r* expressors (B), high versus low *adora2a* expressors (C), and high versus low *adora2b* expressors (D) was analyzed by the log-rank (Mantel-Cox test) method and plotted as Kaplan-Meier curves.

components as a tumor-promoting mechanism, which is supported by previous reports (*8*, *20*).

Adenosine receptor signaling is targeted in the clinic by various methods: blockade of A2AR, A2BR, or both; and blockade or inhibition of ectonucleotidases, such as CD39 and CD73 (*5*). However, so far, these approaches have had very limited success, which may be partially due to the fact that they were tested in heavily pretreated patients, who are refractory to other therapies. One other potential challenge for these approaches is choosing the appropriate patient population. Therefore, a biomarker-driven approach is needed. The discovery of potential biomarkers depends on a better understanding of the mechanisms of adenosine-mediated regulation of the immune system. Our previous observations emphasized the importance of myeloid cells as targets for adenosine-mediated suppression of antitumor immune responses (*8*, *21*, *22*). Accordingly, Fong *et al.* (*9*) identified an adenosine gene signature that consists mainly of myeloid cell–specific genes. The adenosine gene signature successfully predicted responding patient populations to adenosine receptor blockade in renal cancer (*9*). A2AR signaling in both hematopoietic and nonhematopoietic cells also promotes lymphangiogenesis, promoting the disseminations of tumors (*23*), further suggesting that the tumor-promoting effects of A2AR signaling may go beyond the cell-intrinsic effects of adenosine on T cells. On the basis of our work here and a study indicating that microbiome-derived inosine increases checkpoint blockade responsiveness through A2AR signaling (*24*), negative predictors (*adora2a* and IL-7 signature or microbiome/growth factor signature) of therapy response can also be identified to further stratify the correct therapy-responsive populations. Our observations that *il7r* expression is positively correlated with *adora2a* expression and that both *il7r* and *adora2a* expression is associated with a favorable outcome in patients with human melanoma further support this notion and the gene expression analysis of melanoma tumors by Gide *et al.* (*25*), in which *adora2a* is listed as one of the genes that predicts checkpoint blockade responsiveness.

In summary, our work showed that a protumoral metabolic factor (adenosine) can have anti- or protumoral effects depending on the cytokine (for example, IL-7) milieu. Therefore, our research increases our understanding of the interactions between immunosuppressive metabolites and different cytokine receptors regulating FoxO1 signaling in the TME and peripheral tissues. Other agents that increase cAMP abundance may interact with cytokine signaling in a similar manner to that discussed here, resulting in unexpected clinical outcomes for a given therapy. This will help the development of better strategies to treat cancer and potentially other immune-related diseases associated with immunosuppressive metabolite accumulation.

MATERIALS AND METHODS

Mice and reagents

B16F10 cells were obtained from the American Type Culture Collection. Transgenic mice expressing the *il7r* gene under the proximal *Lck* promoter and lacking endogenous *il7r* expression ($il7r^{-/-}$lck*il7r*) were obtained from the Jackson laboratory. The $adora2a^{-/-}$ mice that was produced by Chen *et al.* (*26*) on a mixed genetic background were backcrossed onto C57BL/6 mice. Six-week-old C57BL/6J, CD45.1, and $EGFP^+$ mice were purchased from the Jackson laboratory, crossed with $adora2a^{-/-}$ mice, and used for experiments after being acclimated for 2 to 6 weeks. $adora2a^{-/-}il7r^{-/-}$ mice, $adora2a^{-/-}il7r^{-/-}$lck*il7r* mice, and littermate controls were obtained by crossing $adora2a^{-/-}$ mice with $il7r^{-/-}$lck*il7r* mice. Only littermate controls were used across the study. The antibodies used in this study for flow cytometry analysis are shown in table S1.

Tumor model

B16F10 cells were cultured in R5F [RPMI 1640 containing 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate, penicillin (50 U/ml), and streptomycin (50 mg/ml)]. Tumor cells were injected into mice after reaching 60 to 80% confluence. B16-F10 cells (1×10^5) in suspension were injected subcutaneously into the mice. Tumor volumes were measured with digital calipers and were calculated as height × $width^2/2$.

