Adora2b Adenosine Receptor Signaling Protects during Acute Kidney Injury via Inhibition of Neutrophil-Dependent TNF-α Release

Almut Grenz, Jae-Hwan Kim, Jessica D. Bauerle, Eunyoung Tak, Holger K. Eltzschig and Eric T. Clambey

J Immunol 2012; 189:4566-4573; Prepublished online 1 October 2012; doi: 10.4049/jimmunol.1201651
http://www.jimmunol.org/content/189/9/4566

Why The JI?

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

*average

References This article cites 70 articles, 33 of which you can access for free at:
http://www.jimmunol.org/content/189/9/4566.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

Errata An erratum has been published regarding this article. Please see next page or:
/content/199/1/363.full.pdf
Adora2b Adenosine Receptor Signaling Protects during Acute Kidney Injury via Inhibition of Neutrophil-Dependent TNF-α Release

Almut Grenz,* Jae-Hwan Kim,† Jessica D. Bauerle,‡ Eunyoung Tak,* Holger K. Eltzschig,* and Eric T. Clambey*

Renal ischemia is among the leading causes of acute kidney injury (AKI). Previous studies have shown that extracellular adenosine is a prominent tissue-protective cue elicited during ischemia, including signaling events through the adenosine receptor 2b (Adora2b). To investigate the functional role of Adora2b signaling in cytokine-mediated inflammatory pathways, we screened wild-type and Adora2b-deficient mice undergoing renal ischemia for expression of a range of inflammatory cytokines. These studies demonstrated a selective and robust increase of TNF-α levels in Adora2b-deficient mice following renal ischemia and reperfusion. Based on these findings, we next sought to understand the contribution of TNF-α on ischemic AKI through a combination of loss- and gain-of-function studies. Loss of TNF-α, through either Ab blockade or study of Tnf-α–deficient animals, resulted in significantly attenuated tissue injury and improved kidney function following renal ischemia. Conversely, transgenic mice with overexpression of TNF-α had significantly pronounced susceptibility to AKI. Furthermore, neutrophil depletion or reconstitution of Adora2b+/− mice with Tnf-α–deficient neutrophils rescued their phenotype. In total, these data demonstrate a critical role of adenosine signaling in constraining neutrophil-dependent production of TNF-α and implicate therapies targeting TNF-α in the treatment of ischemic AKI. The Journal of Immunology, 2012, 189: 4566–4573.

Although there are multiple etiologies for AKI, a common occurrence contributing to AKI is obstruction of renal blood flow, which results in renal ischemia (10). The pathogenesis of AKI following renal ischemia is multifactorial, involving the impaired function and apoptosis of tissue-resident epithelial and endothelial cells, followed by the resulting inflammatory cascade that sequentially recruits neutrophils, monocytes, and T cells to the posts ischemic kidney (10). The outcome of AKI is regulated by the balance of pathogenic mechanisms (e.g., prolonged ischemia, cell death, and the elicitation of proinflammatory cytokines including TNF-α) relative to protective mechanisms (e.g., extracellular adenosine, heme oxygenase, and the production of proresolving leukotrienes) (11).

Among tissue-protective factors elicited during ischemia, there is clear evidence that the generation and signaling of extracellular adenosine can function as a primary mechanism that limits ischemic tissue injury and inflammation (11, 12). These suppressive functions of extracellular adenosine signaling are exemplified by seminal studies by Sitkovsky et al. (13), in which the adenosine receptor 2a (Adora2a) was identified as a potent anti-inflammatory receptor in vivo. During tissue ischemia, there is a transient accumulation of extracellular adenosine, generated by the stepwise degradation of precursor molecules (nucleotides including ATP, ADP, or AMP) (14, 15). Extracellular adenosine signals through four distinct adenosine receptors (Adora1, Adora2a, Adora2b, Adora3) (16, 17), and studies show that hypoxia also enhances adenosine signaling by transcriptional increases of adenosine receptor levels (15). Although the mechanisms by which extracellular adenosine mediate protective effects remain only partially understood, adenosine receptor signaling can potentially limit inflammation by influencing both parenchymal and bone marrow-derived compartments (11, 18). In the context of Adora2b, the focus of this research, multiple studies have demonstrated that adenosine generation and signaling via Adora2b protects against ischemic injury in the heart (19, 20), liver (21), and intestine (22, 23).
Previous studies identified that extracellular adenosine generation and signaling through adenosine receptors can potentially limit AKI (24–29). Although protective effects of adenosine involve signaling through multiple adenosine receptors (including Adora1, Adora2a, and Adora2b), each of which influences discrete cell types and phases of AKI (as reviewed in Refs. 26, 30), the hypoxia-inducible nature of Adora2b (also known as the A2B adenosine receptor) places it as a prominent pathway affording renovascular protection during ischemia (31). Recent studies have shown that one protective mechanism elicited by Adora2b signaling is to restore optimal blood flow to the postischemic kidney (32). In this study, we sought to investigate the contribution of Adora2b in limiting inflammation and tissue damage during AKI, with a particular focus on leukocyte regulation. These studies demonstrated that Adora2b has a critical role in specifically regulating TNF-α production and that Adora2b restricts tissue damage mediated by neutrophils via this pathway.

