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A2A Adenosine Receptor May Allow Expansion of T Cells Lacking Effector Functions in Extracellular Adenosine-Rich Microenvironments¹

Akio Ohta,² Akiko Ohta, Manasa Madasu, Radhika Kini, Meenakshi Subramanian, Nipun Goel, and Michail Sitkovsky

Immunosuppressive signaling via the A2A adenosine receptor (A2AR) provokes a mechanism that protects inflamed tissues from excessive damage by immune cells. This mechanism is desirable not only for preventing uncontrolled tissue destruction by overactive immune responses, but also for protecting tumor tissues from antitumor immune responses. In aforementioned circumstances, T cell priming may occur in an environment containing high concentrations of extracellular adenosine. To examine qualitative changes in T cells activated in the presence of adenosine, we asked whether different functional responses of T cells are equally susceptible to A2AR agonists. In this study, we demonstrate that A2AR signaling during T cell activation strongly inhibited development of cytotoxicity and cytokine-producing activity in T cells, whereas the inhibition of T cell proliferation was only marginal. Both CD8⁺ and CD4⁺ T cells proliferated well in the presence of A2AR agonists, but their IFN- γ -producing activities were susceptible to inhibition by cAMP-elevating A2AR. Importantly, the impaired effector functions were maintained in T cells even after removal of the A2AR agonist, reflecting T cell memory of the immunoregulatory effect of adenosine. Thus, although the adenosine-rich environment may allow for the expansion of T cells, the functional activation of T cells may be critically impaired. This physiological mechanism could explain the inefficiency of antitumor T cells in the tumor microenvironment. *The Journal of Immunology*, 2009, 183: 5487–5493.

Extracellular adenosine is recognized to be an important physiological regulator of immune response (1–3). Immune cells express different subtypes of adenosine receptors on their surface, and adenosine can suppress inflammatory responses (1, 4–9). Four distinct subtypes of adenosine receptors have been identified, as follows: A1, A2A, A2B, and A3 receptors (10). On T cells, the predominant subtype expressed is A2A adenosine receptor (A2AR)³ (11, 12). A2AR agonists inhibit T cell activation through the induction of immunosuppressive intracellular cAMP (1–3, 9, 10). Genetic deficiency or pharmacological antagonism of A2AR results in significantly exaggerated inflammatory tissue damage (1, 13, 14). These observations demonstrate that the adenosine-A2AR pathway is one nonredundant, down-regulatory mechanism of inflammation among many negative regulators of immune response (1–3).

Adenosine may be formed as a physiological response to tissue insult due to inflammatory collateral damage to the microvasculature. This response, in turn, causes a decrease in tissue oxygen

tension (2, 3). Extracellular adenosine concentration is regulated by the following: 1) formation of adenosine from ATP/ADP/AMP by apyrase and 5'-nucleotidase; 2) degradation of adenosine to inosine by adenosine deaminase; 3) salvage of adenosine to AMP by adenosine kinase; and 4) flux of adenosine by membrane nucleoside transporter (2, 3, 15). Apyrase and 5'-nucleotidase, i.e., CD39 and CD73, are up-regulated under hypoxic conditions by the activation of hypoxia-inducible factor-1 α (15–17). In addition, hypoxia is known to down-regulate adenosine kinase (18, 19). Taken together, these studies support the view that the increase in extracellular adenosine in the inflamed tissue microenvironment is due to local tissue hypoxia. Further supporting this interpretation, our recent data demonstrate that whole body hypoxia reduced inflammatory tissue damage due to the recruitment of the adenosine-A2AR pathway (20).

This reaction limits excessive inflammatory responses and prevents critical damage to normal tissues. However, the same mechanism may also allow tumors to escape antitumor immunity. Measurement of oxygen tension revealed a state of chronic hypoxia in tumor tissues (21, 22). Accordingly, a gradient of intratumoral adenosine was demonstrated radiating from the center of the solid tumor (23). The presence of adenosine in tumors might play a role in suppressing antitumor immune responses. In support of this hypothesis, A2AR-deficient mice successfully rejected a tumor challenge that no wild-type mice could survive (23). A2AR antagonists also improved the efficacy of the adoptive transfer of antitumor T cells (23).

