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Renal Ischemia-Reperfusion Injury and Adenosine 2A Receptor-Mediated Tissue Protection: The Role of CD4+ T Cells and IFN-γ

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A3A adenosine receptor (A3A-R)-expressing bone marrow (BM)-derived cells contribute to the renal protective effect of A3A agonists in renal ischemia-reperfusion injury (IRI). We performed IRI in mice lacking T and B cells to determine whether A3A-R expressed in CD4+ cells mediate protection from IRI. Rag-1 knockout (KO) mice were protected in comparison to wild-type (WT) mice when subjected to IRI. ATL146e, a selective A3A agonist, did not confer additional protection. IFN-γ is an important early signal in IRI and is thought to contribute to reperfusion injury. Because IFN-γ is produced by kidney cells and T cells, we performed IRI in BM chimeras in which the BM of WT mice was reconstituted with BM from IFN-γ KO mice (IFN-γ KO → WT chimera). We observed marked reduction in IRI in comparison to WT → WT chimeras providing additional indirect support for the role of T cells. To confirm the role of CD4+ A3A-R in mediating protection from IRI, Rag-1 KO mice were subjected to ischemia-reperfusion. The study observed in Rag-1 KO mice was reversed in Rag-1 KO mice that were adoptively transferred WT CD4+ cells (WT CD4+ → Rag-1 KO) or A3A-KO CD4+ cells (A3A-KO CD4+ → Rag-1 KO). ATL146e reduced injury in WT CD4+ → Rag-1 KO mice but not in A3A-KO CD4+ → Rag-1 KO mice. Rag-1 KO mice reconstituted with CD4+ cells derived from IFN-γ KO mice (IFN-γ CD4+ → Rag-1 KO) were protected from IRI; ATL146e conferred no additional protection. These studies demonstrate that CD4+ IFN-γ contributes to IRI and that A3A agonists mediate protection from IRI through action on CD4+ cells.


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Materials and Methods

Renal IRI and treatment with ATL146e

All animals were handled and procedures were performed in adherence to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. C57BL/6 mice (7–8 wk of age; Charles River Breeding...
Laboratories), congenic A2A KO mice (C57BL/6 background, 6–7 wk of age) previously described (7, 17) (a gift from J. Chen, Boston University, Boston, MA), congenic Rag-1 KO mice (on C57BL/6 background), and IFN-γ KO mice (mouse strain 10 wk; The Jackson Laboratory) were used. Rag-1 KO mice were originally derived from B6.129S7-Rag1tm1Mom/J breeders (The Jackson Laboratory) and have been backcrossed 20 generations onto a C57BL/6 background (provided by M. McDuffie, University of Virginia, Charlottesville, VA). Mice were allowed free access to food and water until the day of surgery. Mice were anesthetized with ketamine (100 mg/kg, i.p.), xylazine (10 mg/kg, i.p.), and acepromazine (1 mg/kg, i.m.) and subjected to bilateral flank incisions as previously described (7, 18). Both renal pedicles were cross-clamped for 32 min. Surgical wounds were closed with metal staples and mice were returned to cages for 24 h. Following 24 h of reperfusion, animals were reanesthetized, blood was obtained by cardiac puncture, and kidneys were removed for various analyses.

Mice were anesthetized with vaporized halothane (Halothan Vapor 19.1) before s.c. implantation of osmotic minipumps (model 1003D; ALZA). The pumps released either vehicle (0.01% DMSO in PBS) or ATL146e (10 ng/kg/min). A dose of ATL146e was chosen on the basis of ALZA). The pumps released either vehicle (0.01% DMSO in PBS) or ATL146e (10 ng/kg/min). A dose of ATL146e was chosen on the basis of

Statistical analysis

Unpaired Student’s t test or one-way ANOVA followed by Tukey’s post hoc analysis was used for all comparisons. A value of p < 0.05 was used to define statistical significance.

