Adenosine Acts through A2 Receptors to Inhibit IL-2-Induced Tyrosine Phosphorylation of STAT5 in T Lymphocytes: Role of Cyclic Adenosine 3′,5′-Monophosphate and Phosphatases

Hong Zhang, David M. Conrad, Jared J. Butler, Chuanli Zhao, Jonathan Blay and David W. Hoskin

*J Immunol* 2004; 173:932-944; doi: 10.4049/jimmunol.173.2.932

http://www.jimmunol.org/content/173/2/932

**Why The JI?**

- Rapid Reviews! 30 days* from submission to initial decision
- No Triage! Every submission reviewed by practicing scientists
- Speedy Publication! 4 weeks from acceptance to publication

*average

**References**

This article cites 70 articles, 37 of which you can access for free at:
http://www.jimmunol.org/content/173/2/932.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Adenosine Acts through A₂ Receptors to Inhibit IL-2-Induced Tyrosine Phosphorylation of STAT5 in T Lymphocytes: Role of Cyclic Adenosine 3',5'-Monophosphate and Phosphatases

Hong Zhang,* David M. Conrad,* Jared J. Butler,* Chuanli Zhao,* Jonathan Blay,† and David W. Hoskin²*‡

Adenosine is a purine nucleoside with immunosuppressive activity that acts through cell surface receptors (A₁, A₂a, A₂b, A₃) on responsive cells such as T lymphocytes. IL-2 is a major T cell growth and survival factor that is responsible for inducing Jak1, Jak3, and STAT5 phosphorylation, as well as causing STAT5 to translocate to the nucleus and bind regulatory elements in the genome. In this study, we show that adenosine suppressed IL-2-dependent proliferation of CTLL-2 T cells by inhibiting STAT5a/b tyrosine phosphorylation that is associated with IL-2R signaling without affecting IL-2-induced phosphorylation of Jak1 or Jak3. The inhibitory effect of adenosine on IL-2-induced STAT5a/b tyrosine phosphorylation was reversed by the protein tyrosine phosphatase inhibitors sodium orthovanadate and bpV(phen). Adenosine dramatically increased Src homology region 2 domain-containing phosphatase-2 (SHP-2) tyrosine phosphorylation and its association with STAT5 in IL-2-stimulated CTLL-2 T cells, implicating SHP-2 in adenosine-induced STAT5a/b dephosphorylation. The inhibitory effect of adenosine on IL-2-induced STAT5a/b tyrosine phosphorylation was reproduced by A₂ receptor agonists and was blocked by selective A₂a and A₂b receptor antagonists, indicating that adenosine was mediating its effect through A₂ receptors. Inhibition of STAT5a/b phosphorylation was reproduced with cell-permeable 8-bromo-cAMP or forskolin-induced activation of adenyl cyclase, and blocked by the cAMP/protein kinase A inhibitor Rp-cAMP. Forskolin and 8-bromo-cAMP also induced SHP-2 tyrosine phosphorylation. Collectively, these findings suggest that adenosine acts through A₂ receptors and associated cAMP/protein kinase A-dependent signaling pathways to activate SHP-2 and cause STAT5 dephosphorylation that results in reduced IL-2R signaling in T cells. The Journal of Immunology, 2004, 173: 932–944.
and PI3K signaling pathways. The Grb2/Sos complex is recruited to Shc, and Sos activates the ERK pathway that up-regulates genes such as c-fos and c-jun (25, 26). The PI3K signaling pathway, which includes the serine/threonine kinase Akt (27), is activated through recruitment of the adaptor protein GAB2 (28, 29). The second proliferative signal transduced by the IL-2R complex involves the transcription factor STAT5 (30, 31), which is recruited to phosphorylated Y510 Shc (23), becomes tyrosine phosphorylated, homo- and/or heterodimerizes with other STAT molecules, and then directly translocates to the nucleus, where the complex binds regulatory elements that control gene expression (32). The STAT5 family consists of highly homologous STAT5a and STAT5b proteins that are coded for by two different STAT5 genes (33). STAT5 plays a critical role in IL-2-driven T cell responses because T lymphocytes from mutant mice with targeted deletions (33). STAT5b plays a critical role in IL-2-driven T cell responses and then directly translocates to the nucleus, where the complex heterodimerizes with other STAT molecules, and then directly translocates to the nucleus, where the complex binds regulatory elements that control gene expression (32).

Cell culture

The IL-2-dependent CTLL-2 T cell line was obtained from the American Type Culture Collection (Manassas, VA). CTLL-2 cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (hereafter called complete RPMI 1640 medium), and 20 U/ml IL-2 (R&D Systems, Minneapolis, MN) in 250-ml culture flasks maintained at 37°C in a humidified 5% CO2 incubator. Spleen T cells were obtained from spleens of mice sacrificed by cervical dislocation. Spleens were excised using aseptic technique, and spleen cell suspensions were prepared in cold PBS (pH 7.4). Following erythrocyte removal by osmotic shock, lymphocytes were washed, resuspended in complete RPMI 1640 medium, and passed through a nylon wool column (Cellular Products, Buffalo, NY) to enrich for T lymphocytes. T cells were placed in wells of a 24-well flat-bottom tissue culture plate (8 × 105 cells/well) and induced to up-regulate IL-2R by incubation for 24 h at 37°C in a humidified 5% CO2 incubator in the presence of anti-CD3 mAb (1/20 dilution of hybridoma 145-2C11 culture supernatant) and IL-2. CTLL-2 cells and activated spleen T cells were washed twice with PBS, resuspended in serum-free complete RPMI 1640 medium, and incubated for 4 h at 37°C in a humidified 5% CO2 incubator to remove residual IL-2 from IL-2R. CTLL-2 cells and activated spleen T cells were then washed with PBS and resuspended in complete RPMI 1640 medium before use in experiments. CTLL-2 and spleen T cell viability was verified by trypan blue dye exclusion to be at least 95%.

Cell proliferation assay

CTLL-2 cells were added in triplicate (105 cells/well) to a 96-well, flat-bottom microtiter plate and cultured in the absence or presence of the indicated concentrations of adenosine and/or coformycin for 30 min before the addition of 10 U/ml IL-2. Following 18 h of culture at 37°C in a 5% CO2 humidified incubator, each well was pulsed with 0.5 μCi of tritiated thymidine ([3H]Tdr; sp. act., 60 Ci/mmol; ICN Biomedicals, Mississauga, Canada) for an additional 6 h. Samples were then harvested onto glass fiber filter mats (Skatron, Sterling, VA) using a Titer-Tek multiple sample harvester, and [3H]Tdr incorporation was determined as a measure of DNA synthesis using a liquid scintillation counter. Data are shown as mean cm± SD.