Materials and Methods

Mice generation and breeding

All mice were housed in a 12-h light/dark cycle and were gender-, age-, and weight-matched between 12 and 16 wk. In transcript and pharmacological studies (30), mice obtained from The Jackson Laboratory were used. Mice deficient in TNF-α (33) were obtained from The Jackson Laboratory (B6.129S-Tnfaip3<sup>−/−</sup>/J, stock number 003008). TNFΔ AU-rich element (ARE) mice (34) were kindly provided by Dr. Jesus Rivera-Nieves (University of California, San Diego, La Jolla, CA) and generated, validated, and characterized as previously described (35). Adora2b-deficient (Adora2b<sup>−/−</sup>) mice were characterized as previously described (31).

Murine model for renal ischemia

Mice underwent right nephrectomy followed by left renal artery ischemia (0, 10, 30, and 40 min of ischemia) using a hanging weight system, as previously described (24, 25, 31, 36). Briefly, a right nephrectomy is performed, and then the left kidney is carefully isolated and the left adrenal gland dissected away. The kidney is placed in a lutein trap, a 0-0 nylon suture is threaded under the renal artery. Weights are attached at both ends of the suture, and ischemia is performed for indicated time points. Ischemia is confirmed by color change of the kidney from red to pale white. At the end of the designated ischemic time period, the weights are repositioned and reperfusion ensues. Inulin clearance was performed 6 h following reperfusion.

Inulin clearance

Inulin clearance was measured 1 h after renal ischemia (40 min) as described previously (31). Briefly, mice were anesthetized using 50 mg/kg body weight. Inulin was injected into the retro-orbital vein plexus of a catheter placed in the ureteral bladder for timed urine collection after removal of the right kidney. After surgery, mice were received a bolus of 0.45% sodium chloride solution in an amount equal to 20% body weight. Continuous infusion was maintained at a rate of 800 μL/h/25 g body weight, and FITC-labeled inulin (0.75 g/100 ml; Sigma-Aldrich) was added to the infusion for evaluation of whole-kidney GFR as described before (37, 38). After stabilization of the animals for 20 min, 20-min timed urine collections were performed. Blood was obtained in the middle of every urine collection period for measurement of FITC-inulin. Concentration of inulin in plasma and urine was performed by measurement of wavelength using a spectrophotometer (Biotek Synergy 2), and GFR was calculated by standard formulas.

Renal histology

Kidneys were excised and harvested 24 h following 40 min of renal ischemia. Renal tissues were fixed in 4.5% buffered formalin, dehydrated, and embedded in paraffin. Sections (3 μm) were stained with H&E (31). Examination and scoring of three representative sections of each kidney (n = 4–6 for each condition) were carried out blinded.

Cytokine ELISA

To quantify renal cytokine content, we performed cytokine ELISA and followed the manufacturer’s instructions (Meso Scale Discovery).

Transcriptional analysis

Total RNA was isolated from mouse kidneys using the TRIzol Reagent according to the manufacturer’s instructions (Invitrogen). Therefore, frozen tissue was homogenized in TRIzol Reagent (Invitrogen) and chloroform. After spinning at 12,000 × g for 15 min, the aqueous phase was removed, and the RNA was precipitated with isopropanol. RNA was pelleted, washed with ethanol, treated with DNase, and the concentration was quantified. The PCR reactions contained 1 μM sense and 1 μM antisense oligonucleotides with SYBR Green (Bio-Rad). Each target sequence was amplified using increasing numbers of cycles of 94˚C for 1 min, 58˚C for 0.5 min, and 72˚C for 1 min. Quantification of transcript levels was measured by real-time RT-PCR (iCycler; Bio-Rad). Primers for TNF were: forward 5′-ACC CTG ACC CCT TTA CT-3′ and reverse 5′-TTT GAG TCC TTG ATG GTG GT-3′.