The evidence strongly suggests that, under inflammatory insult, tissues can form an adenosine-rich microenvironment as a self-protective mechanism against attacks by immune cells (2, 3). Therefore, in inflamed tissue or tumor tissue, T cells may have to activate under the influence of high concentrations of adenosine. T cells activated in the presence of adenosine may be functionally

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³ Abbreviations used in this paper: A2AR, A2A adenosine receptor; A2BR, A2B adenosine receptor; CGS, CGS21680; NECA, 5'-N-ethylcarboxamidoadenosine; PKA, protein kinase A.

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different from T cells in normal environment. In this study, we tested the effects of various concentrations of A2AR agonists on T cell proliferation, cytokine production, and cytotoxicity. Although A2AR signaling was inhibitory to all tested T cell functions, the suppression of T cell proliferation was considerably weaker than the inhibitory effect on other T cell functions. In contrast, IFN- γ production was the most sensitive to A2AR agonists. Thus, an adenosine-rich microenvironment may allow T cells to proliferate, but critically impairs important effector functions, i.e., cytokine production and cytotoxicity. Our results suggest that T cell activation in inflamed tissue and tumor microenvironment results in the induction of T cell unresponsiveness by A2AR-dependent mechanism.

Materials and Methods

Mice

C57BL/6 mice were obtained from Charles River Laboratories. The mice were housed in the animal facility of Northeastern University and were used at 8–10 wk of age in accordance with institutional animal care guidelines.

Cytokine production and cell proliferation

Spleen cells (5×10^5 cells) from C57BL/6 mouse were stimulated with anti-CD3 mAb (145-2C11; BD Biosciences) at 0.1 μ g/ml. A2AR agonists, 5'-N-ethylcarboxamidoadenosine (NECA; Sigma-Aldrich) or CGS21680 (CGS; Tocris Cookson), were added at concentrations between 10^{-9} and 10^{-5} M. Culture supernatant was collected after 24 h for subsequent measurement of cytokine levels. IFN- γ , IL-2, and IL-10 levels were quantified by ELISA (R&D Systems), according to manufacturer's instructions. For proliferation assay, cells were incubated with 1 μ Ci of [3 H]TdR (American Radiolabeled Chemicals) for an additional 4 h, and radioactivity of incorporated TdR was counted.

cAMP assay

Measurements of cAMP levels were performed, as described previously (24). Cells (2×10^5) were incubated with NECA or CGS for 15 min at 37°C. The concentration of A2AR agonists was 5 μ M. A2AR antagonist, ZM241385 (Tocris Cookson), was added at 1 μ M. cAMP levels were determined by ELISA (GE Healthcare).

Proliferation assay using CFSE

Spleen cells were washed with PBS and labeled with CFSE (Molecular Probes) at 1 μ M for 8 min. To remove excess CFSE, the cell suspension was diluted with FCS and centrifuged. This washing step was repeated twice. The resulting CFSE-labeled cells were stimulated with anti-CD3 mAb. After the culture, the cells were stained with PE-conjugated anti-CD8 and allophycocyanin-conjugated anti-CD4 mAbs, and cell division was analyzed by flow cytometer.

Apoptosis assay

Spleen cells activated for 24 h in the presence of various concentrations of CGS were examined for the induction of apoptotic cell death. The assay was done using annexin V-FITC apoptosis detection kit (BD Biosciences) and subsequent flow cytometric analysis.

Intracellular cytokine staining

Cytokine production from T cells was analyzed by intracellular staining, as described (25). After the stimulation with anti-CD3 mAb for 24 h, cells were further incubated in the presence of brefeldin A (10 μ g/ml) for 2 h. Cells were surface stained with PE-conjugated anti-CD8 mAb and allophycocyanin-conjugated anti-CD4 mAb. After fixation and permeabilization, intracellular IFN- γ was stained with FITC-labeled anti-IFN- γ mAb and analyzed by FACSCalibur (BD Biosciences). All Abs were obtained from BD Biosciences.