Flow cytometry

CTLL-2 cell surface expression of IL-2R α-, β-, and γ-chains was determined by flow cytometry using FITC-conjugated Ab (BD Pharmping, Mississauga, Canada), as previously described (36).

RT-PCR analysis

Total RNA was isolated from CTLL-2 cells using TRIzol reagent, as recommended by the manufacturer (Invitrogen Life Technologies, Carlsbad, CA). Single-strand cDNA was synthesized from 0.5 μg of RNA with 200 U of Moloney murine leukemia virus-derived reverse transcriptase (Life Technologies) in the presence of 0.2 mM dNTPs, 1 μg of random hexamers, and 10 mM DTT. PCR was performed using an automatic DNA thermocycler (MJ Research, Watertown, MA). Each PCR used equal amounts of cDNA, 2.5 U of Taq polymerase (Life Technologies), 0.2 mM dNTPs, and 0.5 μM each primer pair in a 1/10 dilution of PCR buffer (2 M KCl, 1 M Tris-HCl, pH 8.4, 1 M MgCl2, and 1 mM BSA). The primer pairs described below were used for PCR (amplicon size is given after the reverse primer). F, refers to forward primer; R, refers to reverse primer. All primers were designed to bind intron-bridging exons of the respective gene.

**Materials and Methods**

**Mice**

Adult (6- to 8-wk-old) female C57Bl/6 mice were purchased from Charles River Laboratories (Lasalle, Canada) and housed in the Carleton Animal Care Facility at Dalhousie University. Mice were maintained on standard laboratory chow and water supplied ad libitum.

**Reagents**

RPMI 1640 medium was purchased from Sigma-Aldrich (Oakville, Canada). L-glutamine, penicillin, streptomycin, FCS, and TRIzol reagent were purchased from Life Technologies. L-Glutamine, penicillin, streptomycin, FCS, and TRIzol reagent were purchased from Life Technologies. 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (hereafter called complete RPMI 1640 medium), and 20 U/ml IL-2 (R&D Systems, Minneapolis, MN) in 250-ml culture flasks maintained at 37°C in a humidified 5% CO2 incubator. Spleen T cells were obtained from spleens of mice sacrificed by cervical dislocation. Spleens were excised using aseptic technique, and spleen cell suspensions were prepared in cold PBS (pH 7.4). Following erythrocyte removal by osmotic shock, lymphocytes were washed, resuspended in complete RPMI 1640 medium, and passed through a nylon wool column (Cellular Products, Buffalo, NY) to enrich for T lymphocytes. T cells were placed in wells of a 24-well flat-bottom tissue culture plate (8 × 105 cells/well) and induced to up-regulate IL-2R by incubation for 24 h at 37°C in a humidified 5% CO2 incubator in the presence of anti-CD3 mAb (1/20 dilution of hybridoma 145-2C11 culture supernatant) and IL-2. CTLL-2 cells and activated spleen T cells were washed twice with PBS, resuspended in serum-free complete RPMI 1640 medium, and incubated for 4 h at 37°C in a humidified 5% CO2 incubator to remove residual IL-2 from IL-2R. CTLL-2 cells and activated spleen T cells were then washed with PBS and resuspended in complete RPMI 1640 medium before use in experiments. CTLL-2 and spleen T cell viability was verified by trypan blue dye exclusion to be at least 95%.

**Flow cytometry**

CTLL-2 cell surface expression of IL-2R α-, β-, and γ-chains was determined by flow cytometry using FITC-conjugated Ab (BD Pharmingen, Mississauga, Canada), as previously described (36).

**RT-PCR analysis**

Total RNA was isolated from CTLL-2 cells using TRIzol reagent, as recommended by the manufacturer (Invitrogen Life Technologies, Carlsbad, CA). Single-strand cDNA was synthesized from 0.5 μg of RNA with 200 U of Moloney murine leukemia virus-derived reverse transcriptase (Life Technologies) in the presence of 0.2 mM dNTPs, 1 μg of random hexamers, and 10 mM DTT. PCR was performed using an automatic DNA thermocycler (MJ Research, Watertown, MA). Each PCR used equal amounts of cDNA, 2.5 U of Taq polymerase (Life Technologies), 0.2 mM dNTPs, and 0.5 μM each primer pair in a 1/10 dilution of PCR buffer (2 M KCl, 1 M Tris-HCl, pH 8.4, 1 M MgCl2, and 1 mM BSA). The primer pairs described below were used for PCR (amplicon size is given after the reverse primer). F, refers to forward primer; R, refers to reverse primer. All primers were designed to bind intron-bridging exons of the respective gene. 

**Abbreviations used in this paper:** PTP, protein tyrosine phosphatase; APNEA, N(6)-2-(4-aminophenyl)ethylenediamine; 8-Br-cAMP, 8-bromo-cAMP; CSC, N-(3-chloroanilino)caffeine; DMPX, 8-cyclopentyl-1,3-dipropyloxanthine (DPCPX), Rp-cAMPs, forskolin, and 8-bromo-cAMP (8-Br-cAMP) were purchased from Sigma-Aldrich.
Preparation of cell lysates

CTLL-2 cells were adjusted to a density of 2.5 × 10⁶ cells/ml and aliquoted into sample tubes before treatment for 15 min with adenosine or other reagents. CTLL-2 cells were then exposed to IL-2 (10 U/ml) for another 5 min. At the end of the incubation period, samples were chilled on ice and centrifuged at 400 × g and 4°C for 5 min. The cell pellet was washed once with ice-cold PBS, followed by lysis of the cell pellet with modified radioimmunoprecipitation buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 100 mM sodium orthovanadate, 1 mM PMSF, 5 μg/ml leupeptin, 5 μg/ml pepstatin, and 5 μg/ml aprotinin) for 30 min at 4°C. Insoluble cytoplasmic granules and nuclei were removed by centrifugation for 10 min at 12,000 × g and 4°C. The supernatants were collected and stored at −80°C before analysis by Western blotting. The protein concentrations in cellular protein preparations were determined by Bradford protein assay, according to the manufacturer’s instructions (Bio-Rad, Hercules, CA).