Ab-based studies

To neutralize TNF-α in vivo, mice were treated with infliximab (Remicade; Janssen Biotech), a TNF-α-specific mAb therapy, at a dose of 10 mg/kg body weight by i.p. injection 60 min prior renal ischemia. To deplete neutrophils, mice were injected with 150 μg anti-Gr1 Ab (clone RB6-8C5; BioXCell). This Ab results in >90% depletion of neutrophils for at least 24 h (data not shown and Ref. 39).

Neutrophil adoptive transfer

Neutrophils were enriched from Adora2b<sup>−/−</sup> or Tnfα<sup>−/−</sup> mice using magnetic bead-based enrichment (Ly6G beads; Miltenyi Biotec) for positive enrichment for neutrophils. A total of 3 × 10<sup>6</sup> enriched neutrophils were then adoptively transferred by i.v. transfer into the indicated recipient mice.

Statistical analysis

A grading scale of 0–4, as outlined by Jablonski et al. (40), was used for the histopathological assessment of proximal tubular damage. These renal injury score data are given as median and range; all other data are presented as mean ± SD. Renal injury was analyzed with the Kruskal-Wallis test, with follow-up pairwise comparisons by Wilcoxon Mann-Whitney U test. Jablonski index results are shown as box-and-whisker plots. The lines within the boxes show the median, the bounds of the boxes show the upper and lower median, and the whiskers extend out to the data’s smallest and largest number. For all other outcomes, data were compared by two-factor ANOVA with Bonferroni’s posttest or by two-tailed Student t test when appropriate. Data are expressed as mean ± SD. A p value <0.05 was considered statistically significant. For all statistical analyses, GraphPad Prism 5.0 software (GraphPad) for Windows XP was used.

Study approval

All animal protocols were in accordance with the United States Guidelines of the Animal Institutional Care and Use Committee for use of living animals and approved by the Institutional Animal Care and Use Committee of the University of Colorado guidelines for animal care.

Results

Adora2b<sup>−/−</sup> mice have a selective and profound induction of TNF-α during AKI

Previous studies had implicated Adora2b signaling in tissue protection from ischemia and reperfusion injury (32, 41, 42). Based on these studies, we exposed wild-type C57BL/6J (Adora2b<sup>+/+</sup>) and Adora2b<sup>−/−</sup> mice to ischemic kidney injury using a well-established hanging-weight model (36); following reperfusion, kidney function was assessed by measuring the GFR. As expected, Adora2b<sup>−/−</sup> mice had more pronounced kidney injury, as measured by an impaired GFR following renal ischemia (Fig. 1A). Next, we sought to define how Adora2b regulates inflammatory cytokine profiles following renal ischemia, comparing Adora2b<sup>+/+</sup> and Adora2b<sup>−/−</sup> mice subjected to AKI. Ischemic kidney tissue was then screened for the relative abundance of a panel of cytokines. In this screen, we found that Adora2b<sup>−/−</sup> mice had a pronounced increase in TNF-α protein, whereas other cytokines (IL-1, IL-2, IL-4, and IL-12) had less pronounced differences in expression between Adora2b<sup>−/−</sup> and Adora2b<sup>−/−</sup> mice (Fig. 1B). These data identify elevated levels of TNF-α in Adora2b<sup>−/−</sup> mice as one potential pathogenic player contributing to the worse AKI in these mice.
Adora2b-dependent regulation of TNF-α occurs following prolonged periods of ischemia

To better understand the contribution of Adora2b-dependent inhibition of TNF-α production, we measured the kinetics of TNF-α transcript levels in wild-type (WT) and Adora2b−/− mice subjected to various periods of ischemia. Animals exposed to as little as 10 or 30 min of ischemia had no difference in TNF-α transcript levels between WT and Adora2b−/− mice (Fig. 2). Interestingly, 40 min of renal ischemia produced increased levels of TNF-α relative to their wild-type counterparts (Fig. 2). The temporal regulation of TNF-α following prolonged ischemia, we focused our further studies on 40 min of ischemia, the time at which there is a maximal difference between WT and Adora2b−/− mice.