Results

A2A agonists do not confer additional protection in the absence of T and B cells

Kidneys subjected to IRI in chimeric mice whose BM was replaced with BM from A2A KO mice were resistant to protection usually seen with A2A agonist administration (7). Furthermore in mice whose macrophages were deficient of A2AR and subjected to IRI, A2A agonists were still able to reduce tissue injury (4). These results implicate nonmacrophage BM cells as the target of tissue protection. Given the growing evidence for the role of T cells in IRI we sought to determine whether T cells were necessary for A2A agonist-mediated tissue protection. The kidneys of WT mice or Rag-1 KO mice were subjected to 32 min of ischemia followed by 24 h of reperfusion (Fig. 1). Ischemia-reperfusion produced markedly elevated plasma creatinine of 2.17 ± 0.15 mg/dl (n = 4) in WT mice (see Ref. 18 and dose of ATL146e in Materials and Methods), but a much smaller increase of 0.52 ± 0.08 mg/dl (n = 5) in Rag-1 KO mice treated with vehicle (p < 0.001 compared with WT mice) or 0.57 ± 0.09 mg/dl (n = 5) in Rag-1 KO mice treated with ATL146e (p < 0.001 compared with WT mice) (Fig. 1c).

There was no significant difference in plasma creatinine between Rag-1 KO mice treated with vehicle or ATL146e. Histologically, there was evidence of tissue necrosis in the outer medulla in WT mice (Fig. 1b), an effect that was reduced in Rag-1 KO mice (Fig. 1c).

No additional histological evidence of tissue protective effect was observed in the outer medulla of Rag-1 KO treated with ATL146e (Fig. 1d). Quantitative analysis of histological changes yielded injury scores of 3.25 ± 0.14 (n = 10), 1.79 ± 0.26 (n = 7), and 1.42 ± 0.21 (n = 6) for WT, Rag-1 KO treated with vehicle, and Rag-1 KO treated with ATL146e, respectively (p < 0.001, for vehicle or ATL146e treated Rag-1 KO compared with WT mice). There was no statistically significant difference in the histological scores between Rag-1 KO mice treated with vehicle and those treated with ATL146e. These results indicate that kidneys from T and B cell-deficient mice were protected from IRI and that ATL146e did not provide additional tissue protection,
suggesting that the protective effect of ATL146e could be mediated through T or B cells.

**BM-derived IFN-γ mediates tissue injury in renal ischemia-reperfusion**

T cells are the primary source of BM-derived IFN-γ. Thus we reasoned that the absence of BM-derived IFN-γ would render kidneys resistant to IRI as well as to the normally protective effect of A2A agonists. Because IFN-γ is secreted from BM-derived cells and kidney resident cells, we performed IRI in which the BM of WT mice was ablated and reconstituted with BM from IFN-γ KO mice (IFN-γ KO→WT) (Fig. 2). In control WT→WT chimeric mice, the level of plasma creatinine rose following IRI to 1.98 ± 0.18 mg/dl (n = 4) but was reduced significantly to 0.68 ± 0.10 mg/dl (n = 5) in vehicle (p < 0.001 compared with WT→WT chimera) or to 0.56 ± 0.15 mg/dl (n = 5) in ATL146e-treated IFN-γ KO→WT chimeric mice (p < 0.001 compared with WT→WT chimera) (Fig. 2a). There was no significant difference between vehicle and ATL146e-treated IFN-γ KO→WT chimera. Histological analysis of the outer medulla (Fig. 2, b–d) revealed an injury score of 3.60 ± 0.25 (n = 5), 2.00 ± 0.00 (n = 4), and 1.38 ± 0.23 (n = 4) for WT→WT, IFN-γ KO→WT treated with vehicle, and IFN-γ KO→WT treated with ATL146e, respectively (p < 0.001, IFN-γ KO→WT treated with vehicle or ATL146e compared with WT mice). These results suggest that BM-derived IFN-γ contributes significantly to renal IRI; in its absence, kidneys are protected from IRI and ATL146e does not add any further protection. These results indirectly implicate CD4+ cells and IFN-γ derived from BM-derived cells in mediating renal IRI and tissue protection by A2A agonists.

**CD4+ reconstitution in Rag-1 KO mice**

We sought to determine the specific role of CD4+ A2AR and IFN-γ in IRI. Using a negative selection process we were able to purify CD4+ T lymphocytes for adoptive transfer into Rag-1 KO mice. We injected CD4+ cells into Rag-1 KO mice via jugular

**FIGURE 1.** Effect of IRI in Rag-1 KO mice in the absence and presence of ATL146e. a, Plasma creatinine level is shown for mice treated with vehicle or ATL146e (10 ng/kg/min) beginning 5 h before 32 min of ischemia and continuing for 24 h of reperfusion. Values are mean ± SE in n = 4–5 mice for each group. *p < 0.001 vs WT mice treated with vehicle. H&E stains of the outer medulla are shown for WT C57BL/6 (b) and Rag-1 KO mice treated with vehicle (c) or with ATL146e (d). Magnification is ×200.

