The A2B Adenosine Receptor Promotes Th17 Differentiation via Stimulation of Dendritic Cell IL-6

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Discrete populations of CD4+ T lymphocytes with unique cytokine-expression signatures are important for host health and immunity in the face of a diverse array of pathogenic and commensal microorganisms. Classically divided into just two subsets (1), more recent findings indicated a broader diversity in CD4+ T cell heterogeneity. For instance, it is now well accepted that, in addition to Th1 and Th2 cells, regulatory T cells (Tregs) and Th17 cells represent functionally unique cells that develop from a common naive precursor (2, 3). Development into these subsets is controlled largely by cues derived from APCs during activation of Ag-inexperienced CD4+ T cells. Elaborating on this hypothesis, the factors and processes that govern the differentiation of CD4+ T cells remains a fundamental goal of cellular immunology.

Adenosine is a nucleoside with multitudinous physiological effects. Accumulating during hypoxia and inflammation, it is now clear that adenosine acts on both innate and adaptive immune cells, with the outcome depending on the combinatorial expression of four unique G protein-coupled receptors (reviewed in Refs. 4–6). Signaling via the Gs-coupled A2A adenosine receptor (A2AAR), adenosine regulates T cell activation directly (7–11) and results in the differentiation of IL-17– and IL-22–secreting cells and elevation of RNA that encodes signature Th17-associated molecules, such as IL-23R and RORyt. The observed response was similar when DCs were generated from bone marrow or isolated from small intestine lamina propria. Experiments using adenosine receptor antagonists and cells from A2BAR−/− or A2AAR−/−A2BAR−/− mice indicated that the DC A2BAR promoted the effect. IL-6, stimulated in a cAMP-independent manner, is an important mediator in this pathway. Hence, in addition to previously noted direct effects of adenosine receptors on regulatory T cell development and function, these data indicated that adenosine also acts indirectly to modulate CD4+ T cell differentiation and suggested a mechanism for putative proinflammatory effects of A2BAR. The Journal of Immunology, 2011, 186: 000–000.

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Adenosine is an endogenous metabolite produced during hypoxia or inflammation. Previously implicated as an anti-inflammatory mediator in CD4+ T cell regulation, we report that adenosine acts via dendritic cell (DC) A2B adenosine receptor (A2BAR) to promote the development of Th17 cells. Mouse naive CD4+ T cells cocultured with DCs in the presence of adenosine or the stable nonselective adenosine mimetic 5'-(N-ethylcarboximado) adenosine resulted in the differentiation of IL-17– and IL-22–secreting cells. Experiments using adenosine receptor antagonists and cells from A2BAR−/− or A2AAR−/−A2BAR−/− mice indicated that the DC A2BAR promoted the effect. IL-6, stimulated in a cAMP-independent manner, is an important mediator in this pathway. Hence, in addition to previously noted direct effects of adenosine receptors on regulatory T cell development and function, these data indicated that adenosine also acts indirectly to modulate CD4+ T cell differentiation and suggested a mechanism for putative proinflammatory effects of A2BAR.

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Abbreviations used in this article: A2AAR, A2A adenosine receptor; AR, adenosine receptor; BMDC, bone marrow-derived dendritic cell; DC, dendritic cell; EPAC, exchange proteins directly activated by cAMP; NECA, 5′-(N-ethylcarboximado) adenosine; FRC, fibroblast; FACS, fluorescence-activated cell sorting; GM-CSF, IL-6, IL-12, IL-23, IL-33, IL-35, IL-17, IL-22, CD4, CD20, CD25, CD62L, CD44; Treg, regulatory T cell; TGF-β, transforming growth factor β; Th1, type 1; Th2, type 2; Th17, type 17; Th22, type 22; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor β1; TGF-β3, transforming growth factor β3.

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represents a biological mediator that controls CD4+ T cell differentiation.

Materials and Methods

Mice

C57BL/6 mice were purchased from The Jackson Laboratory. A2AR−/−, A3AR−/−, A2AR−/−/A3AR−/− double knockout mice on a C57BL/6 background were bred and maintained at the University of Virginia (17). All mice were housed and handled in accordance with the Institutional Animal Care and Use Committee of the University of Virginia.

