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A2B adenosine receptor gene deletion attenuates murine colitis

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Abstract

Background and significance—The A_{2B} adenosine receptor (A_{2B}AR) is the predominant adenosine receptor expressed in the colonic epithelia. We have previously shown that A_{2B}AR mRNA and protein levels are upregulated during colitis. In this study we addressed the role of the A_{2B}AR in the development of murine colitis and potential mechanism underlying its effects.

Methods—Dextran sodium sulfate (DSS), 2,4,6-trinitrobenzene sulfonic acid (TNBS) and *Salmonella typhimurium* were used to induce colitis in A_{2B}AR null mice ($A_{2B}AR^{-/-}$). Colitis was determined using established clinical and histological scoring. keratinocyte derived chemokine (KC) measurements were performed using ELISA.

Results—Colonic inflammation induced by DSS, TNBS or *S. typhimurium* was attenuated in $A_{2B}AR^{-/-}$ compared to their WT counterparts. Clinical features, histological score, myeloperoxidase activity were significantly decreased in $A_{2B}AR^{-/-}$ mice. However, $A_{2B}AR^{-/-}$ showed increased susceptibility to systemic *Salmonella* infection. Tissue levels of the neutrophil chemokine, KC was decreased in colitic $A_{2B}AR^{-/-}$ mice. In addition, flagellin-induced KC levels were attenuated in $A_{2B}AR^{-/-}$ mice. Neutrophil chemotaxis in response to exogenous IL-8 was preserved in $A_{2B}AR^{-/-}$ mice, suggesting intact neutrophil migration in response to appropriate stimuli.

Conclusions—These data demonstrate, for the first time, that the A_{2B}AR plays a pro-inflammatory role in colitis. A_{2B} receptor antagonism may be an effective treatment for acute inflammatory intestinal diseases such as acute flare of inflammatory bowel disease.

Introduction

Adenosine, an endogenous purine nucleoside that is involved in a variety of physiological functions, is being increasingly recognized to modulate a wide variety of inflammatory/immune response[1, 2]. Following its release from cells or after being formed extracellularly during inflammation[3–5] adenosine mediates its effect through one of the four receptors: A_1,

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A_{2A}, A_{2B} and A_3. Depending on the tissue or cell type, adenosine receptors mediate pro- or anti-inflammatory responses[6]. With respect to intestinal inflammation, the effect of adenosine mediated through the A_{2A} adenosine receptor (A_{2A}AR) has been the most studied. Multiple studies have demonstrated that A_{2A}AR plays an anti-inflammatory role and the most potent anti-inflammatory and immunosuppressive effects of adenosine are generally attributed to occupancy of A_{2A} receptors expressed on immune cells[7, 8]. A_{2A}AR agonists have been demonstrated to suppress the expression of proinflammatory cytokines while sparing anti-inflammatory activity mediated by IL-10 and TGF-β[9]. A_{2A} agonists also ameliorate bacterial colitis such as inflammation induced by *C. difficile*[10]. Similarly, A_1 agonists have been shown to ameliorate intestinal inflammation[11]. Not much is known regarding the role of the A_{2B}AR in intestinal inflammation.

A_{2B}AR is expressed by immune cells as well as the intestinal epithelium. In contrast to immune cells that express multiple adenosine receptors[6, 12], the A_{2B}AR is the predominant adenosine receptor expressed in the colonic epithelium[13]. We have recently demonstrated that A_{2B}AR mRNA and protein expression is upregulated during human and animal models of IBD and that TNF-α plays an important role in the upregulation of A_{2B}AR[14]. In the colonic epithelium, A_{2B}AR plays a prominent role in regulating vectorial electrogenic ion secretion, a secretory pathway that results in movement of isotonic fluid into the lumen[13, 15, 16]. An upregulation of ion secretion during inflammation is considered to be an important component of inflammation-associated diarrhea [17, 18]. In addition to its effect on ion transport, A_{2B}AR mediates IL-6 secretion by the colonic epithelial cells which is polarized to the luminal compartment and activates neutrophils[5]. Such neutrophil-epithelial interaction initiated by adenosine has been shown to be important for adhesion of *Salmonella typhimurium*[19]. Similarly, adenosine induces apically polarized fibronectin secretion, which potentiates adhesion, invasion of *S .typhimurium* through KC secretion[20]. Although these observations suggest a pro-inflammatory role for A_{2B}AR in intestinal inflammation, the effect of A_{2B}AR in the pathogenesis of intestinal inflammation is unknown. The current study addresses the role of A_{2B}AR in colitis using three different models and examines the relative effects of systemic *vs* localized insults on the pathogenesis of intestinal inflammatory response. Finally, potential mechanism by which A_{2B}AR mediates its effects is addressed.