Purification of T cells

Purification of CD4 $^+$ and CD8 $^+$ cell populations was performed using AutoMACS separator (Miltenyi Biotec). Spleen cells were labeled with FITC-conjugated anti-CD4 or anti-CD8 mAb (BD Biosciences), and followed by anti-FITC microbeads (Miltenyi Biotec). After cell sorting, purity of the cells was more than 98%. The purified cells (2×10^5 cells) were

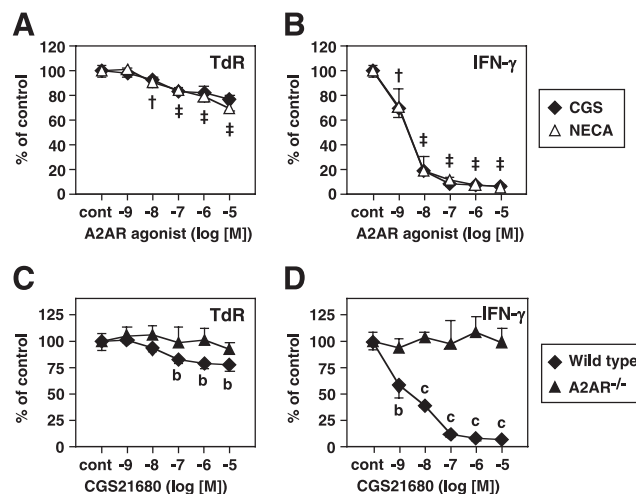


FIGURE 1. A2AR agonists suppress T cell activation, but the magnitude of inhibition is stronger for IFN- γ production than proliferation. **A** and **B**, Spleen cells were stimulated by anti-CD3 mAb with various concentrations of CGS and NECA. Thymidine uptake and IFN- γ levels in the supernatant were examined after 24 h. \dagger , $p < 0.01$; \ddagger , $p < 0.001$ for both control vs CGS and control vs NECA. Data represent average \pm SD of triplicate samples. **C** and **D**, The immunosuppressive effects of CGS were mediated by A2AR. Data represent average \pm SD of triplicate samples. **b**, $p < 0.01$; **c**, $p < 0.001$ wild type vs A2AR $^{-/-}$.

stimulated with anti-CD3 mAb (0.1 μ g/ml) in the presence of feeder cells (5×10^5 cells). The feeder cells were prepared from spleen cells depleted of CD3-positive cells using AutoMACS, and were pretreated with 50 μ g/ml mitomycin C for 30 min at 37°C.

Cytotoxicity

Cytotoxicity of T cells against P815 mastocytoma (H-2 d) was determined by 51 Cr release assay. Effector cells were prepared 36 h after stimulation of C57BL/6 spleen cells with anti-CD3 mAb, and incubated with the target cells for 4 h in the presence of anti-CD3 mAb (1 μ g/ml).

Statistics

Data represent mean \pm SD. Statistical calculations were performed using Student's t test. Statistical significance was accepted for p values less than 0.05.

Results

Expansion of T cells with impaired IFN- γ production in the presence of A2AR agonists

Susceptibility of T cell proliferation and cytokine secretion to A2AR stimulation was compared using anti-CD3 mAb-stimulated spleen cells in the presence or absence of adenosine analogues. Both NECA (nonspecific adenosine receptor agonist) and CGS21680 (A2AR-specific agonist) inhibited T cell proliferation and IFN- γ levels in a concentration-dependent manner (Fig. 1, **A** and **B**). A2AR involvement in this inhibition was demonstrated in genetically controlled experiments in which suppression of T cell proliferation and IFN- γ production by these synthetic adenosine analogues was not observed in T cells from A2AR-deficient mice (Fig. 1, **C** and **D**). Inhibition of T cell proliferation was statistically significant when CGS or NECA was added at 10 nM, but the decrease was limited to only 10% (Fig. 1A). The cells maintained 80% of their original proliferative activity even in the presence of much higher concentrations of CGS and NECA. Indeed, the CFSE labeling of spleen cells confirmed massive T cell proliferation during stimulation in the presence of CGS. This was shown by stepwise dilution of the fluorescence in daughter cells (Fig. 2A). CGS treatment has some tendency to decrease the numbers of the extensively divided T cells (*left peaks* in