Reagents

Adenosine, the adenosine mimetic 5’-(N-ethylcarboxamido) adenosine (NECA), the cAMP inhibitor protein kinase inhibitor, and the exchange proteins directly activated by cAMP (EPAC) agonist 8-pCPT-2’-O-Me-cAMP were purchased from Sigma. The AR antagonists ZM241385 and SCH58261 were purchased from Toxin, and ATLL62 was kindly provided by PGXHealth, a division of Clinical Data (Charlottesville, VA). The following cell-signaling inhibitors were purchased from Santa Cruz Biotechnology: 2’, 5’-dideoxyadenosine, P98059, SB203580, U-73122, LY294002, and bisindolylmaleimide I.

Cell purification

Naive CD4+ T cells were isolated from spleen by physical disaggregation, followed by ACK lysis with ACK buffer and enrichment with CD4 microbeads and LS columns (Milteny Biotech), according to the manufacturer’s instructions. Resultant cells were stained with anti-mouse CD4-FITC (BD Biosciences), CD44-PE (eBioscience), CD62L-PE/Cy5.5 (eBioscience), and CD25-allophycocyanin (BD Biosciences), and CD4+CD44+CD62L−CD25− cells were sorted with a BD FACSVantage cell sorter. In some cases, CD4+ or CD4+CD62L− T cells were enriched with CD4+ or CD4+CD62L− MACS kits (Milteny Biotech), according to the manufacturer’s instructions.

Bone marrow-derived DCs (BMDCs) were generated as described previously (17). Briefly, bone marrow was flushed from femurs and tibias and RBCs were lysed with ACK buffer and cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, 50 μM 2-ME, 25 mM HEPES, 2 mM sodium pyruvate, and 20 ng/ml TNF-α (PeproTech). After 6 days, nonadherent semidifferentiated cells were harvested and enriched with CD11c microbeads and LS columns (Milteny Biotech). Cells were incubated overnight with 5 ng/ml GM-CSF and used the following day in coculture experiments.

APCs from small intestine lamina propria were isolated, as described previously (17, 26). Briefly, small intestines were removed from euthanized donor C57BL/6 mice, opened longitudinally to flush out feces, cut into 5-mm pieces, and incubated for 20 min at 37°C on a shaker in HBSS supplemented with 5% heat-inactivated FBS and 2 mM EDTA. After passing the preparation through a metal filter, intestinal fragments were collected, and the step was repeated. Then intestinal fragments were passing the preparation through a metal filter, intestinal fragments were collected, and the step was repeated. Then intestinal fragments were cut into 5-mm pieces, and incubated for 20 min at 37°C on a shaker in HBSS supplemented with 5% heat-inactivated FBS and 2 mM EDTA. After passing the preparation through a metal filter, intestinal fragments were collected, and the step was repeated. Then intestinal fragments were cut into 5-mm pieces, and incubated for 20 min at 37°C on a shaker in HBSS supplemented with 5% heat-inactivated FBS and 2 mM EDTA. After passing the preparation through a metal filter, intestinal fragments were collected, and the step was repeated.

CD4+ T cell stimulation

DCs (1 × 106) and naive CD4+ T cells (1 × 105) were cultured in 200 μl T cell media in 96-well plates in the presence of 1 μg/ml soluble anti-CD3 and anti-CD28. Cells were treated with 500 nM NECA or vehicle control and, in some cases, with 5 or 100 μM adenosine. Other experiments were conducted with TGF-β1 (R&D Systems), 5 μg/ml neutralizing Ab to IL-6–gp130 (R&D Systems), or 250 nM AR antagonists. Supernatant and cell lysates for real-time RT-PCR were collected at 72 h. Supernatant was assayed using commercial kits, according to manufacturer’s instructions, for IL-17 (BD Biosciences), IL-22 (R&D Systems), IL-10 (BD Biosciences), and IL-6 (BD Biosciences).

