Much Ado about Adenosine: Adenosine Synthesis and Function in Regulatory T Cell Biology

Peter B. Ernst, James C. Garrison and Linda F. Thompson

*J Immunol* 2010; 185:1993-1998; doi: 10.4049/jimmunol.1000108

http://www.jimmunol.org/content/185/4/1993

**References**

This article cites 89 articles, 47 of which you can access for free at:

http://www.jimmunol.org/content/185/4/1993.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
Much Ado about Adenosine: Adenosine Synthesis and Function in Regulatory T Cell Biology

Peter B. Ernst,*†‡ James C. Garrison,§ and Linda F. Thompson‖

Recent studies have reported that adenosine is a significant mediator of regulatory T cell (Treg) function. Indeed, activation of the adenosine receptor subtypes expressed by a broad range of immune and inflammatory cells attenuates inflammation in several disease models. This anti-inflammatory response is associated with an increase in intracellular cAMP that inhibits cytokine responses of many immune/inflammatory cells, including T cells and APCs. Thus, adenosine produced by Tregs can provide a paracrine feedback that shapes the host response following an immunologic provocation. This review discusses the evidence that adenosine is an integral part of Treg biology and presents some of the mechanisms that may account for its contribution to the resolution of inflammation and the regulation of the immune/inflammatory cell phenotype. The Journal of Immunology, 2010, 185: 1993–1998.

Adenosine synthesis and the control of inflammation

Adenosine accumulates in response to inflammation and ischemia. There are several means by which adenosine is produced (4), beginning with the metabolism of intracellular ATP, ADP, and AMP via the action of cytoplasmic 5′-nucleotidases. Second, it can be generated by S-adenosylhomocysteine hydrolase. Finally, adenosine can be generated extracellularly from ATP and ADP via the combined action of ecto-nucleoside triphosphate diphosphohydrolase (ecto-NTPDase-1 [CD39])—or possibly other NTPDases—and ecto-5′-nucleotidase (CD73). Adenosine concentrations are also influenced by adenosine deaminase (ADA), a purine salvage pathway enzyme that degrades adenosine to inosine and adenosine kinase, which phosphorylates adenosine to AMP. The role of adenosine in immune regulation can be appreciated when its concentration is modulated by a deficiency of ADA, CD39, or CD73 expression. Loss of ADA elevates adenosine (5), whereas deletion of CD39 (6) or CD73 (7) results in less extracellular adenosine and impaired control of inflammation. For example, cd73−/− mice exhibit defects in kidney function (8), thromboregulation (9), physiologic responses to hypoxia (7), and enhanced responses to some infections (10).

Evidence for the generation of adenosine by Tregs

The first suggestion that adenosine played a role in Treg biology emerged from an in vivo study in mice in which the adoptive transfer of Tregs prevents the induction of colitis by effector Th cells (Teffs) (11). As elaborated below, Tregs failed to control colitis by Teffs that lacked the adenosine A2A receptor (A2AAR), suggesting that adenosine contributed to the anti-inflammatory role conferred by Tregs. Shortly thereafter, murine Tregs were shown to express CD73, which converts 5AMP into adenosine (12). However, CD73 is expressed by most activated Th cells, and its expression is not enriched on Tregs. In two subsequent studies, Tregs were shown to express CD73 and CD39, thus possessing the full synthetic pathway enabling Tregs to synthesize adenosine from ATP (6, 13). Importantly, these Tregs have been shown to regulate inflammation in an adenosine-dependent manner (6, 13), and translational studies report that CD39 is expressed selectively with CD73 on Tregs from different human tissues (10, 13–16).

Abbreviations used in this paper: A2AAR, adenosine 2A adenosine receptor; A2BAR, adenosine 2B adenosine receptor; ADA, adenosine deaminase; AR, adenosine receptor; DAG, diacylglycerol; ecto-NTPDase-1, ecto-nucleoside triphosphate diphosphohydrolase; Epac, exchange protein directly activated by cAMP; IP3, phosphatidylinositol bisphosphate; PDE, phosphodiesterase; PKA, cAMP-dependent protein kinase; PLC, phospholipase C; SOCS, suppressor of cytokine signaling; Teff, effector Th cell; Treg, regulatory T cell.
Additional evidence associating adenosine production with Tregs comes from experiments in which the presence of Foxp3 is positively associated with CD39 expression (13, 17). The documented ability of Tregs to convert ATP to adenosine provides direct evidence of the functional capacity of these enzymes (6, 13) and supports the notion that adenosine is a mediator of Treg function (Fig. 1).