Materials and Methods

Reagents

Dextran sodium sulfate (DSS, MP Biomedicals Inc, Aurora, OH), Trinitrobenzene sulfonic acid (TNBS, Sigma, St.Louis, MO), X-gal (5-bromo-4-chloro-3indolyl-b-D-galactopyranoside, Invitrogen) Myeloperoxidase, (MPO), SuperScript First strand synthesis system for RT–PCR (Invitrogen, CA), iQ SYBR Green Supermix (Biorad, Hercules, CA), KC Duoset ELISA kit, Recombinant Human IL-8 (R&D Systems Inc, Minneapolis, MN).

Experimental animals

The Animal Care Committee of the Emory University, Atlanta approved all procedures performed on animals. The generation of $A_{2B}AR^{-/-}$ mice and its characterization has been described[21]. Mice lacking A_{2B}AR exhibit a normal phenotype. The mice were on a C57BL/6 background as determined by the PCR-based strain detection method MAX (Charles River Labs, MA)[21]. In all experiments 8–10 week old C57BL/6 wild type (WT) and $A_{2B}AR^{-/-}$ female mice were used. Colitis was induced by oral administration of DSS (3% wt/vol) in water *ad libitum* for 6 days. Age-matched WT and $A_{2B}AR^{-/-}$ receiving regular water served as controls. Mice were observed daily and evaluated for changes in body weight and development of clinical symptoms. β-galactosidase staining in colonic tissue was done as described[21]. Gut-restricted and systemic *S. typhimurium* infection was induced as described previously[22].

Colitis was induced by Trinitrobrenzene sulfonic acid (TNBS) as described previously [23, 24].

Clinical and histological Score

Colitis was quantified with a clinical score, as described by Cooper et al, [25] by using the parameters of weight loss, stool consistency, and fecal blood. The length and weight of the colon were measured, and tissue obtained from each colon was processed for further assays. Colonic specimens obtained as described previously were fixed in formalin and coded for blind microscopic assessment of mucosal lesions (descending colon for DSS colitis and cecum for *S typhimurium* colitis). Sections were stained with haematoxylin and eosin for histological scoring as described by Cooper et al. Neutrophil infiltration into the colon was quantified by measuring myeloperoxidase (MPO) activity as described previously [26, 27]

Measurement of cytokines and myeoloperoxidase assay

The pro-inflammatory cytokines were measured by real time PCR[24]. Total RNA was extracted from colonic tissue of WT and $A_{2B}AR^{-/-}$ mice using TRIzol reagent. After quantification, a reverse transcription (RT) reaction was performed with 2 μg of each sample and oligo-dT primer, using the SuperScript First Strand Synthesis System for RT-PCR (Invitrogen, CA). The real-time iCycler sequence detection system (Bio-Rad) was used for real-time RT-PCR. *In vivo* KC levels were determined in the serum of WT and $A_{2B}AR^{-/-}$ mice. Mice were given flagellin (1.0μg/mouse) intraperitoneally and serum was collected 90 minutes after the injection. The levels of KC in the serum samples were quantified by ELISA as described previously [28].Myeloperoxidase assay was performed as described previously [26]. Colonic organ cultures and cytokines measurements from supernatants of organ culture were done as described previously[29].

Intraperitoneal administration of IL-8

Recombinant Human IL-8 (R&D Systems) 1μg/100μl of 1% BSA/PBS was given intraperitoneally to age and gender matched WT and $A_{2B}AR^{-/-}$ mice. Mice were sacrificed 4 hours after the administration of IL-8. 3.0 ml of HBSS solution (without calcium chloride, magnesium sulfate) was injected intraperitoneally and peritoneal fluid was collected through a catheter. Peritoneal fluid was centrifuged at 10,000 rpm for 10 minutes and cells were resuspended into 50 μl of HBSS and 10 μl of this suspension was smeared on the glass slide and the number of neutrophils were counted after Geimsa staining. MPO activity was measured in the remaining cells to assess the number of neutrophils.