Intracellular cell staining and flow cytometry

For flow cytometric analysis of intracellular markers, cells were harvested on day 4 and washed three times. After resting overnight in complete T cell media, cells were stimulated with 50 ng/ml PMA, 750 ng/ml ionomycin, and GolgiStop (BD Biosciences) for 4–5 h. Cells were incubated for 15 min with FcBlock, stained with anti-CD4 (BD Biosciences), permeabilized, and stained with anti–IL-17, anti–IFN-γ, and anti-Foxp3 using a Cytofix/Cytoperm kit (BD Biosciences). Samples were assayed with a BD FACSCalibur flow cytometer, and data were analyzed with FlowJo (TreeStar) software.

Semiquantitative real-time RT-PCR

Cells from coculture were collected and lysed, and total RNA was isolated with Qiagen mini-prep kits and reverse transcribed into cDNA with SuperScript II (Invitrogen), according to the manufacturer’s recommendations. Transcripts were measured by real-time RT-PCR with primer and dual-labeled probes (H4, Mm0045259_m1; H6, Mm00446190_m1; II10, Mm00439616_m1; II17a, Mm00439619_m1; II21, Mm00517640_m1; II22, Mm00444241_m1; II23r, Mm00519943_m1; Tf, Mm00443258_m1; Ilf5, Mm0081778_m1; Rorc, Mm01261022_m1; and Foxp3, Mm00475156_m1) (all from Applied Biosystems) detected in a SmartCycler (Cepheid) and normalized against 18s rRNA. The fold change in the expression of a given gene of interest was determined using the δΔCt method (27).

Statistical analysis

Data are reported as the mean ± SEM. Unless indicated, all data were pooled from three or more independent experiments. The two-tailed Student t test, assuming unequal variance, was performed using Microsoft Excel software. A p value < 0.05 was considered significant.

Results

Adenosine promotes differentiation of Th17 cells

To determine the role of ARs in controlling CD4+ T cell differentiation, naive CD4+ T cells were activated with DCs and anti-CD3, treated with a stable nonsel ective adenosine mimetic (NECA) or vehicle control, and assayed for factors associated with different Th lineages. Real-time RT-PCR indicated that NECA preferentially upregulated Il17 compared with transcripts associated with other Th cell subsets (Fig. 1A). This IL-17 response was confirmed by ELISA analysis of 72-h supernatant and intracellular cytokine staining following restimulation (Fig. 1B, 1C). IFN-γ responses were modestly augmented by NECA, but to a lesser extent than IL-17. Importantly, adenosine also increased the frequency of IL-17–expressing CD4+ T cells (Fig. 1D). Together, these results demonstrated that, in the presence of DCs, adenosine favors the development of IL-17–secreting CD4+ T cells. Further studies were conducted to determine whether these cells had characteristics consistent with a bona fide Th17 cell. Indeed, IL-22 protein, as well as Il21, Il22, Il23r, and Rorc (RORγt), was significantly elevated (Fig. 2).

Published studies suggest that adenosine is an important molecule in Treg development and function (6). To assess whether ARs promote Tregs in the DC coculture model, we assessed Foxp3 transcript and intracellular protein in cells treated with NECA or vehicle control. NECA treatment had little effect on either expression of a given gene of interest was determined using the δΔCt method (27).

Statistical analysis

Data are reported as the mean ± SEM. Unless indicated, all data were pooled from three or more independent experiments. The two-tailed Student t test, assuming unequal variance, was performed using Microsoft Excel software. A p value < 0.05 was considered significant.