Bone marrow reconstitution

Bone marrow reconstitution of C57BL/6 mice ($CD45.2^+$) with bone marrow cells from congenic $CD45.1^+$ mice and $EGF^+adora2a^{-/-}$ mice was performed as described previously (*10*). Briefly, 6- to 12-week-old mice were fasted for 24 hours and then lethally irradiated (2 × 600 rads). After the second radiation exposure, a 1:1 mixture of 5 to 10×10^6 bone marrow cells from donor mice ($CD45.1^+adora2a^{+/+}$ and $CD45.2^+EGF^+adora2a^{-/-}$) were injected intravenously. Mice were treated with antibiotics from 3 days before until 2 weeks after radiation. We injected 1×10^5 B16F10 melanoma cells subcutaneously into the right flanks 10 weeks after reconstitution of the bone marrow. Single-cell suspensions from spleens and tumors were analyzed by cytofluorometry to determine the proportion of $CD44^+$ cells within the $CD45.1^+adora2a^{+/+}$ or $CD45.2^+EGF^+adora2a^{-/-}$ $CD8^+$ T cell populations.

Flow cytometry

Tumors were removed and used to prepare single-cell suspensions by sequential pressing through 100- and 40-μm cell strainers. Cells were washed and resuspended in R10F medium and counted in a Z2 Coulter particle counter (Beckman Coulter). Single-cell suspensions were preincubated for 10 min in 100 μl of fluorescence-activated cell sorting (FACS) buffer [phosphate-buffered saline (PBS) (pH 7.2), 1% heat-inactivated FBS, and 0.05% NaN_3] with anti-CD16/32 antibodies to block Fc receptors. Each sample tube received 100 μl of fluorescently labeled antibody mixture and was incubated for 30 min at 4°C in the dark. After a subsequent wash, the cells were fixed with Fix/Perm buffer (BD Biosciences) for 15 min in the dark. After a final wash in PBS, cells were resuspended in 350 μl of FACS buffer and were analyzed with an LSR II flow cytometer equipped with four lasers and FACSDiva software (BD Biosciences). Live/dead fixable yellow (Invitrogen) was used to exclude dead cells before analysis. Flow cytometry data were analyzed with FlowJo software (9.0.1 version; Tree Star).

In vitro T cell activation

Tissue culture plates were coated with anti-hamster antibody at room temperature in PBS for 2 hours. Anti-mouse CD3ε, clone 145-2C11 (2 μg/ml), and anti-mouse CD28 clone 37.51 (2 μg/ml) (both antibodies from Tonbo Biosciences) were added in the presence or absence of 1 μM NECA (Tocris) or the stable cell-permeable cAMP analog cAMP-Sp (100 μM, Tocris) with or without the FoXO1 inhibitor AS1842856 (25 nM, Merck Millipore) after blocking the plate in cell culture medium (RPMI 1640, 10% FBS, 10 mM Hepes,

1× penicillin/streptomycin, 1× glutamine, 1× Na-pyruvate, and 50 μM 2-mercaptoethanol). Recombinant adenosine deaminase (1 U/ml, Roche) was added to the culture medium to prevent the effect of endogenously generated adenosine. Freshly isolated T cells (3×10^4 cells per well) were added, and plates were centrifuged at 300*g* using low brake mode. For some experiments, recombinant mouse IL-7 (10 ng/ml, BioLegend) was added on day 0 and during feeding by replacing the medium on day 2. To test the effect of ectopic expression of *il7r* on naïve T cell survival, the concentrations of IL-7 indicated in the figure legends were added to naïve, unstimulated T cell suspensions using T cells from wild-type mice or *adora2a*$^{-/-}$ littermates as controls or *adora2a*$^{-/-}$*il7r*$^{-/-}$lck*il7r* versus *adora2a*$^{+/+}$*il7r*$^{-/-}$lck*il7r* littermates as controls. The responsiveness of naïve T cells to IL-7 was measured by the fold difference between the absolute numbers of T cells compared to vehicle. T cell responses were measured by flow cytometry using a CytoFLEX flow cytometer and NovoExpress Software. CellEvent Caspase-3/7 Green ReadyProbes Reagent (Life Technologies) was used according to the manufacturer's instructions to test for caspase activation as a measure of apoptosis in T cells.