Ab blockade of TNF-α limits AKI following renal ischemia in Adora2b−/− mice

Based on the worse AKI in Adora2b-deficient mice (31, 32) and identification of elevated TNF-α in Adora2b−/− mice, we tested the outcome of blocking TNF-α during renal ischemia in Adora2b−/− mice through treatment of animals with infliximab (Remicade; Janssen), a TNF-α-specific mAb therapy used for the treatment of inflammatory diseases including rheumatoid arthritis and inflammatory bowel disease (43). In untreated animals, infliximab treatment had no effect on renal histology (Fig. 3A-C). Infl iximab-treated Adora2b−/− mice subjected to renal ischemia had reduced renal injury relative to control-treated animals (Fig. 3A-C). Mice treated with infliximab before renal ischemia had reduced kidney injury as demonstrated by reduced acute tubular necrosis, less destruction of the brush border, and attenuated hyaline cast formation (Fig. 3B). Semiquantitative histological analysis demonstrated a reduction in the Jablonski index with infliximab treatment (Fig. 3C). These data identify that TNF-α elicited during renal ischemia in Adora2b−/− mice contributes to the enhanced severity of AKI in these animals.

Ab blockade of TNF-α limits AKI in wild-type mice following renal ischemia

The previous studies focused primarily on the role of TNF-α in the enhanced tissue injury in Adora2b−/− mice. To extend these findings to the context of wild-type C57BL/6J mice, we administered the TNF-α-blocking Ab (infliximab) to either control mice or mice experiencing renal ischemia. Infliximab treatment did not alter baseline renal histology in control animals not subjected to ischemia (Fig. 3D-F). However, when animals were subjected to renal ischemia, infliximab-treated animals had pronounced histological protection from renal ischemia with less tubular acute necrosis, less destruction of the brush border, and attenuated cast formation (Fig. 3D-F).

Genetic ablation of TNF-α limits AKI following renal ischemia

Based on the efficacy of Ab blocking against TNF-α in ameliorating AKI, we next tested the outcome of AKI in mice genetically deficient in TNF-α (Tnf−/−). When Tnf−/− mice were subjected to 40 min of ischemia, Tnf−/− mice had reduced AKI, as measured by
improved kidney function (characterized by a higher GFR) and improved tissue integrity as analyzed by histology (Fig. 4). These genetic data provide compelling evidence for a critical role for elevated TNF-α as a primary driver of renal injury during ischemia/reperfusion injury in mice.

**Mice with genetic overexpression of TNF-α have exacerbated AKI following renal ischemia**

Given the correlation among Adora2b deficiency, increased TNF-α levels, and worse AKI, we tested what consequence elevated levels of TNF-α in normal mice might have on AKI. To do this, we analyzed AKI in TNFΔARE mice, a mouse model in which the ARE that confers TNF-α mRNA instability has been genetically ablated through homologous recombination (34). Notably, these mice have elevated levels of TNF-α both in the resting state and following an inflammatory insult (34). When TNFΔARE mice were exposed to 40 min of ischemia, TNFΔARE mice had significantly worse AKI than wild-type controls, measured by reduced GFR (Fig. 5A), and worse tissue integrity by histology (Fig. 5B, 5C). Based on these studies, mice with a genetic lesion that results in elevated TNF-α levels have worse AKI. These genetic data demonstrate that appropriate control of TNF-α protein expression levels are required to mitigate excessive tissue injury during ischemia/reperfusion and AKI in mice.

**Neutrophils contribute to the increased AKI of Adora2b-deficient mice**

Given the important role of both TNF-α and neutrophils in ischemic injury, we next sought to test the contribution of neutrophils to the improved kidney function (characterized by a higher GFR) and improved tissue integrity as analyzed by histology (Fig. 4). These genetic data provide compelling evidence for a critical role for elevated TNF-α as a primary driver of renal injury during ischemia/reperfusion injury in mice.