Results

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FIGURE 2. An adenosine mimetic promotes development of bona fide Th17 cells. Naive CD4+ T cells were activated with BMDCs and anti-CD3 in the presence of 500 nM NECA or vehicle control. After 72 h, cells were harvested, and mRNA was assessed by real-time RT-PCR, or supernatant was collected and assessed by ELISA for IL-17 and IFN-γ (B). Data are pooled from three or more independent experiments and represent the mean ± SEM. *p < 0.05. C, Day 4 cells were harvested, washed, and replated in complete media overnight prior to restimulation with PMA/ionomycin. Dot plots show intracellular IFN-γ and IL-17 in CD4+ T cells. D, Naive CD4+ T cells were activated with BMDCs and anti-CD3 in the presence of vehicle control or 5 or 100 μM adenosine. Day 4 cells were harvested, washed, and replated in complete media overnight prior to restimulation with PMA/ionomycin. Dot plots show intracellular IFN-γ and IL-17 in CD4+ T cells. Dot plots are representative of three or more independent experiments.

these cocultures, suggesting that Th17 differentiation in this model requires A2BAR expressed by the DCs (Fig. 4B). In contrast, NECA still promoted a significant IL-17 response in cocultures of wild-type DCs with CD4+ T cells lacking A2AAR or both A2BAR and A2AAR. Further reinforcing the importance of DCs in this system, NECA did not affect IL-17 when naive CD4+ T cells were stimulated with anti-CD3/28 in the absence of APCs (data not shown).

A2BAR promotes IL-6 to favor Th17, and IL-6 response is cAMP independent

The development of Th17 cells is controlled largely via factors derived from innate immune cells, such as IL-6 and TGF-β1. Previous reports showed that adenosine could promote IL-6 in a variety of cell types, including DCs (28, 29). To assess the role of IL-6 in the A2BAR-mediated Th17 response, cocultures were conducted in the presence of a neutralizing Ab to the IL-6-gp130 receptor. NECA induction of IL-17 was abrogated in the presence of the IL-6-gp130 inhibitor, thus demonstrating that IL-6 is indeed an important mediator of the Th17 response (Fig. 5A, 5B). Although NECA decreased the number of cells expressing Foxp3 as Th17 cells increased, the decrease in Foxp3 did not occur when IL-6-gp130 was blocked. These findings suggested that IL-6 concentrations are a key determinant of the effects of adenosine in the reciprocal control of Th17 and Treg development (Fig. 5B).

To test whether IL-6 in this system is derived from DC A2BAR, we conducted experiments with DCs generated from wild-type, A2AR−/−, or A2AAR−/− mice. A2AR−/− DCs produced little IL-6 upon NECA treatment, whereas wild-type and A2AAR−/− DCs produced significant amounts of IL-6 in response to NECA (Fig. 5C). Importantly, A2BAR−/− DCs did not have an intrinsic defect in IL-6 production, because LPS provoked abundant IL-6 (data not shown). Pharmacologic blockade with AR-selective antagonists corroborated that the IL-6 response depended on A2BAR (data not shown). A role for A2BAR in TGF-β1 induction was also assessed, but NECA treatment of DCs did not elevate TGF-β1 protein or mRNA, as assayed by ELISA or real-time RTPCR (data not shown). This suggested that low levels of TGF-β1 are constitutively produced by DCs or, alternatively, that TGF-β1 is residually present in the FBS used in cocultures.

As introduced previously, the anti-inflammatory effects of adenosine are often attributed to signaling pathways involving A2AR and the intracellular mediator cAMP. This is consistent with the general anti-inflammatory effects of other cAMP-elevating agents, such as prostaglandin E2 and vasoactive intestinal peptide (30). However, in our model, which incorporates both CD4+ T cells and APCs, we describe a putative proinflammatory effect of adenosine on CD4+ T cells that is A2BAR dependent. A2BAR differs from A2AAR and the other ARs in that it can couple to both Gs and Gq, enabling it to activate both adenyl cyclase and PLC, respectively (6). Thus, we sought to determine whether A2BAR relied on cAMP or whether other signaling modalities were important.