Statistical analysis

The data are presented as mean±SE. Statistical analysis was conducted using Student's t-test where $p<0.05$ was considered significant. ANOVA was used in experiments wherein multiple group comparison was involved.

Results

$A_{2B}AR$ expression is increased during colitis

Our previous studies have shown that $A_{2B}AR$ mRNA and protein expression is increased during colitis in both animal models as well as humans[14]. The $A_{2B}AR^{-/-}$ mice used here were created by replacing exon 1 of the $A_{2B}AR$ gene with a reporter construct containing the gene encoding β-galactosidase (β-gal). In order to verify the upregulation of the $A_{2B}AR$ gene in the colonic epithelium during colitis, we determined β-gal expression before and after the induction of colitis in DSS fed mice. β-gal staining was undetectable in wild type (WT) mice. In contrast, colonic epithelial cells in $A_{2B}AR^{-/-}$ mice showed positive staining for β-gal, indicating the

expression of $A_{2B}AR$ in these cells (Figure 1). As shown in the lower right panel, β-gal expression levels were increased in $A_{2B}AR^{-/-}$ DSS-treated mice (Figure 1).

$A_{2B}AR^{-/-}$ mice are resistant to the development of DSS–induced colitis

To investigate the role of the $A_{2B}AR$ in the pathogenesis of colitis, we administered 3% DSS in drinking water to age- and sex- matched WT and $A_{2B}AR^{-/-}$ mice. The mice were compared for clinical signs, including weight loss, stool consistency and occult blood. All WT mice that received DSS developed clinical signs of colitis after day 6. These mice developed weight loss, diarrhea and frank rectal bleeding with a total clinical score of 9.2±0.73 (Figure 2A). In contrast, $A_{2B}AR^{-/-}$ mice did not exhibit weight loss at the end of the treatment period (Figure 2B). These mice had no diarrhea and significantly reduced rectal bleeding as compared to WT mice. Overall, $A_{2B}AR^{-/-}$ mice showed protection from colitis as evaluated by their clinical score of 3±0.63 (*p<0.0002 Figure 2A). In addition, colon length, and other parameters of inflammation[30] correlated with the clinical results. DSS administration to WT mice resulted in an 18% shortening of the colon length, compared with mice treated with water after the experimental period (WT DSS: 5.3±0.12, WT water 6.5±0.2 cm), However, DSS administration to the $A_{2B}AR^{-/-}$ mice showed no effect on colon length ($A_{2B}AR^{-/-}$ DSS 6.4 ±0.24 cm, $A_{2B}AR^{-/-}$ water: 6.6±0.24 cm).

DSS-induced colitis is characterized by the presence of inflammation in the colon with marked crypt destruction, mucosal damage and epithelial erosions and infiltration of inflammatory cells into the mucosal tissue. Histological scores agreed with the clinical scores and confirmed the protective role of $A_{2B}AR$ gene deletion. As shown in Figure 2C, WT mice treated with DSS showed signs of inflammation and tissue damage. These mice had extensive crypt damage, epithelial erosion/ulceration, and infiltration of inflammatory cells in to the lamina propria and muscularis mucosa of colonic sections. In contrast, histological analysis of the sections from $A_{2B}AR^{-/-}$ mice revealed significantly reduced inflammation and these mice were protected from DSS-associated tissue injury with fewer inflammatory infiltrates and ulcerations (Figure 2C). To confirm the histological finding we further determined granulocyte accumulation by MPO activity in the colonic tissue. As shown in Figure 2E, WT mice that received DSS had significantly increased MPO activity (1.76±0.3 U/mg protein) compared to $A_{2B}AR^{-/-}$ that received DSS (0.26±0.14U/mg protein, *p< 0.003, n=5, Figure 2E). In addition, the levels of the keratinocyte derived chemokine (KC, a murine CXC chemokine, a functional homologue of interleukin (IL)-8 and an essential neutrophil chemoattractant[31]) were measured in organ culture as described in the Methods section. As shown in Figure 2F, WT mice showed significantly higher levels of KC (36.6±10.04 pg/ml media) compared to $A_{2B}AR^{-/-}$ mice (12.59±2.9 pg/ml, *p<0.01,n=5). Taken together, these data demonstrate that mice with a targeted deletion of the $A_{2B}AR$ gene had significantly reduced severity of DSS-induced colitis.