Tregs can be defined by the differential expression of surface markers, and subsets of Tregs appear to control different diseases (18). This diversity may be linked to the ability to synthesize adenosine, because Tregs that express CD39 preferentially control Th17 responses (19). More direct evidence in support of adenosine as a mediator of Tregs includes studies showing that Tregs from cd39/− mice are less efficient at suppressing proliferation of Teffs from A2AAR-deficient mice (6). However, it is impossible to ascertain from this study whether the lack of CD39 on the Treg or the lack of A2AAR on the Teff targets was the most important factor. It is worth noting that the Teffs lacking the A2AAR, which could not be controlled in the colitis model in vivo, were inhibited in the proliferation inhibition assay (20). These studies suggest that Tregs use different mediators, including adenosine, that have varied effects on Teff function in distinct assays.

**Adenosine as an anti-inflammatory mediator in disease models**

The digestive tract is ideal for addressing the biologic relevance of adenosine-mediated immune regulation in vivo, because adaptive Treg control responses to the shifting repertoire of luminal Ags in this organ. For example, Tregs regulate the magnitude of gastritis after infection with *Helicobacter* spp. (21–23). Consistent with a role for adenosine in the control of inflammation, gastritis was more severe when mice lacking the A2AAR predominantly (10, 24, 28–30), whereas APCs express the A2AAR and/or A2BAR (31–35). The A2AAR is important target for Treg-derived adenosine. Adenosine production by Tregs also contributes to immune regulation in other regions of the digestive tract. Tregs prevent the wasting and colitis that follows the adoptive transfer of wild-type pathogenic CD4+ CD45RBhi Th cells into scid mice (20). In this same model, A2AAR agonists alone prevented colitis induced by pathogenic Th cells in the absence of Tregs (11). As mentioned above, wild-type Tregs failed to prevent colitis induced by the adoptive transfer of pathogenic Teffs from A2AAR−/− mice, implicating an anti-inflammatory role for Treg-derived adenosine (11). A2AAR on recipient myeloid cells is also required for optimal control of intestinal inflammation, because the cotransfer of Tregs and pathogenic T cells from wild-type mice into rag1/A2AAR−/− double knockout recipients still leads to colitis (C.C. Kurtz and P.B. Ernst, manuscript in preparation). The importance of adenosine in the control of intestinal inflammation is supported by the increased susceptibility of cd39/− mice to colitis induced by dextran sodium sulfate and the association of mutations in the cd39 gene with Crohn’s disease in humans (26).

Based on these studies in the gastrointestinal tract, we propose that adaptive Tregs are important to cope with the complex microbiota and that the production of adenosine by Tregs is significant in this context. However, details of the distribution and expression of the molecules responsible for the synthesis and response to adenosine in the gut remain to be determined.

**Mechanisms for adenosine-mediated Treg function**

Adenosine binds to four distinct subtypes of adenosine receptors (ARs): A1, A2A, A2B, and A3 (1, 4). Because of a lack of useful Abs for the assessment of cell surface AR expression, investigators must rely on multiple approaches to confirm the role of a specific AR subtype in each experimental model, including the use of cells or mice deficient in specific ARs and/or appropriate concentrations of highly selective agonists or antagonists. Using genetically engineered mice lacking the A2AAR, adenosine has been implicated in the control of a wide range of inflammatory responses (27). It is now widely appreciated that Th cells express the A2AAR predominantly (10, 24, 28–30), whereas APCs express the A2AAR and/or A2BAR (31–35). The A2AAR is induced ~8-fold in naive Th cells upon activation (28, 29), whereas induction in excess of 100-fold occurs in APCs in response to LPS (36). Because it has been known for many years that ARs can mediate anti-inflammatory effects, their induction upon immunologic provocation suggests that adenosine is a broadly relevant mediator providing a negative feedback to limit immune-mediated tissue damage. As such, ARs are an important target for Treg-derived adenosine.

Adenosine regulates T cell function indirectly by reducing the secretion of proinflammatory cytokines, including IL-12 and TNF-α, from stimulated APCs by as much as 80–90% (33, 34, 37). In the case of TNF-α, the impairment in expression is due to a decrease in mRNA stability (38). Adenosine also stimulates production of the anti-inflammatory cytokine IL-10 (31–34). In addition, the expression of CD86 and the immunogenicity of dendritic cells are impaired by adenosine acting through the A2AR (13, 31, 37, 39), mimicking some mechanisms of immune regulation conferred by Tregs (13, 40, 41). Thus, the marked induction of ARs after activation provides a feedback mechanism to control the host response to an infection or immunization.