Immunoprecipitation and Western blotting

For immunoprecipitation, the cell lysates were precleared with normal rabbit IgG bound to protein A-Sepharose 4B beads by rotating overnight at 4°C. SHP-1, SHP-2, PTP1B, and STAT5 were immunoprecipitated from cell lysates with the appropriate Ab bound on protein A-Sepharose 4B beads for 2 h at 4°C. After four washes with lysis buffer, the beads were boiled in SDS sample buffer and analyzed by Western blotting. For Western blotting, cell lysates were boiled in SDS sample buffer, and 20 μg of total protein was loaded into each well of a SDS-PAGE gel for separation by electrophoresis. The protein bands were then transferred onto nitrocellulose membranes, and the resulting blots were blocked for 1 h with PBS-Tween (0.25 M Tris, pH 7.5, PBS, 150 mM sodium chloride, and 0.2% Tween 20) containing 5% powdered skim milk. Blots were probed overnight with the indicated Ab at a 1 μg/ml concentration. Blots were then washed three times with PBS-Tween and probed for 1 h with the appropriate HRP-conjugated secondary Ab (goat anti-rabbit IgG or anti-mouse IgG). Next, the blots were washed three times with PBS-Tween, and the protein bands on the blots were detected using an ECL Western blot detection system (Amersham Pharmacia Biotech, Kirkland, Canada). To confirm equal sample loading, the blots were stripped and reprobed with Ab against the appropriate nonphosphorylated protein. Membrane stripping and reprobing were conducted according to the instructions provided by the manufacturer of the ECL kit.

Results

Adenosine inhibits IL-2-induced CTLL-2 proliferation

The effect of adenosine on IL-2-dependent T cell proliferation was determined by culturing IL-2-dependent CTLL-2 cells in medium containing IL-2 without or with various concentrations of adenosine (plus 2.5 μM coformycin to prevent adenosine degradation by endogenous adenosine deaminase). After 24 h of culture, IL-2-induced CTLL-2 proliferation was assessed by [3H]TdR incorporation. As seen in Fig. 1A, the proliferation of CTLL-2 cells in response to IL-2 was significantly inhibited in a dose-dependent fashion by 5 and 10 μM adenosine (p = 0.0009 and p < 0.0001, respectively). Higher concentrations of adenosine (25 and 50 μM) did not have a substantially greater inhibitory effect (Fig. 1B). Unless otherwise noted, all subsequent experiments used 10 μM adenosine because this adenosine concentration had a maximal inhibitory effect on CTLL-2 proliferation. Coulter counting of CTLL-2 cells cultured over a 120-h time course in the presence of IL-2 without or with adenosine (plus coformycin) revealed that adenosine reduced total cell accumulation (Fig. 1C). No inhibition of CTLL-2 DNA synthesis or cell accumulation was observed in the presence of coformycin alone. To ensure that adenosine did not cause nonspecific cytotoxicity in CTLL-2 cell cultures, the viability of CTLL-2 cells following culture in the presence of adenosine was determined by trypan blue dye exclusion. No effect of adenosine at the concentrations used in these studies was observed on CTLL-2 cell viability, indicating that reduced IL-2-driven proliferation of CTLL-2 cells in the presence of adenosine was not due to diminished cell viability (data not shown). In addition, flow cytometric analysis revealed that adenosine did not significantly affect IL-2R expression by CTLL-2 cells (data not shown), indicating that adenosine-treated CTLL-2 cells retained the capacity to bind IL-2.

Adenosine inhibits IL-2-induced tyrosine phosphorylation of STAT5

We hypothesized that the inhibitory effect of adenosine on IL-2-dependent CTLL-2 proliferation might be due to decreased phosphorylation of tyrosine residues on STAT5a and/or STAT5b, because this is an essential step in IL-2R signaling (30, 31). To test this hypothesis, CTLL-2 cells were treated with 10 μM adenosine...
(plus coformycin) for 15 min, followed by exposure to different concentrations of IL-2 (1, 5, or 10 U/ml) for different lengths of time (5 min-12 h). Cell lysates were prepared, and Western blotting with anti-phospho-STAT5a/b or anti-STAT5a Ab was performed to determine STAT5a/b tyrosine phosphorylation and confirm equal protein loading, respectively. The percentage of OD ratios was calculated by comparing the density of individual phospho-STAT5a bands with the corresponding STAT5a band. As shown in Fig. 2, adenosine dramatically decreased dose-dependent STAT5a/b tyrosine phosphorylation in response to IL-2 over the entire 12-h time course, suggesting that adenosine was acting at or before the level of STAT5 to inhibit IL-2-dependent CTLL-2 proliferation. Fig. 3 shows that adenosine, as well as the adenosine analog NECA, had similar inhibitory effects on IL-2-induced tyrosine phosphorylation of STAT5a/b in mouse spleen T cells, demonstrating the physiological relevance of our findings using CTLL-2 cells.

Adenosine does not affect IL-2-induced Jak1 or Jak3 phosphorylation

To determine whether the inhibitory effect of adenosine on STAT5 tyrosine phosphorylation might be due to decreased activity of Jak1 and/or Jak3, which are upstream kinases required for STAT5 induction (37, 38), CTLL-2 cells were treated with 10 μM adenosine (plus coformycin) or the adenosine analogues NECA or CGS 21680 (both at 1 μM) for 15 min before stimulation for 5 min with IL-2. Cell lysates were then prepared, and Western blotting with anti-phospho-Jak1 or anti-phospho-Jak3 Ab was performed to detect Jak1 or Jak3 tyrosine phosphorylation, respectively. The same blot was then stripped and reprobed with anti-phospho-STAT5a/b Ab for comparison with phospho-Jak1 and phospho-Jak3 or with anti-STAT5a Ab to confirm equal protein loading. In contrast to the marked inhibitory effect of adenosine or adenosine analogues (NECA, CGS 21680) on tyrosine phosphorylation of STAT5a/b, neither adenosine nor the adenosine agonists affected IL-2-induced Jak1 or Jak3 tyrosine phosphorylation (Fig. 4). These data indicate that adenosine inhibits IL-2-induced STAT5 tyrosine phosphorylation in mouse spleen T cells. T cells isolated from mouse spleen were cultured for 24 h in the presence of soluble anti-CD3 mAb, washed extensively, and then treated with medium alone, 10 μM adenosine (Ado; plus 2.5 μM coformycin), or 1 μM NECA for 15 min at 37°C, and then stimulated with exogenous IL-2 (10 U/ml) for 5 min. Control consisted of anti-CD3-treated T cells that were not stimulated with exogenous IL-2. Unstimulated T cells were cultured in the absence of anti-CD3 mAb and were not exposed to exogenous IL-2. Cell lysates were prepared, and Western blotting was performed with Ab to phospho-STAT5a/b or STAT5a (both 1 μg/ml) to detect STAT5 tyrosine phosphorylation and protein loading, respectively. The percentage of OD ratios was calculated by comparing the density of individual phospho-STAT5a bands with the corresponding STAT5a band. Results are representative of two independent experiments.
that adenosine was not mediating its inhibitory effect on IL-2R signal transduction by acting upstream of STAT5.