In the next set of experiments, we examined the effect of $A_{2B}AR$ gene deletion on recovery from DSS-induced colitis. Colitis was induced in WT or $A_{2B}AR^{-/-}$ by the administration of DSS in the drinking water for 6 days (colitic phase), after which the mice were switched to regular drinking water for 6 additional days (recovery phase). As shown in Figure 3A, WT mice given DSS showed lower weight compared to water control (−2.8±2.6%), while $A_{2B}AR^{-/-}$ gained weight (7.8±1.5%), demonstrating recovery from acute colitis. MPO measured at the end of the recovery period showed persistently elevated MPO activity in WT mice, while $A_{2B}AR^{-/-}$ mice showed significantly less MPO (Figure 3B). These data demonstrate that the $A_{2B}AR^{-/-}$ mice were not only protected from DSS-induced colitis but also showed improved recovery.

$A_{2B}AR^{-/-}$ mice are resistant to oral *Salmonella typhimurium*–induced colitis

As an alternative model of colitis, we used oral infection with *S. typhimurium* where *S typhimurium* was administered after pretreatment of mice with streptomycin[22]. In this model, *S. typhimurium* induces clinical and histological features of enterocolitis that predominantly involve the cecum. We used this model since it resembles some of the clinical and histological features of human infection and acute flares of IBD, where epithelial enteric/pathogen interaction is thought to play an important role in the pathogenesis[32 32, 33]. Ceca of all the WT mice treated with *S. typhimurium* looked pale and were smaller compared to the $A_{2B}AR^{-/-}$ mice, indicating protection against *S. typhimurium*-mediated effects (Figure 4A). As shown in Figure 4B, MPO activity was significantly higher in WT mice treated with *S. typhimurium* (2.5-fold) compared to control mice. In contrast, $A_{2B}AR^{-/-}$ mice showed no increase in MPO activity. Histological analysis revealed that there was marked leukocyte infiltration with crypt damage in WT mice treated with *S. typhimurium*. In contrast, neutrophil infiltration was attenuated and crypt architecture was preserved in $A_{2B}AR^{-/-}$ mice (Figure 4C & D). Together, these data demonstrate that $A_{2B}AR^{-/-}$ mice are protected from *S. typhimurium*-induced colitis.

$A_{2B}AR^{-/-}$ mice are resistant to the development of TNBS–induced colitis

We next examined whether our results would extend to a distinct model of colitis, namely that induced by the hapten TNBS[24,23]. WT and $A_{2B}AR^{-/-}$ mice were randomized to receive ethanol or TNBS by rectal enema as described in the Methods section. As shown in Figure 5A, WT mice treated with TNBS showed signs of colon inflammation and tissue damage. These mice had extensive infiltration of inflammatory cells into the lamina propria and muscularis mucosa of colon. (top panel, Figure 5A). In contrast, histological analysis of the sections from $A_{2B}AR^{-/-}$ mice revealed significantly reduced inflammation and these mice were protected from TNBS-associated tissue injury, with fewer inflammatory infiltrates and ulcerations (bottom panel Figure 5A). As noted in Figure 5B, WT mice that received TNBS showed significantly higher levels of MPO activity (Figure 5B) and KC mRNA compared to $A_{2B}AR^{-/-}$ mice given TNBS (Figure 5C). These data are consistent with the protective effect of $A_{2B}AR$ gene deletion on the development of TNBS-induced colitis.