A2AAR signaling on activated Th cells directly inhibits the production of cytokines including IL-2, IL-4, IFN-γ, and TNF-α.

**FIGURE 1.** CD39 and CD73 on Tregs generate extracellular adenosine, which can engage ARs on Teffs, APCs, and myeloid cells. This aspect of Treg function would be dependent on the presence of the substrates (local ATP, ADP, and/or AMP), which accumulate as a consequence of inflammation, hypoxia, metabolic stress, and/or cell death.
by as much as 90% (28, 42), again by decreasing mRNA stability (11). Adenosine acts directly as an anti-inflammatory mediator, and the administration of adenosine analogs increases the number of Tregs (42). A role for adenosine in the optimal development of Treg responses could explain why Tregs from A2AR−/− mice failed to control disease caused by the adoptive transfer of wild-type pathogenic Th cells (11). The fact that Tregs produce and respond to adenosine implies that adenosine acts as an autocrine factor to optimize anti-inflammatory responses. This immunosuppressive action of adenosine may be mediated by increasing the concentration of intracellular cAMP. Indeed, Tregs can transfer intracellular cAMP to Teffs via gap junctions (43), and elevating cAMP in Tregs renders them more suppressive (44, 45).

The transfer of cAMP to a target Teff would occur only when Tregs have direct contact with the target cell. This requirement for intimate cell–cell contact for the optimal expression and function of Treg mediators is shared by membrane-associated TGF-β1 (46) and IL-35 (47). Furthermore, inhibition of host responses mediated by interactions of LAG3 (48) and CTLA-4 (41) on Tregs with their respective receptors on target cells requires intimate contact (40). Because the t_{1/2} of adenosine is measured in seconds, intimacy within the immunologic synapse shared by APCs, Tregs, and Teffs would be essential for any inhibitory role for adenosine (Fig. 2).

Coupling of ARs to the control of inflammatory responses

The A2AR and A2BAR are distinguished by their ability to mediate increases in cAMP. The accumulation of cAMP is believed to be responsible for the anti-inflammatory effects of adenosine delivered to T cells, APCs, or neutrophils (1, 4, 27). The A2ARs are coupled to members of the Gs subfamily of G proteins, and their activation raises the level of cAMP (1, 49) (Fig. 3). Similarly, the activation of phosphodiesterases (PDEs) that degrade cAMP enhances responses of activated Th cells (50, 51). PDE activation is mediated in part through CD4. Mutation of class II MHC molecules on APCs so that they no longer bind CD4 prevents PDE activation in Th cells following stimulation and allows cAMP to accumulate (52). In association with this increase in cAMP, Th cells assume a phenotype resembling a Treg rather than an effector Th cell (53). Furthermore, the inflammatory potential of Th cells is limited by blocking PDE activity pharmacologically (24, 54) or by providing exogenous cAMP analogs (55). These results all indicate the important role for cAMP in the inhibition of immune–inflammatory responses.

The accumulation of cAMP leads to the activation of sensors, such as the cAMP-dependent protein kinase (PKA; Fig. 3). cAMP binds to the regulatory subunit of PKA, leading to the release of the active catalytic subunit and the phosphorylation of PKA substrates (56–58). Using PKA inhibitors such as H-89 suggested that activation of PKA is central to the ability of A2AR to inhibit the oxidative burst in neutrophils (59).

The cAMP response element binding proteins are some of the substrates for PKA. Members of this leucine zipper family of transcription factors include CREB, the cAMP response element modulator and activating transcription factor 1 (60). Following activation of PKA, the free catalytic subunit enters the nucleus and phosphorylates the kinase inducible domain of CREB on Ser133, which increases its transcriptional activity. These transcription factors play multiple and sometimes paradoxical roles in Th cell responses. For example, the proteins are subject to alternative splicing and a particular variant of the cAMP response element modulator, termed inducible cAMP early repressor, attenuates cytokine induction after TCR stimulation (60). The expression of Foxp3, a major regulator of the development and function of Tregs is also regulated by CREB (61). Thus, the inhibition induced by adenosine from Tregs could be mediated, in part, through these pathways. However, raising the level of cAMP in T cells with forskolin followed by analysis with oligonucleotide arrays showed a significant induction of the genes for IL-2, IL-2Rα, and IFN-γ (62). This finding conflicts with evidence that phosphodiesterase inhibitors shift the T cell phenotype toward Th2 cells (63). The best explanation may be that the earliest stages of T cell activation include a role for cAMP while this reliance diminishes over time subsequent to the induction of PDE. This timing would match the induction of the A2AR on Th
cells during activation and allow adenosine to induce cAMP as a negative feedback mechanism subsequent to the initiation of Th cell responses. However, the full complement of PKA substrates responsible for the effects of adenosine in immune/inflammatory cells are not known and need to be defined in detail.