**FIGURE 2.** BM-derived IFN-γ contributes to IRI. Plasma creatinine level is shown for chimeric mice in which the BM cells of WT mice were ablated and reconstituted with IFN-γ KO BM (IFN-γ KO→WT) or WT BM (WT→WT) and subjected to 32-min ischemia and 24-h reperfusion. a, WT→WT chimera and IFN-γ KO→WT chimera were treated with vehicle or ATL146e (10 ng/kg/min) beginning 5 h before 32 min of ischemia and continuing for 24 h of reperfusion. Values are mean ± SE in n = 5 mice for each group. *p < 0.001 vs WT→WT chimera. H&E stains of sections from the outer medulla are shown for WT→WT chimera treated with vehicle (b), IFN-γ KO→WT treated with vehicle (c), or IFN-γ KO→WT treated with ATL146e (d) in n = 4–5 mice for each group. Magnification is ×200.
vein, and blood, kidney, and spleen were analyzed for CD4^+ cells. In comparison to WT mice (Fig. 3, a, d, and g), Rag-1 KO mice do not have appreciable amounts of CD4^+ cells in blood, spleen, and kidney (Fig. 3, b, e, and h, respectively); however CD4^+ cells were detected in blood, spleen, and kidney following adoptive transfer of CD4^+ cells into Rag-1 KO mice (Fig. 3, c, f, and i, respectively).

**Effect of CD4^+ A2AR and IFN-γ on IRI**

To determine the target of A2A agonist mediated tissue protection, we performed IRI on kidneys from WT CD4^+ Rag-1 KO and from A2A KO CD4^+ Rag-1 KO mice (Figs. 4 and 5). Compared with Rag-1 KO (as shown in Fig. 1), WT CD4^+→Rag-1 KO reconstituted injury as plasma creatinine rose to 1.63 ± 0.10 mg/dl (n = 8), and ATL146e led to a reduction of plasma creatinine to 0.84 ± 0.12 mg/dl (n = 8; p < 0.001). Histological analysis of the outer medulla showed that the injury score paralleled plasma creatinine and was 3.90 ± 0.08 and 1.63 ± 0.13 mg/dl for WT CD4^+→Rag-1 KO treated with vehicle or ATL146e, respectively (p < 0.0001) (Fig. 5, a and b). Adoptive transfer of CD4^+ cells from A2A KO mice into Rag-1 KO mice also reconstituted injury (A2A KO CD4^+→Rag-1 KO mice subjected to ischemia-reperfusion), but ATL146e had no effect. Plasma creatinine was 1.54 ± 0.11 mg/dl for A2A KO→Rag-1 KO mice treated with vehicle; plasma creatinine was 1.24 ± 0.12 mg/dl for A2A KO→Rag-1 KO mice treated with ATL146e (p < 0.001).

**FIGURE 3.** Flow cytometric analysis of blood, spleen, and kidney from WT, Rag-1 KO, and CD4^+ adoptively transferred into Rag-1 KO mice. Freshly isolated leukocytes from blood (a–c), spleen (d–f), and kidney (g–i) from WT mice (a, d, and g), Rag-1 KO mice (b, e, and h), and Rag-1 KO mice after adoptive transfer of CD4^+ cells (WT CD4^+→Rag-1 KO) (c, f, and i) were subjected to flow cytometric analysis. Density plots are shown for CD4^+ cells after gating for CD3 cells. CD4^+ cells were enriched by negative selection (see Materials and Methods).

**FIGURE 4.** ATL146e-induced tissue protection is mediated through CD4^+ cells. WT CD4^+→Rag-1 KO, A2A KO, or IFN-γ KO CD4^+→Rag-1 KO cells were adoptively transferred into Rag-1 KO mice (2 × 10^7 cells injected via internal jugular vein) and subjected to IRI 7 days later. Mice were treated with vehicle or ATL146e (10 ng/kg/min) beginning 5 h before 32 min of ischemia and continuing for 24 h of reperfusion. Values are mean ± SE in n = 3–8 mice for each group.