**PTP inhibitors rescue STAT5 tyrosine phosphorylation in the presence of adenosine**

We next determined whether the inhibitory effect of adenosine on tyrosine phosphorylation of STAT5 might involve activation of a PTP. CTLL-2 cells were treated for 15 min with sodium orthovanadate (1 mM) or bpV(phen) (100 μM) to inhibit PTP (39, 40), or with 50 nM okadaic acid, which inhibits protein phosphatase 2A, but not PTPs (41), and were then exposed to 10 μM adenosine (plus coformycin) for another 15 min, followed by stimulation for 5 min with IL-2. Cell lysates were then prepared, and Western blotting was performed using anti-phospho-STAT5a/b Ab. Fig. 5A shows that the inhibitory effect of adenosine on IL-2-induced STAT5a/b tyrosine phosphorylation was dramatically reversed in the presence of both sodium orthovanadate and bpV-(phen), but not by okadaic acid. Equal protein loading was confirmed by stripping and reprobing the blot with anti-STAT5a Ab. These data indicate that exposure of CTLL-2 cells to adenosine induced a PTP that targeted STAT5a/b.

The blot was then stripped and reprobed with anti-phospho-ERK1/2 Ab to determine whether adenosine affected IL-2-driven ERK1/2 phosphorylation under our experimental conditions. The

**FIGURE 4.** Adenosine does not prevent Jak1 or Jak3 phosphorylation. CTLL-2 cells were treated with medium alone or 10 μM adenosine (Ado; plus 2.5 μM coformycin), 1 μM NECA, or 1 μM CGS 21680 for 15 min at 37°C, followed by stimulation with IL-2 (10 U/ml) for 5 min. Control consisted of cells that were exposed to only medium. Cell lysates were prepared, and Western blotting with Ab to phospho-Jak1 (1 μg/ml) or phospho-Jak3 (10 μg/ml) was used to detect Jak1 and Jak3 tyrosine phosphorylation, respectively. The same blot was stripped and reprobed with anti-phospho-STAT5a/b Ab for comparison with Jak1/Jak3 and with anti-STAT5a Ab to confirm equal protein loading. Results are representative of four individual experiments.

**FIGURE 5.** PTP inhibitors rescue STAT5 phosphorylation in the presence of adenosine. CTLL-2 cells were treated with 100 μM bpV(phen), 1 mM sodium orthovanadate (Na₃VO₄), or 50 nM okadaic acid (OA) for 15 min at 37°C before treatment with medium alone or 10 μM adenosine (Ado) plus 2.5 μM coformycin for an additional 15 min, followed by stimulation with IL-2 (10 U/ml) for 5 min (A). CTLL-2 cells were treated with medium alone or 10 μM adenosine (Ado) plus 2.5 μM coformycin for 15 min at 37°C, followed by stimulation with IL-2 (20 U/ml) for 5 min (B). Control (A and B) consisted of cells that were exposed to only medium. Cell lysates were prepared, and Western blotting with Ab to phospho-STAT5a/b, phospho-ERK1/2, STAT5a, or ERK1/2 (all 1 μg/ml) was used to detect STAT5a/b and ERK1/2 phosphorylation and confirm equal protein loading, respectively. The percentage of OD ratios was calculated by comparing the density of individual phospho-STAT5a bands with the corresponding STAT5a band. Results are representative of four different experiments.
shows that treatment of IL-2-stimulated CTLL-2 cells with 10 nM adenosine plus PTP1B results in the activation of these PTPs (45–47). Phosphorylation of PTP1B in CTLL-2 cells was increased in the presence of adenosine under these conditions. Although tyrosine phosphorylation induced by IL-2 alone (2.19 vs 1.04 OD ratio of phospho-SHP-1/SHP-1), indicating that SHP-1 is not activated by adenosine (plus coformycin) caused a dramatic increase in SHP-2 tyrosine phosphorylation in comparison with the level of tyrosine phosphorylation in SHP-2/SHP-2), suggesting that adenosine increased the phosphorylation induced by IL-2 alone (2.19 vs 1.04 OD ratio of phospho-SHP-1/SHP-1). We next determined the effect of adenosine on the phosphorylation status of SHP-1, SHP-2, and PTP1B because these PTPs have been shown to dephosphorylate and deactivate growth factor receptor-activated STAT5a and STAT5b in other experimental systems (42–44). Phosphorylation of tyrosine residues on SHP-1, SHP-2, and PTP1B results in the activation of these PTPs (45–47). Fig. 6A shows that treatment of IL-2-stimulated CTLL-2 cells with 10 nM adenosine (plus coformycin) caused a dramatic increase in SHP-2 tyrosine phosphorylation in comparison with the level of tyrosine phosphorylation induced by IL-2 alone (2.19 vs 1.04 OD ratio of phospho-SHP-2/SHP-2), suggesting that adenosine increased the phosphatase activity of SHP-2. In contrast, there was little difference between the effect of IL-2 alone or IL-2 in combination with adenosine on tyrosine phosphorylation of SHP-1 (0.82 vs 0.95 OD ratio of phospho-SHP-1/SHP-1), indicating that SHP-1 is not activated by adenosine under these conditions. Although tyrosine phosphorylation of PTP1B in CTLL-2 cells was increased in the presence of IL-2, PTP1B tyrosine phosphorylation was only slightly increased following treatment with IL-2 plus adenosine in comparison with treatment with IL-2 alone (1.14 vs 0.87 OD ratio of phospho-PTP1B/PTP1B), suggesting that adenosine failed to substantively increase the phosphatase activity of PTP1B.