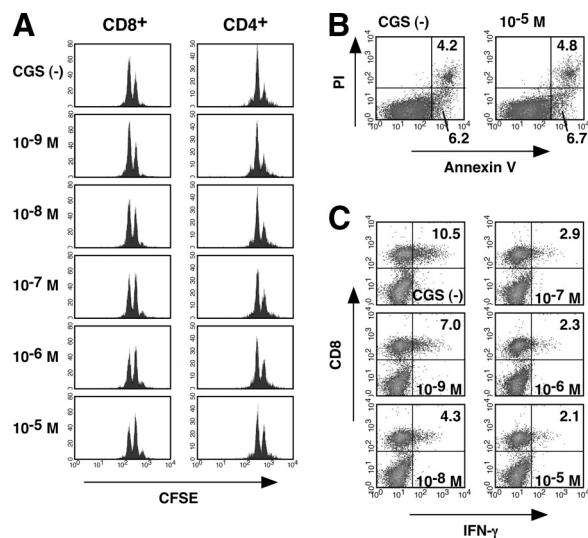


FIGURE 2. Activated T cells proliferated well in the presence of A2AR agonist, but the number of IFN- γ -producing T cells was largely decreased. **A**, Proliferation of T cells in the presence of CGS. Spleen cells were labeled with CFSE and cultured with anti-CD3 mAb for 36 h. The cells were analyzed by flow cytometry after surface staining of CD4 and CD8. The decreased fluorescence intensity indicates dilution of CFSE in proliferated cells. **B**, There was no significant increase of apoptotic cells in CGS-treated cells. Spleen cells were activated, as described above, and stained with FITC-annexin V and propidium iodide (PI). The numbers represent percentage of cells in quadrants. CGS did not increase apoptotic cells at any concentrations. Only results from control and the highest concentration of CGS were shown as representative. **C**, Decrease of IFN- γ -producing cells by CGS. Intracellular IFN- γ expression in activated T cells was analyzed after 36 h of stimulation with anti-CD3 mAb and subsequent incubation with brefeldin A for 2 h. The numbers represent percentage of CD8⁺ IFN- γ ⁺ cells.

Fig. 2A) in both CD4⁺ and CD8⁺ cells. However, the inhibitory effect of CGS on proliferation was not strong enough to abolish the appearance of the extensively divided T cells, and many T cells proliferated well even at the highest concentration of CGS (Figs. 1A and 2A). During T cell activation, CGS did not increase apoptotic cell death, indicating that the inhibition of cell proliferation was not due to augmented cell death (Fig. 2B).

In contrast to the effect on cell proliferation, the inhibitory effect on IFN- γ production in the same cell culture was much more remarkable even at 10 nM CGS and NECA. More than 90% of IFN- γ was suppressed by higher concentrations of A2AR agonists (Fig. 1B). This drastic change could not be explained by a decrease in the T cell number, because there was only a slight decrease in cell proliferation in CGS-treated T cells. Indeed, intracellular IFN- γ staining directly confirmed that CGS strongly down-regulated the frequency of IFN- γ -producing T cells (Fig. 2C). The amount of IFN- γ from each producer was also decreased, as indicated by the attenuation of fluorescence intensity. These results show that T cells are able to proliferate under an immunosuppressive concentration of A2AR agonists, but IFN- γ production in these cells is critically impaired.

Both CD4⁺ and CD8⁺ T cells are sensitive to A2AR-mediated immunosuppression

To confirm the expression of functional A2AR on T cells, we monitored cAMP production in response to A2AR stimulation. When exposed to CGS or NECA, purified CD4⁺ and CD8⁺ cells up-regulated cAMP levels (Fig. 3A). The increase was blocked by A2AR-selective antagonist ZM241385, thereby confirming that