Originally, cAMP was thought to activate PKA exclusively, but it is now apparent that it regulates other cAMP sensors including the exchange protein directly activated by cAMP (Epac) (58, 64). Epac initiates a large number of cAMP-mediated events, including cell proliferation, cell survival, secretion, and Ca\textsuperscript{2+} metabolism (58, 64). There are two isoforms of Epac—Epac1 and Epac2, with Epac1 being more widely expressed and present in T cells (58, 64). Epac1 is a guanine nucleotide exchange factor for the small GTP-binding protein Rap (60), with major roles in integrin-mediated cell adhesion and the formation of cell–cell junctions (58, 64). Epac1 can signal dependently or independently from PKA to inhibit proinflammatory responses, in part through its effects on suppressor of cytokine signaling proteins (SOCS) (65). SOCS1 and SOCS3 both inhibit responses in Th cells and APCs by multiple mechanisms (66). Moreover, adenosine stimulates the accumulation of IL-10 (32, 34, 37, 67), which can act in an autocrine manner to induce SOCS3 (66).

A significant finding is that Epac1 and Rap activate a novel member of the phospholipase family—phospholipase (PLC) C-ε (64). PLC-ε is widely expressed, contains the requisite domains responsible for the phospholipase activity that hydrolyzes phosphatidylinositol bisphosphate to inositol trisphosphate (IP\textsubscript{3}) and diacylglycerol (DAG), and is a CDC25 binding domain with guanine nucleotide exchange factor activity for small GTP-binding proteins in the Ras family (64). Thus, in addition to raising Ca\textsuperscript{2+} levels in cells, PLC-ε, Epac, and Rap initiate signaling cross-talk and potential links between cell membrane receptors and signaling pathways regulated by small GTP-binding proteins (64). This pathway of activation resembles that mediated through the Gq α subunit associated with the A\textsubscript{2B}AR expressed by APCs (see below and Fig. 3). The roles of Epac1, Rap, and PLC-ε in the regulation of immune function by all of the immune/inflammatory cells are important subjects for future work.

The A\textsubscript{2B}AR couples to two different G protein α subunits, the Gs α subunit, and the Gq α subunit (68). In cells where the A\textsubscript{2B}AR is coupled to the Gs α subunit, activation of Gs increases the activity of adenylylase, raises the level of cAMP, and can potentially activate PKA and Epac1 as well as their downstream targets. Although cAMP is associated with the inhibition of inflammatory responses, it should be remembered that cholecalciferol, which stimulates large amounts of cAMP, is also one of the most potent adjuvants ever described (69). Thus, cAMP induction does not always equate with inhibition. Moreover, some proinflammatory cytokines, such as IL-6, are induced through the activation of the A\textsubscript{2B}AR in dendritic cells (39), possibly by cAMP-sensitive promoters that can be activated by adenosine (70, 71). It will be important to identify the targets for cAMP that confer inhibition of host responses.

In settings in which the A\textsubscript{2B}AR is coupled to the Gq α subunit, it can increase the activity of PLC-β, causing the breakdown of phosphatidylinositol bisphosphate to IP\textsubscript{3} and DAG. This activity briefly elevates the IP\textsubscript{3}/Ca\textsuperscript{2+} and DAG signals in the activated cells, thereby activating Ca\textsuperscript{2+}-calmodulin–dependent enzymes, especially the calmodulin-sensitive protein kinases, such as Ca\textsuperscript{2+}–calmodulin kinase II (72) and the serine-threonine kinase, protein kinase C. Because protein kinase C has been implicated in the activation of T cells and APCs (73), it is possible that adenosine signaling through the A\textsubscript{2B}AR induces potentially proinflammatory effects.