Because a substrate-trapping mutant of SHP-2 has recently been shown to physically interact with and form a complex with cytokine tyrosine-phosphorylated STAT5 from IL-2-stimulated CTLL-20 mouse T cells (43), it was important to determine whether adenosine also increased the association of SHP-2 with STAT5 in our system. CTLL-2 cells were exposed to 10 nM adenosine (plus coformycin) for 15 min, followed by stimulation with IL-2 for 5 min. Immunoprecipitation of cell lysates with anti-STAT5a Ab, followed by Western blotting with anti-SHP-2 Ab, revealed that adenosine and NECA both caused at least a 2-fold increase in the amount of SHP-2 that coprecipitated with STAT5 relative to the IL-2 control (Fig. 6B), indicating that adenosine promotes the formation of SHP-2-STAT5 complexes.

**Adenosine-receptor expression by CTLL-2 cells**

Adenosine mediates its effects through four receptor subtypes designated A1, A2A, A2B, and A3, which are G protein-coupled membrane-associated molecules (48). We used RT-PCR analysis to determine the adenosine receptor subtypes expressed by CTLL-2 cells. Mouse brain was used as a positive control because this tissue expresses all known adenosine receptor subtypes (49). Fig. 7 shows that CTLL-2 cells expressed mRNA coding for A1, A2A, A2B, and A3 receptors, but little detectable mRNA coding for A1 receptors.

**FIGURE 6.** Adenosine induces SHP-2 phosphorylation and SHP-2 association with STAT5 in IL-2-stimulated CTLL-2 cells. A, CTLL-2 cells were treated with medium alone or 10 nM adenosine (Ado; plus 2.5 μM coformycin) for 15 min at 37°C, followed by stimulation with IL-2 (10 U/ml) for 5 min. Control consisted of cells that were exposed to only medium. Cell lysates were prepared; immunoprecipitation with anti-SHP-1, anti-SHP-2, or anti-PTP1B Ab (2 μg/sample) and Western blotting with anti-phosphotyrosine Ab (1 μg/ml) were used to detect SHP-1, SHP-2, and PTP1B tyrosine phosphorylation, respectively. Blots were stripped and reprobed with Ab to STAT5a, Shp-2, and STAT5b in other experimental systems. B, CTLL-2 cells were treated with medium alone or 10 nM adenosine (Ado; plus 2.5 μM coformycin) for 15 min at 37°C, followed by stimulation with IL-2 (10 U/ml) for 5 min. Control consisted of cells that were exposed to only medium. Cell lysates were prepared; immunoprecipitation with anti-STAT5a Ab (2 μg/sample) and Western blotting with anti-SHP-2 Ab (1 μg/ml) were performed. Blots were stripped and reprobed with Ab to STAT5a band. Results are representative of two independent experiments.
A2 receptor agonists inhibit IL-2-induced STAT5a/b tyrosine phosphorylation

To determine whether the inhibitory effect of adenosine on tyrosine phosphorylation of STAT5 was mediated through a particular cell surface adenosine receptor, we compared the effect of treating CTLL-2 cells for 15 min with 10 μM adenosine (plus coformycin) with that of treatment either with the stable adenosine analog NECA at a concentration able to stimulate both A2a and A2b receptors, or with the A2a receptor-selective agonist CGS 21680, or with the A1/A2 receptor-selective agonist APNEA (all at 1 μM) before stimulation with IL-2 for 5 min. Fig. 8 shows that both NECA and CGS 21680 mimicked the inhibitory effect of adenosine on STAT5a/b phosphorylation, suggesting that the effect was mediated through A2a and, possibly, A2b receptors. In contrast, the A1/A2 receptor-selective agonist APNEA did not prevent IL-2-induced STAT5a/b tyrosine phosphorylation, ruling out a role for A1 or A2 receptors. As before, the inhibitory effect of adenosine, NECA, and CGS 21680 was reversed in the presence of the PTP inhibitor bpV(phen).

A2 receptor agonists block the inhibitory effect of adenosine and adenosine receptor agonists on IL-2-induced tyrosine phosphorylation of STAT5a/b

To confirm that A2 receptors mediated the inhibitory effect of adenosine on STAT5a/b tyrosine phosphorylation, we treated CTLL-2 cells for 15 min with the A1 receptor antagonist DPCPX (20 μM), the A2 receptor antagonist DMPX (20 μM), and the A2a receptor-selective antagonist CSC (2 μM), the A2a receptor-selective antagonist alloxazine (2 μM), CSC in combination with alloxazine (both at 2 μM), or the A1, A2a, A2b, and A3 adenosine receptor expression was determined by RT-PCR.

FIGURE 7. Adenosine receptor expression by CTLL-2 cells. Total RNA was isolated from mouse brain (lanes 2, 5, 8, and 11) or from CTLL-2 cells (lanes 3, 6, 9, and 12), and A1, A2a, A2b, and A3 adenosine receptor expression was determined by RT-PCR.

FIGURE 8. A1 adenosine receptor agonists inhibit IL-2-induced STAT5 phosphorylation. CTLL-2 cells were treated with medium alone, 10 μM adenosine (Ado; plus 2.5 μM coformycin), 1 μM NECA (A2a/A2b receptor agonist), CGS 21680 (A2a receptor agonist), or APNEA (A1 receptor agonist) for 15 min at 37°C, followed by stimulation for 5 min with IL-2 (10 U/ml). Control consisted of cells that were exposed to only medium. Cell lysates were prepared, and Western blotting with anti-phospho-STAT5a Ab (both 1 μg/ml) was used to detect STAT5a tyrosine phosphorylation and confirm equal protein loading, respectively. The percentage of OD ratios was calculated by comparing the density of individual phospho-STAT5a bands with the corresponding STAT5a band. Results are representative of four different experiments.

cAMP/PKA-dependent signaling pathways regulate SHP-2 and STAT5 tyrosine phosphorylation