$A_{2B}AR$ is not required for systemic *S. typhimurium* sepsis

The attenuated colonic inflammation in response to DSS, *S. typhimurium* or TNBS might result from a systemic defect in immune response, or limited to inflammatory response in the colon in response to luminal insults. We explored these possibilities in the next set of experiments. To determine whether the immune response to *S. typhimurium* was impaired in $A_{2B}AR^{-/-}$ mice, we examined the response to orally administered *S. typhimurium* without pretreatment with streptomycin. *S. typhimurium*, under these circumstances, leads to systemic invasion through jejunum and ileum[34] resulting in the colonization of liver and spleen with progression to generalized sepsis. Mice succumb to *S. typhimurium* sepsis and die within 10–14 days after the administration of *S. typhimurium*. WT and $A_{2B}AR^{-/-}$ were administered *S. typhimurium* (SL3201; 10^6 colony-forming units per mouse by gavage). Mice were weighed daily and observed for clinical signs of sepsis and for mortality. As shown in Figure 6A, 90% of $A_{2B}AR^{-/-}$ mice died within 10 days compared to 20% of WT mice. Consistent with the mortality data, $A_{2B}AR^{-/-}$ mice showed signs of weight loss earlier than WT mice. However, overall mortality was comparable in both groups at the end of the experiment (Figure 6B). These data demonstrate that immune response to systemic *Salmonella* infection was actually heightened in $A_{2B}AR^{-/-}$ mice.

$A_{2B}AR^{-/-}$ mice exhibit defective chemokine response

The foregoing data suggest that attenuation of inflammatory response as a result of $A_{2B}AR$ gene deletion was limited to the colon. We hypothesized that colonic epithelial $A_{2B}AR$ may regulate inflammatory response through secretion of the chemokine, KC. Consistent with this notion is the reduced level of IL-8-like chemokine (KC) neutrophilic infiltrate, and MPO activity in the $A_{2B}AR^{-/-}$ mice compared to WT mice in all three models of acute colitis. To determine whether $A_{2B}AR$ is required to mediate KC secretion, we administered flagellin, a potent inducer of epithelial KC response[35] to WT and $A_{2B}AR^{-/-}$ mice. $A_{2B}AR^{-/-}$ mice showed significantly lower levels of KC (60±23.3 ng/ml), compared to WT mice treated with flagellin (153.3±22.42 ng/ml, *p<0.004, n=3, Figure 7A).

$A_{2B}AR$ is not required for neutrophil migration

We next determined whether $A_{2B}AR$ is required for neutrophil migration in the presence of appropriate chemotactic stimulus, i.e. IL-8. WT and $A_{2B}AR^{-/-}$ mice were given recombinant human IL-8 (1.0 μg) intraperitoneally as described in the Methods section. Four hours after the administration of IL-8, mice were sacrificed and neutrophils in the peritoneal fluid were determined by MPO activity. As shown in Figure 7B, the administration of IL-8 induced in a brisk neutrophil migration into the peritoneum in the WT mice (302.5±133.2 U/mg protein) which was similar in $A_{2B}AR^{-/-}$ mice (217±105.7, n=4).

Discussion

In this study we demonstrate that $A_{2B}AR$ plays a pro-inflammatory role in the intestine. Using three mouse models of colitis that have distinct inciting factors, we demonstrate that the $A_{2B}AR$ gene deletion ameliorates inflammatory response in the colon. DSS elicits an acute mucosal inflammatory response that mimics several features of acute colitis and flares of inflammatory bowel disease [25,36]. In the injury phase, mice develop weight loss, blood in stool and diarrhea similar to the symptoms in acute flares of inflammatory bowel disease (IBD). Our data demonstrate that $A_{2B}AR^{-/-}$ mice develop significantly reduced clinical symptoms, including weight loss, blood in stool and diarrhea associated with the administration of DSS. The reduced severity of colitis is also reflected in a reduced histological score wherein $A_{2B}AR^{-/-}$ mice showed less inflammatory infiltrates, ulcers and epithelial damage. When DSS treatment was terminated after the first week, most wild type mice are unable to heal injury as reflected by continued weight loss and persistently high inflammatory infiltrate, while $A_{2B}AR^{-/-}$ mice showed weight gain indicating mucosal healing and recovery. The efficient healing of $A_{2B}AR^{-/-}$ mice likely reflects reduced DSS-induced injury in the colitic phase. However, it is also possible that $A_{2B}AR$ receptor may play a role in inhibiting wound healing. Our data also show that the $A_{2B}AR$ gene deletion protects against the development of tiffilitis induced by *S. Typhimurium a* in streptomycin-pretreated mice as well as colitis induced by TNBS. Together, these data demonstrate that the A_{2B} receptor mediates a pro-inflammatory response in the intestine. Based on our previous data that showed upregulation of $A_{2B}AR$ mRNA and protein expression in human and animal models of IBD[14], $A_{2B}AR$ antagonism may be an effective treatment for acute flares of IBD and infectious colitis.