**Mice with genetic overexpression of TNF-α have exacerbated AKI following renal ischemia**

Given the correlation among Adora2b deficiency, increased TNF-α levels, and worse AKI, we tested what consequence elevated levels of TNF-α in normal mice might have on AKI. To do this, we analyzed AKI in TNFΔARE mice, a mouse model in which the ARE that confers TNF-α mRNA instability has been genetically ablated through homologous recombination (34). Notably, these mice have elevated levels of TNF-α both in the resting state and following an inflammatory insult (34). When TNFΔARE mice were exposed to 40 min of ischemia, TNFΔARE mice had significantly worse AKI than wild-type controls, measured by reduced GFR (Fig. 5A), and worse tissue integrity by histology (Fig. 5B, 5C). Based on these studies, mice with a genetic lesion that results in elevated TNF-α levels have worse AKI. These genetic data demonstrate that appropriate control of TNF-α protein expression levels are required to mitigate excessive tissue injury during ischemia/reperfusion and AKI in mice.

**Neutrophils contribute to the increased AKI of Adora2b-deficient mice**

Given the important role of both TNF-α and neutrophils in ischemic injury, we next sought to test the contribution of neutrophils to the
worse AKI in Adora2b−/− mice. To do this, Adora2b−/− mice were subjected to Ab-mediated depletion of neutrophils, following treatment with an anti-Gr1 Ab (39). Following deletion of neutrophils, Adora2b−/− mice were subjected to renal ischemia. In these studies, we found that neutropenic mice had improved renal function (measured by increased GFR relative to controls, Fig. 6A) and reduced histological damage (Fig. 6B, 6C). Further, when we analyzed TNF-α levels in animals depleted of neutrophils, we found that neutrophil depletion resulted in a profound decrease in the amount of TNF-α detected during AKI (Fig. 6D). These data indicate that neutrophils contribute to the exacerbated AKI following renal ischemia in Adora2b−/− mice. Moreover, these data identify neutrophils as a major source of TNF-α during AKI following prolonged renal ischemia.

To investigate the role of neutrophil-derived TNF-α as a factor contributing to Adora2b−/− AKI, we next performed adoptive transfer studies of magnetically enriched neutrophils from either Adora2b−/− or Tnf−/− mice into Adora2b−/− recipients previously depleted of neutrophils. Notably, whereas adoptive transfer of neutrophils from Adora2b−/− mice into Adora2b−/− mice had impaired renal function (as measured by GFR, Fig. 6E), adoptive transfer of neutrophils from Tnf−/− mice was associated with attenuated AKI and a relatively improved GFR (Fig. 6E). Based on these data, neutrophil-derived TNF-α is an important contributor to AKI in Adora2b−/− mice. In total, these studies demonstrate a central role for TNF-α as a molecular mediator of tissue injury during renal ischemia in both wild-type and Adora2b−/− mice.

Discussion

The pathogenesis of AKI following renal ischemia is a complex process, involving the impaired function and apoptosis of tissue-resident epithelial and endothelial cells, coupled with a resulting inflammatory cascade that recruits multiple leukocyte subsets to the posts ischemic kidney (10). The outcome of renal ischemia is tightly regulated by the relative balance of pathogenic versus protective mechanisms (10). One potent protective mechanism elicited during ischemia is extracellular adenosine generation (generated by the stepwise degradation of extracellular ATP through the extracellular enzymes CD39 and CD73) and signaling through adenosine receptors (24–26). Notably, evidence for the potent anti-inflammatory properties mediated by adenosine receptor signaling came from pioneering studies by Sitkovsky’s group (13, 44), in which Adora2a signaling was identified as a primary anti-inflammatory pathway that constrains tissue injury during inflammation and ischemia in multiple organ systems. Although the adenosine receptor can be a potent, tissue-protective pathway, it is important to note that excess extracellular adenosine signaling, whether in the context of adenosine deaminase deficiency or in states of repeated ischemic events (e.g., sickle cell disease) can induce profound pathologies (45–47).