Because adenosine-mediated inhibition of some T cell functions has been linked to increased intracellular cAMP accumulation caused by the activation of adenylyl cyclase that is coupled to A2a and A2b adenosine receptors (13, 50), we examined the effect of both adenosine receptor ligands and indirect methods of eliciting cAMP on concurrent STAT5a/b and SHP-2 phosphorylation in CTLL-2 cells. Stimulation of adenylyl cyclase activity with forskolin (25 μM) or exposure to the stable, cell-permeable cAMP analog 8-Br-cAMP (100 μM) inhibited IL-2-induced tyrosine phosphorylation of STAT5a/b to an extent that was similar to that...
caused by adenosine (plus coformycin) or NECA (Fig. 10). Forskolin and 8-Br-cAMP also caused a marked increase in SHP-2 tyrosine phosphorylation in IL-2-stimulated CTLL-2 cells that was well above that caused by IL-2 alone, and was comparable to that caused by adenosine (plus coformycin) or NECA. The increase in tyrosine phosphorylation of SHP-2 closely paralleled the inhibitory effect of adenosine or NECA on STAT5a/b tyrosine phosphorylation, consistent with a role for SHP-2 in mediating STAT5 dephosphorylation. To determine whether cAMP-dependent activation of PKA was linked to the inhibitory effect of adenosine on tyrosine phosphorylation of STAT5a/b, we treated CTLL-2 cells with Rp-cAMPS (100 μM), which is a specific membrane-permeable inhibitor of cAMP-dependent protein kinase I and II (51), before exposure to adenosine (plus coformycin) and subsequent stimulation with IL-2. Fig. 11 shows that Rp-cAMPS almost completely prevented adenosine-mediated inhibition of STAT5a/b tyrosine phosphorylation, suggesting that PKA induces SHP-2-mediated dephosphorylation of STAT5a/b. In addition, adenosine-induced tyrosine phosphorylation of SHP-2 was dramatically reduced in the presence of Rp-cAMPS (data not shown). Collectively, these data implicate cAMP/PKA-dependent signaling pathways coupled to A2a and A2b adenosine receptors in the activation of SHP-2 and the subsequent dephosphorylation of STAT5.

Adenosine does not affect IL-2-induced phosphorylation of Akt

Finally, we determined whether altered signaling through the PI3K pathway might also contribute to the inhibitory effect of adenosine on IL-2-dependent growth of CTLL-2 cells. CTLL-2 cells were treated for 15 min with 10 μM adenosine (plus coformycin) or 1 μM NECA or CGS 21680 before stimulation with IL-2 for 5 min. Cell lysates were then prepared, and a Western blot was probed with anti-phospho-Akt Ab to detect tyrosine phosphorylation of Akt, a serine/threonine kinase that is activated by phosphorylation in a PI3K-dependent fashion (27). The same blot was then stripped and reprobed with anti-phospho-STAT5a/b Ab for comparison with phospho-Akt, as well as with anti-STAT5a Ab to confirm equal protein loading. In comparison with the inhibitory effect of adenosine on STAT5a/b tyrosine phosphorylation, neither adenosine nor adenosine analogues affected IL-2-induced Akt phosphorylation (Fig. 12). Note that coformycin alone did not affect IL-2-induced tyrosine phosphorylation of STAT5a/b in CTLL-2 cells.

Discussion

In the present investigation, we show that adenosine inhibited IL-2-driven DNA synthesis and accumulation of mouse CTLL-2 T cells (Fig. 1). This observation is consistent with our earlier finding that adenosine interferes with the proliferation of mouse primary T cells in response to TCR stimulation with anti-CD3 mAb (11). Cell viability and flow cytometric analysis established that the inhibitory effect on CTLL-2 cells was not due to nonspecific toxicity by adenosine or adenosine-induced down-regulation of IL-2R (data not shown). Adenosine-mediated inhibition of CTLL-2 cell proliferation was in sharp contrast to a previous study in which adenosine was reported to enhance IL-2-dependent proliferation of CTLL-2 cells. Antonsamy et al. (52) based their conclusion on
the observation that elimination of endogenous adenosine with exogeneous adenosine deaminase inhibits the IL-2-dependent growth of CTLL-2 cells, which can then be restored by the addition of the stable adenosine analog R-phenylisopropyladenosine. However, there are several possible explanations for the apparent contrast with our results. First, the concentration of R-phenylisopropyladenosine that augments IL-2-driven CTLL-2 cell proliferation in the presence of exogenous adenosine deaminase is 3 logs lower than the adenosine concentration that we showed inhibited IL-2-dependent growth of CTLL-2 cells (10 nM vs 10 μM). Adenosine would most likely affect CTLL-2 cells differently at these concentrations because the A₁ adenosine receptor subtype (which is detectable, although not abundant on our CTLL-2 cells) that transduces a stimulatory signal has a higher affinity for ligand than the A₂b adenosine receptor that transduces an inhibitory signal (49). Consistent with this hypothesis, Antonyasamy et al. (52) report low A₁, A₂a, and A₃ adenosine receptor expression by CTLL-2 cells, while our own data also indicated A₂b adenosine receptor expression by CTLL-2 cells (Fig. 7). Moreover, Antonyasamy et al. (52) exclude any growth-enhancing role for A₂a adenosine receptors on CTLL-2 cells. In addition, commercial preparations of adenosine deaminase are variably contaminated with superoxide dismutase (53), which is known to protect T cells from activation-induced cell death (54). It is therefore possible that CTLL-2 cell numbers might be increased in the presence of exogenous adenosine deaminase due to the protective effect of contaminating superoxide dismutase.

Finally, adenosine deaminase facilitates R-phenylisopropyladenosine binding to A₁ adenosine receptors and subsequent signalling through a mechanism that involves the physical interaction of adenosine deaminase with A₁ adenosine receptors (55). The addition of exogenous adenosine deaminase plus low concentrations of R-phenylisopropyladenosine to IL-2-stimulated CTLL-2 cell cultures may, therefore, have favored stimulatory A₁ adenosine receptor signaling over inhibitory A₂b adenosine receptor signaling in the experiments performed by Antonyasamy et al. (52).

Proliferative signaling through the IL-2R can occur via two distinct routes, one involving STAT5 (30, 31) and the other using the adaptor protein Shc that activates ERK and PI3K signaling pathways (56). STAT transcription factors are cytoplasmic proteins that are tyrosine phosphorylated and then translocate to the nucleus following activation of associated kinases (32). Our studies revealed that adenosine exerted a potent inhibitory effect on IL-2-induced tyrosine phosphorylation and activation of STAT5a and STAT5b in both CTLL-2 and primary T cells (Figs. 2 and 3), which was not due to inhibition of IL-2-induced phosphorylation and activation of the upstream kinases Jak1 or Jak3 (Fig. 4). This finding is consistent with a recent report that UV radiation, which suppresses cellular immune responses (57), also inhibits IL-2-induced tyrosine phosphorylation and activation of STAT5 in T cells without affecting IL-2-induced tyrosine phosphorylation of Jak1 or Jak3 (58).