**FIGURE 5.** Kidney histology after ischemia-reperfusion. Representative H&E-stained sections of outer medulla from WT CD4^+→Rag-1 KO mice (a and b), A2A KO CD4^+→Rag-1 KO mice (c and d), or IFN-γ KO CD4^+→Rag-1 KO mice (e and f) whose kidneys were subjected to IRI and treated with either vehicle (a, c, and e) or with ATL146e (b, d, and f). Magnification is ×200.
Histological analysis of the outer medulla showed that the injury score paralleled plasma creatinine and was $2.90 \pm 0.43$ (n = 5) and $2.90 \pm 0.46$ (n = 6) for A2AR KO CD4$^+$→Rag-1 KO treated with vehicle and ATL146e, respectively (p = NS) (Fig. 5, c and d). These results underscore the critical role of A2AR expressed on CD4$^+$ cells. In the absence of A2AR on CD4$^+$ cells, A2AR agonists are ineffective in protecting kidneys from IRI.

To determine the role of CD4$^+$ IFN-γ in IRI we adoptively transferred IFN-γ KO CD4$^+$ cells into Rag-1 KO mice (IFN-γ KO; CD4$^+$→Rag-1 KO) and administered vehicle or ATL146e. Injury was not reconstituted in IFN-γ KO CD4$^+$→Rag-1 KO mice subjected to renal ischemia-reperfusion and ATL146e had no effect on the extent of IRI. Plasma creatinine was 0.52 ± 0.02 mg/dl (n = 6) and 0.64 ± 0.01 (n = 3) mg/dl, for vehicle and ATL146e treated IFN-γ→Rag-1 KO, respectively (p = NS) (Fig. 4). Histological analysis of the outer medulla showed that the injury score paralleled plasma creatinine and was $1.42 \pm 0.20$ (n = 6) and $1.33 \pm 0.17$ (n = 3) for vehicle and ATL146e, respectively (p = NS) (Fig. 5, e and f).

Kidney sections were stained with a mAb to neutrophils to determine the extent of infiltration following IRI. Fig. 6 shows representative photographs of the outer medulla of kidney sections following IRI in the presence and absence of ATL146e. Neutrophil infiltration score was $3.11 \pm 0.31$ (n = 4) and $0.56 \pm 0.46$ (n = 4) for WT CD4$^+$→Rag-1 KO treated with vehicle and ATL146e, respectively (p < 0.001) (Fig. 6, a and b); $2.60 \pm 0.183$ (n = 4) and $2.70 \pm 0.17$ (n = 4) for A2AR KO CD4$^+$→Rag-1 KO treated with vehicle and ATL146e, respectively (p = NS) (Fig. 6, c and d) and $0.23 \pm 0.08$ (n = 3) and $0.32 \pm 0.04$ (n = 3) for IFN-γ KO CD4$^+$→Rag-1 KO treated with vehicle and ATL146e, respectively (p = NS) (Fig. 6, e and f).

**FIGURE 6.** Kidney neutrophil infiltration after ischemia-reperfusion. Kidney sections were stained with anti-neutrophil Ab. Representative sections of outer medulla from WT CD4$^+$→Rag-1 KO mice (a and b), A2AR KO CD4$^+$→Rag-1 KO mice (c and d), or IFN-γ KO CD4$^+$→Rag-1 KO mice (e and f) whose kidneys were subjected to IRI and treated with either vehicle (a, c, and e) or with ATL146e (b, d, and f). Magnification is ×200.

### Discussion

The impact of A2AR as a negative feedback modulator of the innate immune system has contributed to our understanding of the mechanism of tissue protection. Our previous studies demonstrated that A2AR-mediated protection from renal IRI was mainly achieved by activating those receptors expressed on BM-derived lineages (7) but not monocytes/macrophages (4). In this study, we extend previous observations by demonstrating the importance of CD4$^+$ T cell in A2AR-mediated protection and describe several findings that may contribute toward understanding the functional role of A2AR in tissue protection. We confirmed findings by Rabb and colleagues (14) that CD4$^+$ T lymphocytes are important in the early stage of reperfusion-induced inflammation. Activation of A2AR on the CD4$^+$ T lymphocytes by A2AR agonists reduces IRI by attenuating signals for inflammation in response to reperfusion. Moreover, IFN-γ is an essential mediator of CD4$^+$ T cell-induced reperfusion injury and A2AR-mediated tissue protection.