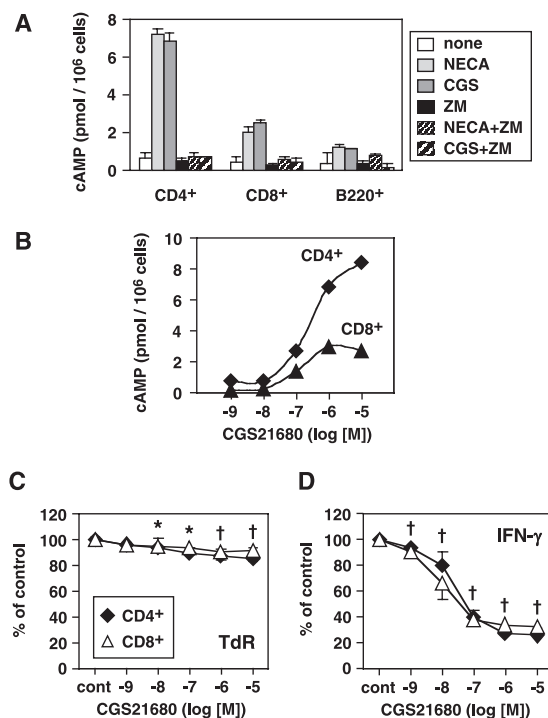


FIGURE 3. Both CD4⁺ and CD8⁺ T cells respond well to A2AR agonists and are susceptible to immunosuppressive effects. **A**, NECA- or CGS-inducible cAMP levels in purified CD4⁺, CD8⁺ T cells, and B cells. ZM241385 (ZM) is an A2AR antagonist. **B**, Concentration-dependent increases of cAMP levels in CD4⁺ and CD8⁺ cells. **C** and **D**, Suppression of proliferation and IFN- γ production from purified CD4⁺ and CD8⁺ cells stimulated in the presence of CGS. Data represent average \pm SD of triplicate samples. *, $p < 0.05$; †, $p < 0.01$ vs control in both CD4⁺ and CD8⁺ cells.

the increase of cAMP was due to A2AR-mediated responses. CD4⁺ cells produced higher levels of cAMP than CD8⁺ cells, but the concentration of CGS required for the remarkable cAMP induction was similar in CD4⁺ and CD8⁺ cells (Fig. 3B). cAMP response in B cells was even lower than in CD8⁺ cells (Fig. 3A).

The vigorous cAMP response of CD4⁺ and CD8⁺ cells suggested that both of these T cell populations might be targets of an A2AR-mediated immunosuppressive mechanism. We examined the effects of A2AR agonist on the activation of purified CD4⁺ and CD8⁺ T cells. As in similar observations with unseparated cell cultures, T cell stimulation in the presence of CGS resulted in slightly decreased proliferative activities in both CD4⁺ and CD8⁺ cells (Fig. 3C). In contrast to the weak effect on cell proliferation, CGS strongly suppressed IFN- γ production from CD4⁺ and CD8⁺ cells (Fig. 3D). These results show that T cell activation is inhibited by an A2AR-mediated signal in both CD4⁺ and CD8⁺ T cells. Indeed, strong susceptibility of the IFN- γ -producing mechanism and the resistance of cell proliferation to A2AR agonist were common characteristics in both CD4⁺ and CD8⁺ T cells.

Effects of A2AR agonist on cytokine production

We next examined the effects of A2AR agonist on other cytokines. In addition to IFN- γ , IL-2 was sensitive to the immunosuppressive effect of CGS. Although the inhibition of IL-2 was not as potent as that of IFN- γ , IL-2 levels decreased up to 40% by CGS (Fig. 4A). Because IL-2 plays an important role in activated T cells, we tested whether the inhibition of IL-2 production was responsible for the immunosuppressive effect of A2AR agonist. rIL-2 was added together with CGS at the start of T cell stimulation; however, IL-2

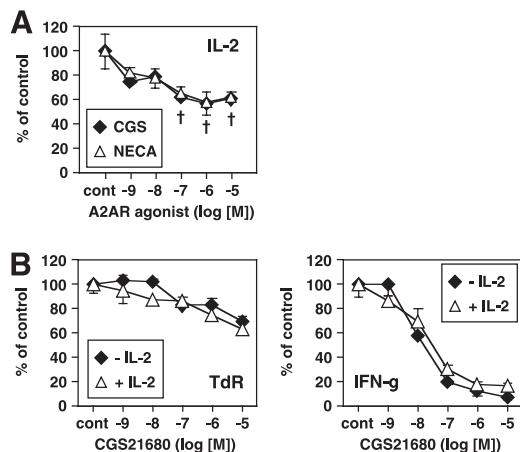


FIGURE 4. IL-2 supplementation did not reverse A2AR-mediated immunosuppression. **A**, Decrease of IL-2 levels by A2AR agonists. Spleen cells were cultured as in Fig. 1. The panel shows IL-2 levels in the supernatant after 24 h. †, $p < 0.01$ vs control in both CGS- and NECA-treated cells. **B**, Mouse rIL-2 (5 ng/ml) was added to compensate decrease of spontaneous IL-2 production. There was no significant improvement in levels of thymidine uptake and IFN- γ production by IL-2 supplementation. Data represent average \pm SD of triplicate samples.

supplementation failed to reverse T cell inhibition by CGS. Inhibition of cell proliferation and IFN- γ production remained unchanged by the addition of IL-2 (Fig. 4B).