We also examined the effect of adenosine on ERK1/2 and Akt phosphorylation in IL-2-stimulated CTLL-2 cells. The PI3K signaling pathway (which includes the serine/threonine kinase Akt) and the ERK signaling pathway are both activated in response to IL-2R signaling (56). Surprisingly, we found that the concentration of IL-2 (10 U/ml) that was used to induce CTLL-2 proliferation and STAT5 phosphorylation in our experiments failed to induce phosphorylation of ERK1/2 (Fig. 5A), suggesting that activation of the ERK signaling pathway is not essential for IL-2-driven T cell proliferation. This result is contrary to recent reports that IL-2 induces ERK1/2 phosphorylation in CTLL-2 cells (56, 59). However, in both cases, the investigators used a concentration of IL-2 that was 10-fold higher (100 U/ml) than the concentration of IL-2 (10 U/ml) that we used in our experiments. Consistent with these earlier reports, we observed that at least 20 U/ml IL-2 was required to induce ERK1/2 phosphorylation in CTLL-2 cells (56, 59). However, adenosine failed to inhibit ERK1/2 phosphorylation induced by a higher concentration of IL-2. We also observed that ERK1/2 is phosphorylated in the presence of the PTP inhibitor bpV(phen) (Fig. 5A), suggesting that constitutive ERK1/2 phosphorylation in CTLL-2 cells is regulated by a PTP. Our findings also indicate that the Jak/STAT5 pathway of CTLL-2 cells is more sensitive than the ERK pathway to IL-2R signaling. In addition, adenosine did not affect IL-2-induced tyrosine phosphorylation of Akt (Fig. 12). Because phosphorylation plays a major role in Akt activation (60) and Akt is an important intermediate in the PI3K signaling pathway (27), this finding suggested that IL-2-induced...
PI3K signaling in CTLL-2 cells was not impaired in the presence of adenosine. Collectively, these results indicate that adenosine-mediated inhibition of IL-2-induced CTLL-2 proliferation was due to impaired activation of the IL-2-induced Jak/STATS pathway and did not involve perturbation of ERK or PI3K signaling pathways.

Protein tyrosine phosphorylation events that are crucial for the control of T cell activation are closely regulated by the opposing actions of protein tyrosine kinases and PTPs (16). PTPs have been shown to participate as either positive or negative regulators of signal transduction in a wide range of physiological processes, which include cellular growth and proliferation, migration, differentiation, and survival (66). Despite the important roles played by PTPs in a wide range of physiological and pathological processes, the mechanism by which PTPs exert their regulatory effects is not well understood. Because adenosine did not inhibit IL-2-induced Jak1 or Jak3 phosphorylation in CTLL-2 cells, we considered the possibility that adenosine-mediated inhibition of STAT5a/b tyrosine phosphorylation might result from the activation of a PTP that targets STAT5. PTPs are known to closely regulate kinases that are associated with various cytokine signal transduction pathways to maintain cellular homeostasis (62). More recently, a PTP associated with the nucleus was shown to specifically dephosphorylate and deactivate STAT5a and STAT5b in COS-7 cells, as well as in mammary epithelial COMMA-1D cells (63). Our results show that the inhibitory effect of adenosine on STAT5a/b tyrosine phosphorylation in CTLL-2 cells was abrogated in the presence of the PTP inhibitors bpV(phen) and sodium orthovanadate (Fig. 5A), suggesting that adenosine activated a PTP that dephosphorylated STAT5.

SHP-1, SHP-2, and PTP1B were all possible candidates to mediate adenosine-induced PTP activity that targeted IL-2R-associated STATs in CTLL-2 cells because these PTPs have been reported to dephosphorylate and deactivate STAT5a and STAT5b associated with growth factor receptors in other experimental systems (42–44). Activation of SHP-1, SHP-2, and PTP1B requires phosphorylation of PTP tyrosine residues (45–47). Tyrosine phosphorylation of SHP-2, but not SHP-1, was strongly enhanced in IL-2-stimulated CTLL-2 cells that were exposed to adenosine, indicating that exposure to adenosine activated SHP-2, but not SHP-1, in CTLL-2 cells (Fig. 6A). This finding is in good agreement with a recent report that SHP-2, rather than SHP-1, mediates tyrosine dephosphorylation of IL-2R-associated STAT5 in the cytosolic compartment of mouse T cells, thereby maintaining STAT5 activity at a steady state basal level (43). SHP-2, but not SHP-1, has also been shown to specifically interact with tyrosine-phosphorylated STAT5a and accelerate STAT5a dephosphorylation in IL-3-stimulated myeloid cells (64). Importantly, the same study showed that dephosphorylation of STAT5a is dramatically delayed in SHP-2-deficient mouse fibroblast cells, providing direct evidence that SHP-2 is an important mediator of STAT5a dephosphorylation. Although IL-2 caused a modest increase in tyrosine phosphorylation of PTP1B, there was no substantial change in phosphorylation following treatment with adenosine (Fig. 6A), which argued against a role for PTP1B in adenosine-induced STAT5a/b dephosphorylation in IL-2-stimulated CTLL-2 cells. It is also important to note that the effect of tyrosine phosphorylation on the phosphatase activity of PTP1B is still somewhat controversial because phosphorylation of PTP1B tyrosine residues has been reported by different groups to increase or inhibit phosphatase activity (47, 65). Protein phosphatase 2A is also able to dephosphorylate STAT5a following IL-3 stimulation of myeloid progenitor cells (66). However, the inhibitory effect of adenosine on STAT5a/b phosphorylation in IL-2-stimulated CTLL-2 cells was not diminished in the presence of okadaic acid, a well-known inhibitor of protein phosphatase 2A (Fig. 5A). This observation excluded a role for protein phosphatase 2A in our experimental system. We also showed that treatment with adenosine or the adenosine analog N6-cyclopentyladenosine resulted in a substantial increase in the amount of SHP-2 that coimmunoprecipitated with STAT5 from IL-2-stimulated CTLL-2 cells (Fig. 6B), suggesting that adenosine promoted the formation of SHP-2–STAT5 complexes that have been implicated in the efficient dephosphorylation and deactivation of STAT5 by SHP-2. This result is consistent with the ability of a substrate-trapping mutant of SHP-2, but not SHP-1, to form a stable complex with tyrosine-phosphorylated cytosolic STAT5 from IL-2-stimulated mouse CTLL-20 cells, thereby showing that STAT5 can serve as a direct substrate of SHP-2 (43). STAT5 tyrosine dephosphorylation and deactivation do not require nuclear translocation of the SHP-2–STAT5 complex because SHP-2 cannot be detected in the nucleus of IL-2-stimulated CTLL-20 cells. Taken together, our findings provide strong evidence that adenosine-induced dephosphorylation of STAT5a/b in IL-2-stimulated CTLL-2 T cells was mediated by SHP-2. However, we cannot at the present time rule out a possible contribution by one or more as yet unidentified STAT5-reactive phosphatases to this process. It may be possible to use SHP-2 deletion/inactivation approaches (e.g., dominant-negative mutant of SHP-2, small interfering RNA knockdown) to resolve this issue, although the compound signaling functions of SHP-2 may complicate the interpretation of results obtained from such experiments (67).