Can there be too much of a good thing?

In some circumstances, adenosine can exacerbate immunopathology as illustrated in the murine model of multiple sclerosis—experimental autoimmune encephalomyelitis, (EAE). Disease in this model depends on the migration into the CNS of pathogenic T cells sensitized by prior immunization with a peptide derived from myelin oligodendrocyte protein. Contrary to expectations, cd73\textsuperscript{−/−} mice were found to be highly resistant to EAE (74); this was not caused by a lack of T cell responsiveness to immunization with myelin oligodendrocyte protein, because T cells of cd73\textsuperscript{−/−} mice were activated, secreted increased quantities of proinflammatory cytokines, and caused severe disease when adoptively transferred into tat\textsuperscript{−/−} mice, which are cd73\textsuperscript{−/−}. These findings strongly suggested that CD73-derived adenosine and perhaps AR signaling were needed for T cell entry into the CNS. This notion was confirmed by the observation that an A\textsubscript{2A}AR antagonist protected wild-type mice from EAE (74). Thus, this model demonstrates that the anti-inflammatory effects of adenosine can be offset by the effect of adenosine on the access of pathogenic T cells to the CNS.

Adenosine, like other Treg mediators, can provoke disparate responses. For example, TGF-β1 acting alone is anti-inflammatory and favors the emergence of adaptive Tregs (75); however, in combination with IL-4 or IL-6, it stimulates Th9 (76) and Th17 (77) cells, respectively. Similarly, the inhibition of IL-12 coupled with the concomitant induction of IL-6 in dendritic cells after exposure to adenosine analogs is sufficient to convert the anti-inflammatory potential of TGF-β1 into a proinflammatory milieu that induces Th17 cells (J.M. Wilson and P.B. Ernst, submitted for publication). We propose that adenosine complements TGF-β1 as a mediator of suppression and an inducer of Th17 cells.

Additional evidence suggests that adenosine exacerbates intestinal inflammation in response to bacteria or chemicals via its effects on the A\textsubscript{2B}AR (78). However, other investigators have reported the opposite (79). In the lung, adenosine is believed to contribute to inflammation, largely through the A\textsubscript{2B}AR (80). Other data indicate that adenosine contributes to fibrosis in the liver or lung through the A\textsubscript{2A}AR (81) or A\textsubscript{2B}AR (80). The stimulation of fibrosis may be an attempt to "wall off" inflammation, but the widespread fibrotic atrophy of functional tissue is not usually desirable.

Anti-inflammatory mediators from Tregs can also contribute to immunosuppression that limits the clearance of infections (10, 82) or tumors (83). For example, the absence of the A\textsubscript{2A}AR enhances immune reactivity, allowing the regression of tumors in most A\textsubscript{2A}AR-deficient mice tested, whereas no tumors were resolved in wild-type mice (84). Similarly, knockdown of CD73 expression in ovarian or breast tumors or treatment of mice with a specific CD73 inhibitor or anti-CD73 Ab slowed tumor growth (85–87). Adenosine has also been suggested to exacerbate sepsis (88), although its anti-inflammatory effects complement antibiotic treatment to improve survival in other models (89). Undoubtedly, there are sit-
utions in which adenosine production provides a premature anti-inflammatory feedback that competes with the host’s ability to mount a sufficient response.

Conclusions
Adenosine is one of several mediators that accumulate in the inflammatory milieu, conferring pleiotropic effects in a paracrine or autocrine manner that may be either beneficial or undesirable. These diverse outcomes reflect patterns of AR expression associated with distinct cellular lineages and differentiation states, as well as linkage to divergent G protein-coupled signaling pathways. Although the outcome of AR signaling is sometimes paradoxical, this inconsistency points to the complexity of biology rather than arguing against a role for adenosine in Treg function. Similar to other mediators (e.g., TGF-β1, IL-10) adenosine is not produced solely by Tregs, nor is it always beneficial. Clearly, a deeper understanding of the effects of adenosine and its interaction with other anti-inflammatory mediators will be required before we are able to translate current findings into novel anti-inflammatory therapies for the treatment of human disease.

Acknowledgments
We thank Dr. Ken Tung and Jeff Wilson (University of Virginia) and Paul Kincade (Oklahoma Medical Research Foundation) for sharing constructive suggestions for the content of this review.

Disclosures
P.B.E. is the principal investigator on a grant that has a subcontract with PGXhealth, which makes adenosine analogues.

References