Despite extensive T cell proliferation, A2AR agonists strongly suppressed IFN- γ and IL-2 production in these activated T cells. Down-regulation of IFN- γ and IL-2 might correlate with functional differentiation of T cells into Th2/Tc2-type effectors. We tested the expression of Th2 cytokines; however, the increase of IL-4 was not detected at any concentration of CGS (data not shown). Likewise, there was no notable increase in IL-10 levels (data not shown). Changes in the Th1/Th2 balance are not likely a mechanism for A2AR-mediated down-regulation of IFN- γ .

Induction of activated T cells lacking significant effector functions

The suppression of T cell effector functions by A2AR signaling is not limited to cytokine production. Cytotoxic activity was also suppressed in T cells activated in the presence of CGS (Fig. 5). The decrease in cytotoxicity was $\sim 50\%$ of control. The magnitude of inhibition was less potent than that of IFN- γ , comparable to IL-2, and stronger than proliferation.

We further examined whether the A2AR-mediated inhibition is a temporary change only during the presence of A2AR agonist or

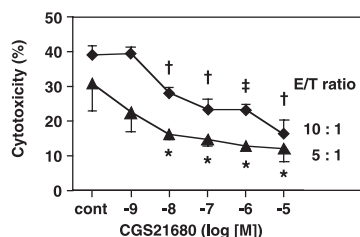


FIGURE 5. Decreased cytotoxicity in activated T cells developed under influence of A2AR agonist. T cell activation was induced as in Fig. 1 with various concentrations of CGS. After extensive wash to remove CGS, cytotoxicity against P815 cells was examined by 4-h standard ^{51}Cr release assay. Data represent average \pm SD of triplicate samples. *, $p < 0.05$; †, $p < 0.01$; ‡, $p < 0.001$ vs control.

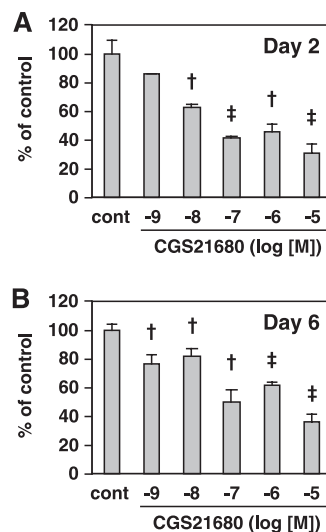


FIGURE 6. Impaired IFN- γ -producing activity in activated T cells developed in the presence of A2AR agonist. T cell activation was induced by anti-CD3 mAb with various concentrations of CGS. After 36 h, CGS was removed by extensive wash. Cells were then restimulated by immobilized anti-CD3 and anti-CD28 mAbs either immediately after removal of CGS (day 2, **A**) or after culturing for 4 more days in the absence of CGS (day 6, **B**). When cells were rested for 4 days, IL-2 (5 ng/ml) was added to the culture. IFN- γ levels in the supernatant were quantified 24 h after restimulation. Data represent average \pm SD of triplicate samples. †, $p < 0.01$; ‡, $p < 0.001$ vs control.

the persistent impairment of T cell functions. After T cell activation for 2 days, cells were examined for the IFN- γ -producing potential by subsequent restimulation in the absence of CGS (Fig. 6A). Interestingly, CGS-pretreated cells produced a significantly smaller amount of IFN- γ even after the removal of CGS. This result suggests that CGS not only inhibits IFN- γ production during T cell activation, but also impairs establishment of fully functional effector T cells. We further tested whether the inferior IFN- γ -producing activity in CGS-pretreated cells would persist for a longer time. CGS was removed after 2 days of T cell activation, and the cells were rested for 4 days in the absence of CGS. Subsequent restimulation of these cells showed persistently inferior IFN- γ production in CGS-pretreated cells (Fig. 6B). Taken together, activated T cells developed under the influence of A2AR stimulation are not fully mature as strong effector cells.