The role of T lymphocytes in renal IRI has been under-appreciated in reperfusion studies until recently. Rather, emphasis was placed on the role of neutrophils in the pathogenesis of IRI (20–22). Previously, we have shown that expression of chemokines, such as RANTES, IP-10, MIP-1α, and MIP-1β, was up-regulated in both murine liver and renal IRI models using C57BL/6 and congenic A2AR KO mice (7, 23). These chemokines have been demonstrated to recruit T lymphocytes to the region of inflammation. Furthermore, RANTES activates T lymphocytes directly without Ag-mediated TCR activation (24). Similar patterns of chemokine expression were also observed in our BM chimera studies, wherein RANTES and IP-10 were the most prominent up-regulated chemokine transcripts found in BM chimeras subjected to ischemia-reperfusion (7, 23). This result implied that T lymphocytes may be involved in the complex interplay at the early stage of ischemia-reperfusion-induced inflammation.

Recent evidence has clearly demonstrated that T lymphocytes may participate in the immediate response of renal IRI. However, inasmuch as our experiments parallel those with murine nu/nu CD4$^+$ lymphocyte reconstitution studies (14), the current findings in addition provide evidence that A2AR-mediated ischemia-reperfusion protection is also restored in these models, suggesting that CD4$^+$ T lymphocytes are essential for both the full development of ischemia-reperfusion-induced inflammation and A2AR-mediated ischemia-reperfusion protection. These results are in line with our previous studies that show that A2AR-mediated ischemia-reperfusion protection was abolished in BM chimera studies in which A2AR KO marrow cells were used to reconstitute BM of WT mice (7). These studies however did not provide information on the specific BM-derived cells that were the target of A2AR agonist-mediated tissue protection. In follow-up studies we determined that macrophages were important for the full expression of renal IRI; however A2AR expressed on macrophages were not important for tissue protection conferred by A2AR agonists. The current study tested the hypothesis that A2AR on the CD4$^+$ T lymphocytes are critical for the tissue protection. For this purpose, mice without T and B cells (Rag-1 KO) were reconstituted with WT or A2AR genetically ablated CD4$^+$ T cells before subjecting to kidney ischemia-reperfusion. The tissue protection observed in Rag-1 KO mice was reversed with adoptive transfer of WT or A2AR KO CD4$^+$ cells. Although ATL146e induced tissue protection in Rag-1 KO reconstituted with WT CD4$^+$ cells (WT CD4$^+$→Rag-1 KO), this protective effect was absent in Rag-1 KO mice reconstituted with A2AR KO CD4$^+$ cells (A2AR KO CD4$^+$→Rag-1 KO).
These results demonstrate that activation of A$_{2A}$R on CD4$^+$ T cells is a major target for A$_{2A}$R-mediated renal tissue protection.

It is interesting to note that others have reported that Rag-1 KO mice have similar severity of renal tissue damage as WT mice after exposure to renal ischemia-reperfusion (25, 26). It has been suggested that an increase in NK cell activity in Rag-1 KO serves as a compensatory mechanism for loss of T and B cells and is also responsible for the restoration of the reperfusion-induced tissue damage. To what extent NK cell activity has to be up-regulated in the Rag-1 KO mice to compensate for both T and B cell loss is not known. Differences do exist in the method of pedicle clamping, use of heparin and anesthetic agents, and degree of injury, which are variables that could potentially contribute to these differences (25, 26). Furthermore the mice that were used in the current study were derived from B6.129S7-Rag1tm1Mom/J breeders (The Jackson Laboratory). These mice are isogenic and have been backcrossed 20 generations onto a C57BL/6 background. In other studies mice solely without B or T cells have been demonstrated to have less tissue damage upon ischemia-reperfusion challenge (15, 27). Other studies similar to ours show that the extent of IRI is lower in Rag-1 KO mice than in WT mice. Cardiac IRI (28) and liver IRI (Y. J. Day and J. Linden, unpublished observations) are reduced in Rag-1 KO mice. Rag-2 KO mice skeletal muscle is protected from injury phenotype following ischemia-reperfusion (29). Despite differences in published studies on Rag-1 KO, the observation that adoptive transfer of CD4$^+$ T cells reconstitutes injury clearly implicates the role of T lymphocytes in IRI and is consistent with previously published results (14).