To elucidate key pathways that control the NECA-mediated responses observed in our study, we treated DCs with NECA in the presence of various pharmacologic inhibitors known to inhibit G protein pathways and assessed IL-6 production. At the concentrations studied, the inhibitors did not impair DC viability, as assessed by trypan blue exclusion (data not shown). Inhibition of two distinct targets of the adenyl cyclase/cAMP axis had no effect on NECA-mediated IL-6 induction, whereas inhibition of the PLC/B

FIGURE 2. AR activation promotes differentiation of IL-17–secreting CD4+ T cells. A, Naive CD62LhiCD44loCD25loCD4+ T cells were activated with BMDCs and anti-CD3 in the presence of 500 nM NECA or vehicle control. After 72 h, cells were harvested, and mRNA was assessed by real-time RT-PCR, or supernatant was collected and assessed by ELISA for IL-17 and IFN-γ (B). Data are pooled from three or more independent experiments and represent the mean ± SEM. *p < 0.05.

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PKC axis with either U-73122 or bisindolylmaleimide I impaired the IL-6 response significantly (Fig. 5D). Because MAPKs, including p38 and ERK, also play a role in mediating A2BAR effects in some cells, additional inhibitors targeting other pathways were used. We found that the ERK inhibitor PD98509 and the PI3K inhibitor LY294002 both blocked NECA-mediated IL-6, but the p38 inhibitor SB203580 had little effect. Lastly, treating DCs with the cAMP-elevating agent forskolin or the EPAC agonist 8-pCPT-2’’O-Me-cAMP provoked little IL-6, suggesting that stimulating cAMP or EPAC pathways is not sufficient to drive DC production of IL-6 (data not shown). Taken together, this indicated that NECA promoted IL-6 in DCs independently of cAMP but that PLC/PKC, ERK, and PI3K are involved, and it explains why A2BAR, but not A2AAR, stimulates IL-6.

**Intestinal DCs support NECA-mediated Th17 response**

Th17 cells are especially abundant at mucosal sites, such as the small intestine, where they are important in controlling neutrophil accumulation and epithelial barrier integrity (31). The intestine is subject to frequent hypoxia and, thus, is thought to be an adenosine-rich site (32). Therefore, to model the effects of adenosine on T cell activation in the gut, we isolated highly purified subsets of gut APCs and cultured them with naive CD4+ T cells (Fig. 6). We found that the adenosine mimetic NECA significantly enhanced IL-17 in cocultures containing a population of CD11chiCD11b+ gut DCs, a subset previously associated with Th17 responses (26). Additionally, Il22 was significantly upregulated by NECA treatment with this DC subset. Conversely, NECA had no such effect in cultures with a population of CD11c loCD11b+ macrophages, which is consistent with previous studies suggesting this population of APCs promotes Tregs (26). Foxp3 was not altered in any of the NECA-treated groups.

**Discussion**

Previous work showed that direct engagement of A2AAR on T cells inhibited proliferation, as well as Th1 and Th2 responses (9–11). A2AAR also contributes to the generation and function of Tregs (10, 12–14, 33). However, T cell activation and differentiation do not occur in isolation but require APCs and a cadre of extracellular factors. Moreover, the accumulation of endogenous adenosine at inflammatory sites does not exclusively target A2AAR; it stimulates other available AR subtypes. To more faithfully model how adenosine impacts CD4+ T cell differentiation, we studied the effects of a nonselective adenosine mimetic in cocultures with DCs and highly purified naive CD4+ T cells. In this study, we found that adenosine preferentially promoted de novo differentiation of naive T cells into Th17-like cells.
differentiation of CD4+ T cells that express IL-17 and IL-22 and have a transcriptional profile consistent with Th17 cells (i.e., upregulation of genes encoding for IL-23R and the transcription factor RORγt) (34). Our data indicated that activation of A2BAR on DCs stimulated IL-6 production in a cAMP-independent manner, and this IL-6 is an important intermediary that favors the Th17 response. Thus, adenosine represents a biological mediator that can enhance the development of Th17 cells.