In the context of AKI following renal ischemia, the adenosine receptors Adora1 (A1), Adora2a (A2a), and Adora2b (A2b) have each been demonstrated to play protective roles to limit AKI (27, 28, 31). In contrast, Adora3 (A3) has a detrimental role in this context, such that Adora3-deficient mice are protected against renal ischemia-induced AKI (48). Although multiple adenosine receptors participate in protection of the kidney against ischemia, it is notable that different adenosine receptors are thought to elicit distinct mechanisms of protection, ranging from primary modulation of parenchymal cells within the kidney (via Adoral signaling) (26) to inhibition of the recruitment and activation of multiple bone marrow-derived cell types including neutrophils, NK T cells, and T cells (via Adora2a signaling) (11, 49, 50). In this context, it is worth noting that Adora2a signaling has previously been demonstrated to regulate TNF-α production from isolated human neutrophils in vitro and in murine neutrophils in an air pouch model of inflammation (49). Adora2a can also influence the extent of adenosine receptor signaling beyond its own signaling capacity. In particular, careful studies by Sitkovsky’s laboratory (51) have demonstrated that optimal cell-surface expression of Adora2b relies on coinexpression with Adora2a, revealing an unanticipated interconnectedness between these two receptors. Whether the Adora2b-dependent regulation observed in this current study is influenced by integration with Adora2a-dependent signaling during AKI remains to be determined. In the context of AKI following ischemia/reperfusion, Adora2a constrains tissue damage both by limiting neutrophil transmigration and inhibiting T cells (29, 52).

Although Adora2a is thought to primarily target leukocytes in its anti-inflammatory actions, Adora2b signaling appears to be more multifactorial, with examples of Adora2b inhibition in both parenchymal and leukocyte subsets. Adora2b is expressed by multiple cell types and tissues, including expression in the vasculature and in leukocyte subsets such as macrophages (31, 53). To date, studies
analyzing the protective effects of Adora2b in limiting AKI have focused primarily on a renovascular function for Adora2b in limiting AKI (31, 32); this focus was based largely on bone marrow chimera studies that demonstrated Adora2b can mediate protective effects when it is expressed on nonhematopoietic cells (31). Notably, however, there is clear evidence that Adora2b signaling can regulate diverse components of the inflammatory response, including neutrophils, macrophages, dendritic cells, and even lymphocytes (53–57). In this manuscript, we have focused on effector mechanisms regulated by Adora2b within the hematopoietic system, with a focus on the role of Adora2b following a longer ischemic insult (40 min relative to previous studies using 30 min of ischemia). In these studies, we found that Adora2b has a pronounced effect, limiting the production of TNF-α following renal ischemia, and that neutrophil-derived TNF-α contributes to AKI in Adora2b−/− mice. These data are consistent with reports identifying that Adora2b limits TNF-α production from macrophages (53–55), as well as a recent report demonstrating Adora2b can restrict TNF-α production from neutrophils in the context of myocardial ischemia (58).

Though our studies have focused on the protective role of adenosine receptor signaling in AKI following renal ischemia, it is important to note that the etiology of AKI can profoundly influence the contribution of adenosine receptor signaling in either protecting against or exacerbating AKI. For example, studies by Lee et al. (27) have clearly shown that the Adora1 adenosine receptor has divergent effects in AKI with different modes of renal injury. In the context of renal ischemia, Adora1 has a protective role, and Adora1-deficient mice have worse AKI following renal ischemia (27). Conversely, in the context of nephrotoxic and radiocontrast agent-induced AKI, Adora1 has a detrimental role such that Adora1-deficient mice are protected against this form of AKI (59). These data exemplify the complex interplay between AKI etiology and adenosine receptor signaling to ultimately influence kidney function.

Our data in this study, and elsewhere, demonstrate a protective role for Adora2b in limiting acute ischemic tissue injury (23, 31, 58). In these contexts, the hypoxia-inducible nature of Adora2b places it as a central mediator affording tissue-protective effects during ischemia (31). However, it is important to note that in other contexts, Adora2b signaling has been associated with proinflammatory roles, such as the induction of IL-6 in dendritic cells (60). Moreover, depending on the duration and context of Adora2b signaling, this receptor can be a primary driver of pathobiology. Notably, rigorous studies from the laboratories of Xia and Blackburn (47, 61, 62) have clearly demonstrated that Adora2b is responsible for pathogenic outcomes in priapism, sickle cell disease, and bleomycin-induced lung fibrosis.