Although a transient wave of serum IFN-γ level has been found in the early stage of murine liver IRI (10, 30, 31) the origin of this transient wave has not been defined in a renal IRI model. One of the plausible explanations for the appearance of an early transient wave of serum IFN-γ is that lymphocytes might participate in the initiation of reperfusion-induced inflammation. This possibility is supported by our findings that adoptive transfer of WT CD4$^+$ T cells but not IFN-γ KO CD4$^+$ T cells into Rag-1 KO mice reconstituted the injury phenotype following ischemia-reperfusion. Furthermore adoptive transfer of A$_{2A}$R KO CD4$^+$ T cells into Rag-1 KO mice reconstituted injury following IRI and ATL146e did not protect kidneys in the absence of A$_{2A}$R KO CD4$^+$ T cells. These in vivo studies compliment in vitro studies by Lappas et al. (32). In these studies anti-CD3 mAb activation of CD4$^+$ T cells led to an increase in IFN-γ release from CD4$^+$ T cells, an effect that was blocked by 98% following incubation with an A$_{2A}$R agonist. Furthermore the ability of A$_{2A}$R agonists to inhibit IFN-γ release was blocked by 100% in CD4$^+$ T cells obtained from A$_{2A}$R KO mice. These data provide strong support that the mechanism by which A$_{2A}$R agonists mediate renal tissue protection from IRI is due in part to the ability of A$_{2A}$R agonists to suppress IFN-γ release.

Our studies also demonstrate that kidney neutrophil infiltration parallels injury. Pronounced neutrophil infiltration of the outer medulla was observed following IRI of WT CD4$^+$ → Rag-1 KO mice and A$_{2A}$R KO CD4$^+$ → Rag-1 KO mice. However the ability of A$_{2A}$R agonists to block neutrophil infiltration depended on the presence of A$_{2A}$R expressed on CD4$^+$ T cells. When reconstitution of Rag-1 KO mice was conducted with CD4$^+$ T cells from IFN-γ KO mice there was marked reduction of neutrophil infiltration. These results confirm the relationship between T cells and neutrophils (15) and suggest the participation of neutrophils in the early phase (~24 h) of ischemia-reperfusion.

Thus our data support other studies (14) that suggest early activation of T cells. The mechanism by which this occurs is unknown but could be the result of Ag-dependent or Ag-independent activation (33). H$_2$O$_2$ and RANTES are known to activate T lymphocytes through Ag-independent mechanisms. H$_2$O$_2$ is generated at the first few minutes of reperfusion (34, 35) and directly activates CD4$^+$ T lymphocytes (36) through oxidation of cysteine residues that inactivate protein tyrosine phosphatases (37). Cytokines including RANTES mediate Ag-independent T lymphocyte activation (24). Alternatively, T lymphocytes can be activated through classical mechanisms that include TCR response to Ag peptides presented by APCs followed by costimulation by CD80 (B7-1) and/or C86 (B7-2) (33). However Ag-dependent activation in response to IRI has yet to be demonstrated.

Interestingly, A$_{2A}$R have been identified as the predominantly expressed G protein-coupled receptors in T lymphocytes, especially on CD4$^+$ T lymphocytes (38), and as the critical signaling pathway to suppress all TCR-triggered effector function of T lymphocytes (39, 40). Studies suggest that A$_{2A}$R could inhibit TCR-triggered CD25 up-regulation and block the proliferation of T lymphocytes by inducing apoptosis (41). These results suggest that the A$_{2A}$R signaling pathway acts as an intracellular negative regulatory mechanism in T cell proliferation and TCR-triggered responses (41). Furthermore, A$_{2A}$R may suppress intercellular interactions by interfering with cytokines released from Th cells during the inflammation process (42).

In conclusion, CD4$^+$ T lymphocytes play an important role in A$_{2A}$R-mediated tissue protection. This role may involve complex interplay between CD4$^+$ T lymphocytes and other lineages such as macrophage/monocyte, platelet, and endothelium in recruitment of neutrophils at the first few hours of reperfusion. However, by using the adoptively transferred T cell- or B cell-deficient mouse model, our studies demonstrate that IFN-γ may be a critical mediator for this complex interplay. It will be interesting in future studies to determine how the CD4$^+$ T lymphocyte interact with other BM cells during reperfusion injury and by which mechanisms A$_{2A}$R activation may provide protection from tissue injury.

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Disclosures

J. Linden and M. D. Okusa own equity in Adenosine Therapeutics, which provided ATL313 and ATL146e for this study. All other authors have no financial conflict of interest.

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