Western blotting

Single-cell suspensions were prepared from mouse spleens, and CD8$^+$ T cells were isolated using a CD8$^+$ T cell enrichment kit (STEMCELL). For Western blotting analysis, 1×10^6 isolated T cells in cell culture medium were incubated on ice in Eppendorf tubes in the presence of anti-CD3 and anti-CD28 antibodies for 10 min. Cross-linking anti-Syrian hamster antibody and NECA or dimethyl sulfoxide (DMSO) control was added before the transfer of cells to 37°C for an additional 10 min. After incubation, the cells were centrifuged twice at 4°C and lysed in radioimmunoprecipitation assay (RIPA) buffer. The BCA Protein Assay (Thermo Scientific) was used to measure the protein concentrations. Equal amounts of protein lysates for each treatment group were resolved by SDS–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The membranes were blocked in the presence of 5% nonfat dry milk or 5% bovine serum albumin (BSA) for 1 hour at room temperature and were incubated with primary antibodies at 4°C overnight. Blots were developed with Clarity Western ECL substrate (Bio-Rad) after being incubated with horseradish peroxidase–conjugated secondary antibody, and bands were then visualized by film developer.

Measurement of intracellular cAMP

Intracellular cAMP concentration was determined on the basis of the modified protocol from LANCE Ultra cAMP Kit (PerkinElmer). Briefly, T cells activated in the presence of anti-CD3/anti-CD28 for 24 hours were resuspended in freshly made stimulation buffer (1× Hanks' balanced salt solution, 5 mM Hepes, and 0.1% BSA). Cells were preincubated with 0 to 1000 nM FSPTP at 37°C for 60 min before the addition of 10 μM Rolipram or 1 μM CGS 21680. After incubating the cells for 10 min, ice-cold 0.6 M perchloric acid was added to stop the reaction and to precipitate the protein fraction of the cells. We then added 2.5 M potassium carbonate to neutralize the lysate. Clear lysate was obtained by collecting the supernatants after centrifugation at 12,000*g* for 10 min. Working Eu cAMP tracer solution and ULight-anti-cAMP solution were added to the supernatants and incubated for 1 hour at room temperature. Time-resolved fluorescence energy transfer emission was measured with a SpectraMax plate reader using an emission wavelength of 665 nm and a correction wavelength of 615 nm.

Bioinformatics analysis

Survival and gene expression data were downloaded from the TCGA database using the OncoLnc tool (www.oncolnc.org). Km plots were drawn for high expressors (the top 30%) and low expressors (the bottom 30%) of the patient population. For correlation analysis, linear regression of log-transformed expression values was used to calculate Pearson's correlations coefficient (*r*) value and statistical significance.

Statistical methods

Data were analyzed by GraphPad Prism Software (version 8.4.3). For datasets involving two groups, Student's *t* tests were performed; for datasets involving more than two groups, one-way analysis of variance (ANOVA) was performed; and for samples involving more than one variable, two-way ANOVA tests were performed. ANOVA tests also included post hoc multiple comparisons analysis. The choice of post hoc analysis was determined by whether the comparison was made between experimental groups and control group or between all of the groups. For tumor growth experiments, normal distribution was tested using the Shapiro-Wilk test and two-way ANOVA test together with post hoc analysis for multiple comparisons for each day.

SUPPLEMENTARY MATERIALS

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Fig. S1. Adenosine signaling inhibits T cell activation but sustains CD127 expression in vitro.

Fig.S2. Cell-intrinsic adenosine signaling is associated with reduced accumulation of CD8$^+$ TILs in tumors.

Fig. S3. A2AR deficiency does not change the proportion of tumor-infiltrating immune cells after ectopic expression of *il7r*.

Fig. S4. Ectopic expression of *il7r* restores the percentage of SP thymocytes in A2AR-deficient mice.

Fig. S5. Ectopic expression of *il7r* restores the numbers of T cells in the thymus and spleen of A2AR-deficient mice.

Fig. S6. Vβ selection of T cells is similar between *adora2a*$^{+/+}$ and *adora2a*$^{-/-}$ or *adora2a*$^{+/+}$*il7r*$^{-/-}$lck*il7r* and *adora2a*$^{-/-}$*il7r*$^{-/-}$lck*il7r* mice.

Fig. S7. Expression of IL-2R, IL-4R, and IL-15R is similar between *adora2a*$^{+/+}$ and *adora2a*$^{-/-}$ or *adora2a*$^{+/+}$*il7r*$^{-/-}$lck*il7r* and *adora2a*$^{-/-}$*il7r*$^{-/-}$lck*il7r* mice.

Table S1. Antibodies used for flow cytometry.