Discussion

The adenosine-A2AR pathway is one of the important physiological regulators of the inflammatory response (2, 3), as demonstrated by the exacerbation of inflammatory tissue damage in A2AR-deficient mice (1, 13, 14, 26). T cells are targets of adenosine-mediated immunoregulation because they predominantly express A2AR (11, 12). Both CD4 $^{+}$ and CD8 $^{+}$ T cells respond to A2AR stimulation and are susceptible to immunosuppression (Fig. 3).

When adenosine binds to A2AR, the subsequent increase in intracellular cAMP leads to protein kinase A (PKA) activation (1, 10). The accumulated studies of cAMP- and PKA-mediated inhibition of T cell functions have demonstrated that cAMP-elevating A2AR agonists inhibit cytotoxic activity through down-regulation of Fas ligand and granule exocytosis (27–30). A2AR-mediated signaling may down-regulate the very early events of TCR signaling because PKA phosphorylates COOH-terminal Src kinase to inhibit T cell activation by interrupting Lck activation (31). These more

recent data explain further how the A2AR-mediated increase of cAMP may inhibit general T cell responses such as proliferation (32) and cytokine production (33, 34).

Motivating this study was the novel question whether different T cell responses are equally susceptible to inhibition by A2AR signaling. We compared A2AR-mediated inhibition of T cell proliferation, cytokine production, and cytotoxicity in T cells from the same cell culture. There was preferential down-regulation of effector functions such as IFN- γ /IL-2-producing and cytotoxic activities, whereas proliferative activity was resistant to inhibition. Our results suggest that adenosine does not totally suppress T cell functions, but T cell activation in the presence of adenosine may result in proliferation of T cells that express only limited cytotoxicity and cytokine production (Figs. 1, 2, 4, and 5).

Because we observed a strong inhibitory effect of A2AR agonists on IFN- γ and IL-2, which are representative Th1 cytokines, we also tested whether A2AR agonist may facilitate functional differentiation of T cells into Th2/Tc2-type effectors. Because Th1- and Th2-type immune responses are regulated in a mutually exclusive manner, the decrease of Th1 cytokines might indicate polarization of T cells into Th2/Tc2 cells. Indeed, acting on APCs, adenosine may direct a T cell response toward Th2-type immunity by an increase of IL-10 and a decrease of IL-12 (35, 36). This response was not demonstrated, however, because in our experimental system the A2AR-mediated decrease of IFN- γ and IL-2 was not accompanied by an increase in Th2-type cytokines, IL-4 and IL-10.

Adenosine may exert its anti-inflammatory activity by up-regulating IL-10, as observed in LPS-stimulated macrophages (35, 37). However, in our T cell culture, there was no increase in IL-10 levels, suggesting that the anti-inflammatory activity of IL-10 does not account for the A2AR-mediated T cell inhibition in this study. Although the IL-10 gene has functional cAMP-responsive elements in the untranslated region (38), the regulation mechanism of IL-10 gene in T cells seems to be different from that of macrophages.

The expansion of functionally poor T cells is similar to results reported in a recent publication by Zarek et al. (34) that demonstrate the A2AR-mediated induction of anergic T cells. In their study using a Th1 clone, suppression of IL-2 production played a key role in the induction of T cell anergy. Indeed, CGS critically impaired IL-2 production rather than IFN- γ production, and IL-2 supplementation reversed the inhibition of T cells (34). In contrast to this published result, our experiments show that IL-2 production was only moderately inhibited by CGS, and IL-2 supplementation failed to reverse the decrease of T cell proliferation and IFN- γ production (Fig. 4). This inconsistency could be due to differences in cell types used in the studies. Although we started from resting T cells freshly prepared from the mouse spleen, Zarek et al. (34) used functionally differentiated Th1 clone. Activated T cells may respond to the increase in intracellular cAMP in a different manner from resting T cells (39). In addition, activated Th and Tc effectors were shown to be more inhibited in their IL-2 than IFN- γ production in response to A2AR agonist (40).