In the murine system, TGF-β1 and proinflammatory cytokines, such as IL-6, are critical for Th17 differentiation, whereas a host of other cytokines, such as IL-2 and IFN-γ, are negative regulators (35). The data reported in this article suggest that adenosine alters the balance of these cytokines in a way that favors Th17 differentiation at the expense of Treg development. For instance, adenosine directly impairs IL-2 and IFN-γ production by Th cells (9–11, 14), and we previously showed that an adenosine mimetic impaired IL-12 by DCs (17). Conversely, adenosine was reported to promote or remain permissive for DC production of TGF-β1 (29). The importance of specific cytokines in controlling adenosine-mediated Th17 differentiation is evidenced by the fact that A2AR stimulates IL-6 and that neutralization of the IL-6–gp130 receptor abrogates the Th17 response. The necessary role for IL-6 in this system implies that Th17 cells are induced in a Stat3-dependent mechanism (36); however, that has yet to be

FIGURE 5. IL-6, stimulated in a cAMP-independent manner, is important in A2BAR-mediated Th17 responses. A, Naive CD4+ T cells were activated with BMDCs and anti-CD3 in the presence of a neutralizing Ab to IL-6–gp130 and 500 nM NECA or vehicle control. Day 4 cells were harvested and washed, and supernatants were collected and assayed for IL-17. B, Naive CD4+ T cells were activated with BMDCs and anti-CD3 in the presence of a neutralizing Ab to IL-6–gp130 and 500 nM NECA or vehicle control. Day 4 cells were harvested, washed, replated in complete media overnight prior to restimulation with PMA/ionomycin, and assayed for the production of IL-17 or Foxp3 by flow cytometry. Data are representative of three or more independent experiments. C, BMDCs derived from wild-type, A2AAR−/−, and A2BAR−/− mice were treated with 1 μM NECA or vehicle control, and 18-h supernatant was collected and assessed by ELISA for IL-6. D, BMDCs were treated with 1 μM NECA in the presence of 10 μM signaling inhibitors or vehicle control, and 18-h supernatant was collected and assessed by ELISA for IL-6. Bis I, bisindolylmaleimide I; DAdo, 2′-5′ dideoxyadenosine; LY, LY294002; PD, PD98059; SB, SB203580. Data were pooled from three or more independent experiments and represent the mean ± SEM. *p < 0.05.

FIGURE 6. Gut CD11c+CD11b+ DCs, but not macrophages, support adenosine mimetic-mediated Th17 T cell response. A, Small intestinal lamina propria APCs were isolated by FACS based on forward light scatter/side scatter and CD11c/CD11b expression. B, MACS-naive CD4CD62L-enriched splenocytes were activated with CD11chiCD11b+ DCs or CD11cloCD11b+ macrophages and anti-CD3 in the presence of 500 nM NECA or vehicle control. After 72 h, lysates were collected and assessed by real-time RT-PCR. Data were pooled from three or more independent experiments and represent the mean ± SEM. C, MACS-naive CD4CD62L-enriched splenocytes were activated with CD11chiCD11b+ DCs or CD11cloCD11b+ macrophages and anti-CD3 in the presence of 500 nM NECA or vehicle control. After 72 h, supernatant was collected and assessed by ELISA for IL-17, IL-22, and IL-10. Data were pooled from three or more independent experiments and represent the mean ± SEM. *p < 0.05.
confirmed. The role of TGF-β1 in this system is less clear, because we did not find direct evidence that A2B AR stimulation was sufficient to generate TGF-β1. However, the fact that neutralizing Ab to TGF-β1 diminished IL-17 production indicated that adenosine-mediated Th17 differentiation requires the presence of TGF-β1.

Although NECA favors Th17 cells and not Tregs, upon IL-6 neutralization, the opposite result was observed: Th17 expansion was abrogated as was the decrease in cells expressing Foxp3. These data suggested that IL-6 induced by NECA was predominantly, if not solely, responsible for the decrease in Foxp3-producing Th cells and that it is a key intermediate controlling the effects of adenosine and not solely, responsible for the decrease in Foxp3-producing Th cells. These neutralization experiments indicated that ARs modulate CD11c+CD11b+ lamina propria DCs to control intestinal Th responses, which agrees with other studies on intestinal DCs (26). The difference in A2BAR expression on intestinal DCs to control intestinal Th responses, which agrees with other studies on intestinal DCs (26). The difference in A2BAR expression on intestinal DCs to control intestinal Th responses, which agrees with other studies on intestinal DCs (26). The difference in A2BAR expression on intestinal DCs to control intestinal Th responses, which agrees with other studies on intestinal DCs (26). The difference in A2BAR expression on intestinal DCs to control intestinal Th responses, which agrees with other studies on intestinal DCs (26).