While $A_{2B}AR^{-/-}$ mice were resistant to an inflammatory response to luminally administered toxins or bacteria (DSS, *S. typhimurium*, or TNBS) in the colon, they were highly susceptible to systemic challenge *S. typhimurium* when administered without antibiotic pretreatment. This resulted in systemic invasion, likely through its entry through jejunal and ileal mucosa and colonization of mesenteric lymph node, spleen and liver leading to sepsis and mortality within two weeks of infection[37] . Interestingly, our data show that systemic immune response to *Salmonella* is heightened in $A_{2B}AR^{-/-}$ mice leading to rapid weight loss and death significantly earlier than their WT counterparts. These data are consistent with the increased susceptibility

of $A_{2B}AR^{-/-}$ mice to lipopolysaccharide (LPS)[21]. This study also found that the $A_{2B}AR^{-/-}$ mice displayed a mild but significant increase in the level of the proinflammatory cytokine, TNF-α, under baseline conditions. These data were taken to suggest that the $A_{2B}AR$ plays a systemic anti-inflammatory role. Another explanation for the increased susceptibility of A2bAR−/− mice to systemic Salmonellosis is the lack of neutrophil recruitment in the absence of A2BAR. This notion is supported by a recent study which demonstrated that neutrophil depletion increased bacterial translocation [38] Other studies have corroborated with the anti-inflammatory effects of $A_{2B}AR$ in some tissues[39–41]. However, the role of $A_{2B}AR$ in mediating effects on macrophage TNF-α was clarified by another study wherein the investigators demonstrated that deletion of the $A_{2B}AR$ gene increases TNF-α, but the receptors do not have a direct role in the macrophage TNF-α response. Rather, $A_{2B}AR$ mediates a pro-inflammatory response through secretion of cytokines such as IL-6 from macrophages [42, 43]. This is consistent with other studies that showed a pro-inflammatory role for $A_{2B}AR$ in some tissues [20, 43–48]. Such disparate effects of adenosine receptors, depending on the tissue, may be explained by its signaling partners in the tissue. We and others have shown that the $A_{2B}AR$ may exist in a multiprotein complex[49, 50] in the lung and intestinal epithelia, and interaction with its partners may determine its functional effects. Further, the $A_{2B}AR$ activates other signaling pathways through Gq coupling[51, 52] and similarly, the signaling pathway it activates may also play a role in its function.

A common denominator of the three models of colitis used in this study is the predominant neutrophilic infiltrate that characterize the histology and as evident by MPO. KC is the major neutrophil chemoattractant secreted by the epithelium in response to a variety of luminal agents, including *Salmonella* [53,54,35]. Our data suggest that one of the underlying mechanisms for decreased inflammatory infiltrates in the $A_{2B}AR^{-/-}$ mice is the reduced ability of the epithelia to secrete KC. Indeed, our data show that KC secretion in response to flagellin is attenuated in $A_{2B}AR^{-/-}$ mice. With respect to *S. typhimurium*, KC has been demonstrated to play a critical role in the inflammatory response elicited by this pathogen[54,55] . The neutrophil emigration into the peritoneum in response to the administration of IL-8 in $A_{2B}AR^{-/-}$ mice is consistent with the notion that neutrophil migration into the colon may be impaired in the $A_{2B}AR^{-/-}$ mice due to defective KC secretion by the colonic epithelia. Although $A_{2B}AR^{-/-}$-mediated KC secretion may play an important role in the development of colonic inflammation, inflammatory response is complex and other factors may contribute to the $A_{2B}AR$ receptor-mediated pro-inflammatory response. Additional studies, such as microarray analysis of colonic mucosa from WT versus $A_{2B}AR^{-/-}$ mice may give further insight into additional pro-inflammatory pathways triggered by the activation of $A_{2B}AR$ in intestinal inflammation.