The outcome of AKI is regulated at multiple stages, from mechanisms that function rapidly (within minutes to hours of injury) to the later involvement of components of the adaptive immune response including T cells that can contribute to tissue injury and repair days after the initial insult (10). In this larger context, it is important to note that Adora2b induces multiple protective effects during ischemic injury. For example, by analyzing the role of Adora2b within the renal vasculature, we recently showed that Adora2b promotes optimal postischemic blood flow within the kidney, thereby ensuring maximal return of blood flow, tissue oxygenation, and the removal of waste products from the ischemic kidney (32). This mechanism functions through cross talk of Adora2b with ENT1, the equilibrative nucleoside transporter, a transporter protein that controls the extent and timing of extracellular adenosine. Based on the data presented in this study, demonstrating that Adora2b-regulated TNF-α directly impacts AKI, it is worth noting that TNF-α can have profound effects on the vasculature, raising the possibility that the mechanism identified in this study may integrate with the Adora2b–ENT1 pathway to regulate postischemic blood flow to the kidney (32). Although Adora2b signaling can mediate protective effects during renal ischemia [using either nephrectomy and unilateral ischemia or simultaneous, bilateral renal ischemia (32)], it remains to be seen what contribution Adora2b has in the context of AKI following nephrotoxin exposure [e.g., cisplatin (63)] or following sepsis [in a context in which Adora2b deficiency is beneficial in limiting bacterial colonization (55)]. This is an important question for future investigation because different forms of AKI are characterized by distinct kinetics and effector mechanisms (e.g., ischemia/reperfusion, nephrotoxin, or sepsis) (2, 10, 63). A notable contrast between our model and that of cisplatin-induced AKI is that parenchymal cells are the prominent source of TNF-α (64, 65), in contrast to our renal ischemia model in which neutrophils are a prominent source of TNF-α. Because Adora2b can mediate protective effects on both parenchymal and bone marrow-derived cell types, it is possible that the contribution of Adora2b signaling in AKI will vary depending on the etiology of AKI under study.

Beyond the kidney, Adora2b has been shown to mediate additional tissue-protective effects in models of acute inflammation. For example, Adora2b signaling can elicit cardioprotective effects through the circadian rhythm protein Period2, which in turn promotes metabolic adaptation in the ischemic heart (41). In acute colitis and following hypoxia, Adora2b also functions as an essential receptor for the anti-inflammatory properties of netrin-1, a neuronal guidance molecule (66). Adora2b signaling can also regulate the superoxide burst in neutrophils (67), which would limit neutrophil-derived production of potentially toxic reactive oxygen species. Finally, Adora2b has been shown to limit the inflammatory properties of macrophages and T cells (53, 55, 57). Given that these cell types can influence regulate AKI (52, 68, 69), Adora2b-mediated protection in AKI may arise by altering the function of multiple leukocyte subsets.

The multiple etiologies of AKI, combined with the paucity of therapeutic interventions, continue to be a significant unmet medical challenge. Given the weight of evidence that extracellular adenosine generation and signaling are tissue-protective during AKI, this pathway is of significant therapeutic interest. To optimally harness the potential of Adora2b in limiting AKI, it will be important for future studies to define how the multiple protective mechanisms of Adora2b integrate to induce maximal protection during AKI. In addition, it will be equally important to determine how to therapeutically activate the beneficial effects of Adora2b signaling without triggering deleterious consequences of prolonged Adora2b signaling (47, 70).

Acknowledgments
We thank Dr. Jesus Rivera-Nieves for kindly providing the TNFΔARE mice and members of the Eltzschig laboratory for critical discussion.

Disclosures
The authors have no financial conflicts of interest.

References


Letter of Retraction

A request was received from the Research Integrity Officer of the University of Colorado Denver, Anschutz Medical Campus, and by Dr. Clambey, to retract this article: “Adora2b Adenosine Receptor Signaling Protects during Acute Kidney Injury via Inhibition of Neutrophil-Dependent TNF-α Release” by Almut Grenz, Jae-Hwan Kim, Jessica D. Bauerle, Eunyoung Tak, Holger K. Eltzschig, and Eric T. Clambey, The Journal of Immunology, 2012, 189: 4566–4573.

The Editor-in-Chief of The Journal of Immunology was informed that the University of Colorado Denver had conducted a review of the work and concluded that data used for the upper right hand panel of Figure 3B (−Infliximab +Ischemia in a homozygous Adora2b−/− mouse) was duplicated with the upper right hand panel of Figure 3E (−Infliximab +Ischemia in a WT mouse). The authors were not able to provide the original data for these figures, and thus they cannot be validated. There were no findings of scientific misconduct made against any of the coauthors relating to the article. The identified errors appear to be the result of honest error, but due to the lack of original data, the article is hereby retracted.