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. C. Cekic, J. Linden, Purinergic regulation of the immune system. *Nat. Rev. Immunol.* **16**, 177–192 (2016).
2. D. M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* **12**, 252–264 (2012).
3. B. Allard, D. Allard, L. Buisseret, J. Stagg, The adenosine pathway in immuno-oncology. *Nat. Rev. Clin. Oncol.* **17**, 611–629 (2020).
4. T. Maj, W. Wang, J. Crespo, H. Zhang, W. Wang, S. Wei, L. Zhao, L. Vatan, I. Shao, W. Szeliga, C. Lyssiotis, J. R. Liu, I. Kryczek, W. Zou, Oxidative stress controls regulatory T cell apoptosis and suppressor activity and PD-L1-blockade resistance in tumor. *Nat. Immunol.* **18**, 1332–1341 (2017).
5. D. Vijayan, A. Young, M. W. L. Teng, M. J. Smyth, Targeting immunosuppressive adenosine in cancer. *Nat. Rev. Cancer* **17**, 709–724 (2017).
6. J. Linden, C. Cekic, Regulation of lymphocyte function by adenosine. *Arterioscler. Thromb. Vasc. Biol.* **32**, 2097–2103 (2012).
7. C. Cekic, J. Linden, Adenosine A2A receptors intrinsically regulate CD8+ T cells in the tumor microenvironment. *Cancer Res.* **74**, 7239–7249 (2014).
8. C. Cekic, Y. J. Day, D. Sag, J. Linden, Myeloid expression of adenosine A2A receptor suppresses T and NK cell responses in the solid tumor microenvironment. *Cancer Res.* **74**, 7250–7259 (2014).
9. L. Fong, A. Hotson, J. D. Powderly, M. Sznol, R. S. Heist, T. K. Choueiri, S. George, B. G. M. Hughes, M. D. Hellmann, D. R. Shepard, B. I. Rini, S. Kummar, A. M. Weise,