The data obtained with BMDCs were consistent with observations made using DCs isolated from the intestinal lamina propria, a site rich in Th17 cells. When used in coculture experiments, the CD11c+CD11b+ DC subset preferentially increased IL-17 and IL-22 mRNA in the presence of the adenosine mimetic. These findings indicated that ARs modulate CD11c+CD11b+ lamina propria DCs and suggested that endogenous adenosine acts via lamina propria DCs to control intestinal Th responses, which agrees with other studies on intestinal DCs (26). The difference in A2BAR-mediated effects between these two intestinal APC populations may reflect the fact that resting macrophages express fewer A2B AR (17), and this receptor leads to the production of IL-6, whereas the A2A AR does not. Although we showed that adenosine is one factor that can promote Th17 development, there are clearly redundant pathways that contribute to the in vivo response. For instance, A2B AR−/− mice have numbers of Th17 cells in small intestine lamina propria comparable to wild-type controls; moreover, the abundance of Th17 cells in our A2B AR−/− mice could be augmented by manipulating commensal flora (data not shown), presumably due to segmented filamentous bacteria (SFB), which persist in some, but not all, mouse facilities (39). Although ruling out a necessary role for A2B AR in the SFB-mediated Th17 response, this does not discount the possibility that local accumulation of adenosine can act to promote Th17 cells. The role of A2A AR in the promotion of Th17 cells, and possibly inflammation, is somewhat surprising but not entirely unexpected. For example, some studies implicated A2B AR in pulmonary inflammation (40). This is at least consistent with other evidence suggesting that intestinal inflammation is reduced in the absence of normal A2B AR function (23, 24). However, it is important to note that another group investigating adenosine in the gut found the exact opposite (41). One interpretation of the conflicting results of A2A AR regulation on intestinal inflammation is that the microbiome differs in the animal facilities of the respective investigators. Although the A2B AR-dependent induction of Th17 could be consistent with these receptors promoting inflammation, emerging thought suggests that Th17 cells may confer protective/anti-inflammatory responses. For example, mice harboring SFB have a marked increase in intestinal Th17 cells but no disease (39). In addition, the coexpression of IL-22 by Th17 cells can be protective (42). Similarly, IL-17R knockout mice have worse intestinal disease in some models (43). Because Th1, Th2, and Th17 cells contribute to the protection of the host, which is why they exist, their contribution to the pathogenesis of disease may require the presence of other factors. For example, in Crohn’s disease, Th17 responses are implicated in the pathogenesis but usually are the result of their heightened activation by accessory cytokines, such as IL-23 (44). Thus, the presence of IL-17-producing cells in the absence of other cues may not be sufficient for inflammation to ensue.

Factors that calibrate the magnitude and quality of CD4+ T cell responses are critical for host health. Although previous work emphasized an inhibitory role for adenosine in controlling CD4+ Th cells, the current study suggests that adenosine promotes Th17 cells. Thus, adenosine represents a biological mediator that regulates CD4+ T cell development and function, albeit in a manner that was not predicted. Adenosine shares its pleiotropic functions with other factors, such as IL-2 and TGF-β1. For example, IL-2 drives the expansion of effector T cells, but it is required for the maintenance of Tregs (45). Similarly, TGF-β1 is important for the induction of Foxp3 and the accumulation of induced Tregs, and it contributes to Th17 cell development (46). The relative concentration of a nucleoside, the types of cells that it targets, and the specific ARs available for engagement may be important determinants of the final outcome. The current study is significant because it adds to the regulatory effects of adenosine and expands the current thinking on its role in the control of host responses.

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Disclosures

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References