Comparing with the suppression in effector T cells, adenosine-rich microenvironment may not be hostile to the immunosuppressive regulatory T cells. CD39/CD73 expression on the surface of regulatory T cells at high levels indicates their capability to increase local extracellular adenosine concentration by ATP catabolism (16, 17). Indeed, regulatory T cells use adenosine as one of their immunosuppressive mechanisms (17). Upon T cell activation, in contrast to the decrease in CD4⁺ and CD8⁺ T cells (Fig. 2), FoxP3⁺ cell population within CD4⁺ cells slightly increased from $1.93 \pm 0.21\%$ in normal condition to $3.07 \pm 0.15\%$ in the

presence of CGS (10^{-5} M) after 2 days (data not shown). Taking general inhibition of T cell proliferation into consideration, the change in the number of T regulatory cells may be so small to explain the drastic down-regulation of T cell effector functions. However, their regulatory activity will need to be compared in the presence or absence of A2AR signaling. This is because A2AR stimulation was reported to up-regulate FoxP3 mRNA (34), and the regulatory activity may be enhanced in T regulatory cells activated in adenosine-rich environment.

The presented data may be relevant to better understanding immunosuppression near or within tumors (41–43) because tumor tissues contain higher concentrations of extracellular adenosine (23). Tumor-infiltrating T cells isolated from cancer patients are not cytotoxic to tumor cells as they are, but they regain tumor-specific cytotoxicity after culturing in vitro with IL-2 (44). This implies that priming of antitumor T cells has taken place in cancer patients, but the effector functions are disabled in the tumor microenvironment (45). Although otherwise strongly responsive to the tumor Ag, it is important to note that the same antitumor T cells are poorly responsive in the tumor. As indicated by diminished T cell proliferation and cytokine production, antitumor T cells are rapidly tolerized when they move into the tumor microenvironment (46, 47).

A variety of mechanisms has been shown to be involved in tumors that evade antitumor immune responses, as follows: FoxP3⁺ regulatory T cells (48, 49), myeloid suppressor cells (50, 51), IL-10 (52), TGF- β (53, 54), programmed death ligand-1 (55), indoleamine-2,3-dioxygenase (56, 57), and glucose deprivation (58). Adenosine is a newly identified immunoregulatory molecule in tumors. Dramatic regression of immunogenic tumors in A2AR-deficient mice suggests the significance of the adenosine-A2AR pathway in suppressing antitumor T cells (23). This study demonstrates a substantial decrease in effector activities, but not in proliferation, of T cells stimulated in the presence of A2AR agonist. It therefore suggests that inactivation of the adenosine-A2AR pathway may enhance effector activities of antitumor T cells. Thus, antiadenosinergic therapy may be beneficial in tumor immunotherapy when combined with treatments that promote the number and/or function of antitumor T cells, e.g., adoptive T cell transfer and tumor vaccine.

In addition to A2AR, T cells are expressing A2B adenosine receptor (A2BR), which is also coupled to Gs protein and increases intracellular cAMP (23, 59, 60). Recent studies revealed A2BR expression in other immune cells and involvement of A2BR in the regulation of inflammation, but the results are somewhat controversial. A2BR antagonists are shown to inhibit pathogenesis of airway sensitivity, pulmonary inflammation, and colitis, suggesting proinflammatory role of A2BR (61–63). However, studies using A2BR-deficient mice have shown exacerbation of vascular inflammation, lung injury, ischemia/reperfusion injury, and colitis (64–67). The immunosuppressive role of A2BR is correspondent to the production of anti-inflammatory molecules such as IL-10, TGF- β , and indoleamine-2,3-dioxygenase from dendritic cells in the presence of A2BR stimulation (68). Furthermore, hypoxia-inducible factor-1 α -inducible netrin-1 was found to down-regulate inflammatory responses by A2BR-dependent mechanism (69). These findings suggest cooperation of adenosine and netrin-1 in the hypoxia-induced immunosuppressive mechanism through adenosine receptors. Therefore, it is speculated that adenosine-A2BR interaction may inhibit T cell activation, but details of direct effect of A2BR stimulation on T cells remain to be elucidated.

A2AR-mediated immunoregulation may represent the fundamental mechanism that has evolved to protect normal tissues from collateral damage during the antipathogen response (1–3, 13, 14,

26). The presented study suggests that this A2AR-mediated mechanism may have evolved to limit excessive collateral damage in local tissue environments without a global inhibition of T cell expansion. In addition, because A2AR stimulation did not induce apoptosis or strongly suppress proliferation of activated T cells (Figs. 1 and 2), the mechanism may also play a role in the long-term preservation of Ag-specific T cells, i.e., immunological memory.

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Disclosures

The authors have no financial conflict of interest.

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