M. J. Riese, B. Markman, L. A. Emens, D. Mahadevan, J. J. Luke, G. Laport, J. D. Brody, L. Hernandez-Aya, P. Bonomi, J. W. Goldman, L. Berim, D. J. Renouf, R. A. Goodwin, B. Munneke, P. Y. Ho, J. Hsieh, I. McCaffery, L. Kwei, S. B. Willingham, R. A. Miller, Adenosine 2A receptor blockade as an immunotherapy for treatment-refractory renal cell cancer. *Cancer Discov.* **10**, 40–53 (2020).
10. C. Cekic, D. Sag, Y. J. Day, J. Linden, Extracellular adenosine regulates naive T cell development and peripheral maintenance. *J. Exp. Med.* **210**, 2693–2706 (2013).
11. Y. M. Kerdiles, D. R. Beisner, R. Tinoco, A. S. Dejean, D. H. Castrillon, R. A. DePinho, S. M. Hedrick, Foxo1 links homing and survival of naive T cells by regulating L-selectin, CCR7 and interleukin 7 receptor. *Nat. Immunol.* **10**, 176–184 (2009).
12. S. M. Hedrick, R. Hess Michelini, A. L. Doedens, A. W. Goldrath, E. L. Stone, FOXO transcription factors throughout T cell biology. *Nat. Rev. Immunol.* **12**, 649–661 (2012).
13. C. E. Martin, D. S. Spasova, K. Frimpong-Boateng, H. O. Kim, M. Lee, K. S. Kim, C. D. Surh, Interleukin-7 availability is maintained by a hematopoietic cytokine sink comprising innate lymphoid cells and T cells. *Immunity* **47**, 171–182.e4 (2017).
14. L. X. Wang, R. Li, G. Yang, M. Lim, A. O'Hara, Y. Chu, B. A. Fox, N. P. Restifo, W. J. Urba, H. M. Hu, Interleukin-7-dependent expansion and persistence of melanoma-specific T cells in lymphodepleted mice lead to tumor regression and editing. *Cancer Res.* **65**, 10569–10577 (2005).
15. K. Adachi, Y. Kano, T. Nagai, N. Okuyama, Y. Sakoda, K. Tamada, IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat. Biotechnol.* **36**, 346–351 (2018).
16. M. Habibi, M. Kmieciak, L. Graham, J. K. Morales, H. D. Bear, M. H. Manjili, Radiofrequency thermal ablation of breast tumors combined with intralesional administration of IL-7 and IL-15 augments anti-tumor immune responses and inhibits tumor development and metastasis. *Breast Cancer Res. Treat.* **114**, 423–431 (2009).
17. A. Andersson, S. C. Yang, M. Huang, L. Zhu, U. K. Kar, R. K. Batra, D. Elashoff, R. M. Strieter, S. M. Dubinett, S. Sharma, IL-7 promotes CXCR3 ligand-dependent T cell antitumor reactivity in lung cancer. *J. Immunol.* **182**, 6951–6958 (2009).
18. L. Li, L. Wang, J. Li, Z. Fan, L. Yang, Z. Zhang, C. Zhang, D. Yue, G. Qin, T. Zhang, F. Li, X. Chen, Y. Ping, D. Wang, Q. Gao, Q. He, L. Huang, H. Li, J. Huang, X. Zhao, W. Xue, Z. Sun, J. Lu, J. J. Yu, J. Zhao, B. Zhang, Y. Zhang, Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer. *Cancer Res.* **78**, 1779–1791 (2018).
19. F. Carrette, C. D. Surh, IL-7 signaling and CD127 receptor regulation in the control of T cell homeostasis. *Semin. Immunol.* **24**, 209–217 (2012).
20. A. Young, S. F. Ngiow, Y. Gao, A. M. Patch, D. S. Barkauskas, M. Messaoudene, G. Lin, J. D. Coudert, K. A. Stannard, L. Zitvogel, M. A. Degli-Esposti, E. Vivier, N. Waddell, J. Linden, N. D. Huntington, F. Souza-Fonseca-Guimaraes, M. J. Smyth, A2AR adenosine signaling suppresses natural killer cell maturation in the tumor microenvironment. *Cancer Res.* **78**, 1003–1016 (2018).
21. C. Cekic, D. Sag, Y. Li, D. Theodorescu, R. M. Strieter, J. Linden, Adenosine A_{2B} receptor blockade slows growth of bladder and breast tumors. *J. Immunol.* **188**, 198–205 (2012).
22. S. Chen, I. Akdemir, J. Fan, J. Linden, B. Zhang, C. Cekic, The expression of adenosine A2B receptor on antigen-presenting cells suppresses $CD8^+$ T-cell responses and promotes tumor growth. *Cancer Immunol. Res.* **8**, 1064–1074 (2020).
23. B. Allard, I. Cousineau, D. Allard, L. Buisseret, S. Pommey, P. Chrobak, J. Stagg, Adenosine A2a receptor promotes lymphangiogenesis and lymph node metastasis. *Onco. Targets. Ther.* **8**, 1601481 (2019).
24. L. F. Mager, R. Burkhard, N. Pett, N. C. A. Cooke, K. Brown, H. Ramay, S. Paik, J. Stagg, R. A. Groves, M. Gallo, I. A. Lewis, M. B. Geuking, K. D. McCoy, Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* **369**, 1481–1489 (2020).
25. T. N. Gide, C. Quek, A. M. Menzies, A. T. Tasker, P. Shang, J. Holst, J. Madore, S. Y. Lim, R. Velickovic, M. Wongchenko, Y. Yan, S. Lo, M. S. Carlino, A. Guminski, R. P. M. Saw, A. Pang, H. M. McGuire, U. Palendira, J. F. Thompson, H. Rizos, I. P. D. Silva, M. Batten, R. A. Scolyer, G. V. Long, J. S. Wilmott, Distinct immune cell populations define response to anti-PD-1 monotherapy and anti-PD-1/anti-CTLA-4 combined therapy. *Cancer Cell* **35**, 238–255.e6 (2019).
26. J. F. Chen, Z. Huang, J. Ma, J. Zhu, R. Moratalla, D. Standaert, M. A. Moskowitz, J. S. Fink, M. A. Schwarzschild, A_{2A} adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. *J. Neurosci.* **19**, 9192–9200 (1999).

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Interleukin-7 protects CD8 T cells from adenosine-mediated immunosuppression

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Averting antitumor immunity

Within the tumor microenvironment, the nucleoside adenosine accumulates and acts through its cell surface receptors to inhibit the activity of antitumor CD8 T cells. To alleviate this immunosuppression, therapeutics that target either adenosine production or adenosine receptor signaling are being developed. Koyas *et al*. showed in mice that the actions of adenosine on tumor-infiltrating CD8 T cells depended on the milieu within the tumor. In particular, signaling by the cytokine interleukin-7 (IL-7) counteracted the immunosuppressive effects of adenosine, leading to enhanced antitumor immunity in a melanoma model. Together, these findings suggest that targeting IL-7 signaling may alleviate the inhibition of tumor-infiltrating T cells.

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