Adenosine mediates its effects on responsive cells through cell surface receptors that are divided into A₁, A₂a, A₂b, and A₃ subtypes (48). Activation of A₁ or A₃ adenosine receptors inhibits...
adenosine receptor stimulation promotes the accumulation of intracellular cAMP. A3 adenosine receptor stimulation also activates the phospholipase C/inositol trisphosphate/diacylglycerol pathway (68). Our analysis showed that CTL2-2 cells predominantly expressed A2a, A2b, and A3 adenosine receptor subtypes, although a low level of A1 adenosine receptor expression was also present (Fig. 7). We used specific adenosine receptor antagonists and agonists to demonstrate that the inhibitory effect of adenosine on IL-2-induced STAT5 tyrosine phosphorylation was mediated through A2a and A2b adenosine receptors. This conclusion was based on the finding that A2a/A2b adenosine receptor agonist NECA and the A2a adenosine receptor agonist CGS 21680 mimicked the effect of adenosine (Fig. 8), whereas the A2 receptor antagonist DMPX completely blocked the effect of adenosine (Fig. 9A). In addition, the A2a adenosine receptor antagonist CSC and the A2b adenosine receptor antagonist alloxazine each partially blocked the adenosine effect, whereas a combination of the two antagonists completely blocked the adenosine effect. The selectivity of CSC and alloxazine was confirmed by the observation that CSC blocked the inhibitory effect of A2a adenosine receptor agonist CGS 21680 on STAT5a and STAT5b tyrosine phosphorylation, while alloxazine was without effect (Fig. 9B). Furthermore, the A1/A3 adenosine receptor antagonist APNEA failed to inhibit STAT5 phosphorylation, and neither the A1 adenosine receptor antagonist DPCPX nor the A2 adenosine receptor antagonist MRS-1220 reversed the inhibitory effect of adenosine on STAT5 phosphorylation.

Because A2a and A2b adenosine receptors are coupled to adenyl cyclase and receptor stimulation leads to increased levels of intracellular cAMP (13, 50), it was likely that adenosine-induced SHP-2 activation was mediated through a cAMP-dependent mechanism. As expected, both 8-Br-cAMP and forskolin treatment of IL-2-stimulated CTL2-2 cells increased SHP-2 phosphorylation and, at the same time, decreased STAT5 phosphorylation (Fig. 10). Similar results were obtained when IL-2-stimulated CTL2-2 cells were treated with adenosine or NECA. Taken together, these results suggested that adenosine-induced dephosphorylation of STAT5 was due to adenyl cyclase activation in response to A2a and A2b adenosine receptor stimulation, which resulted in the accumulation of intracellular cAMP and activation of SHP-2, leading to STAT5a/b dephosphorylation. Moreover, the inhibitory effect of adenosine on tyrosine phosphorylation of STAT5a/b involved the cAMP-dependent activation of PKA because inhibition was lost when CTL2-2 cells were treated with Rp-cAMPS, which is a specific membrane-permeable inhibitor of PKA (51), before exposure to adenosine (Fig. 11). In addition, treatment of CTL2-2 cells with Rp-cAMPS prevented adenosine-induced phosphorylation of SHP-2 tyrosine residues (data not shown). Our findings are therefore in good agreement with a recent report that SHP-2 in bovine adrenocortical cells is phosphorylated and activated by cAMP-dependent PKA in response to stimulation with adrenocorticotropic hormone (69).

In conclusion, we have demonstrated for the first time that adenosine mediated an inhibitory effect on IL-2-induced tyrosine phosphorylation of STAT5a and STAT5b in CTL2-2 T lymphocytes, as well as in primary T cells. This interruption of a key proliferative signaling pathway that is associated with the IL-2R most likely accounts for the reduced capacity of adenosine-treated CTL2-2 T cells to proliferate in response to IL-2. In addition, we showed that the inhibitory effect of adenosine on tyrosine phosphorylation and activation of STAT5a and STAT5b was not due to a reduction in Jak1 or Jak3 phosphorylation, but rather was the result of adenosine-induced activation of a PTP. Increased SHP-2 activation and enhanced formation of SHP-2-STAT5 complexes in the presence of adenosine strongly suggested that the adenosine-induced PTP responsible for the inhibitory effect was SHP-2. We have previously reported that adenosine is an important immunosuppressive constituent of the microenvironment of solid carcinomas (10). Because T cells play a major role in antitumor immunity (70), the adenosine-induced defect in IL-2-stimulated proliferative signaling through STAT5 caused by PTP activation would be expected to result in impaired T cell-mediated antitumor immunity. The inhibitory effect of adenosine on STAT5a/b tyrosine phosphorylation was mediated via cAMP/PKA-dependent signaling pathways coupled to A2a and A2b adenosine receptors. This finding opens up the possibility of pharmacological intervention with appropriate adenosine receptor antagonists that would be expected to
prevent adenosine inhibition of IL-2R signal transduction and, thereby, potentiate T cell-based immunotherapy of solid cancers.

Acknowledgment

We thank A. Bennett for expert technical assistance.

References


The Journal of Immunology

943

Downloaded from http://www.jimmunol.org/ on November 13, 2017