In summary, we demonstrate that $A_{2B}AR^{-/-}$ mice develop reduced inflammation in response to luminal toxins (DSS, TNBS) and *Salmonella*. We present data that suggest that attenuated KC secretion by the colonic epithelium may play a role in the decreased inflammatory response seen in $A_{2B}AR^{-/-}$ mice. Taken together, our study focuses on the $A_{2B}AR$ as a therapeutic target for treatment of acute intestinal inflammatory disorders such as acute flares of IBD.

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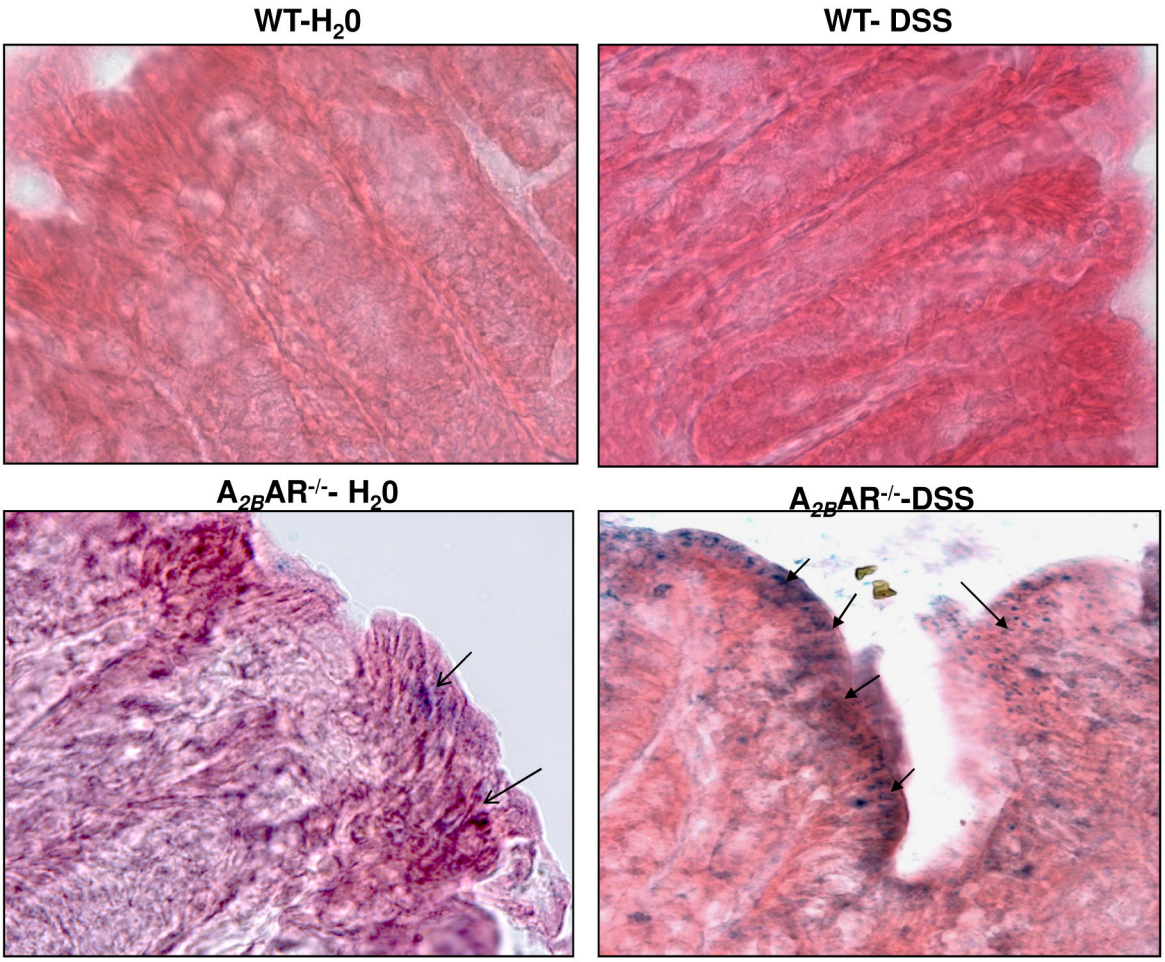
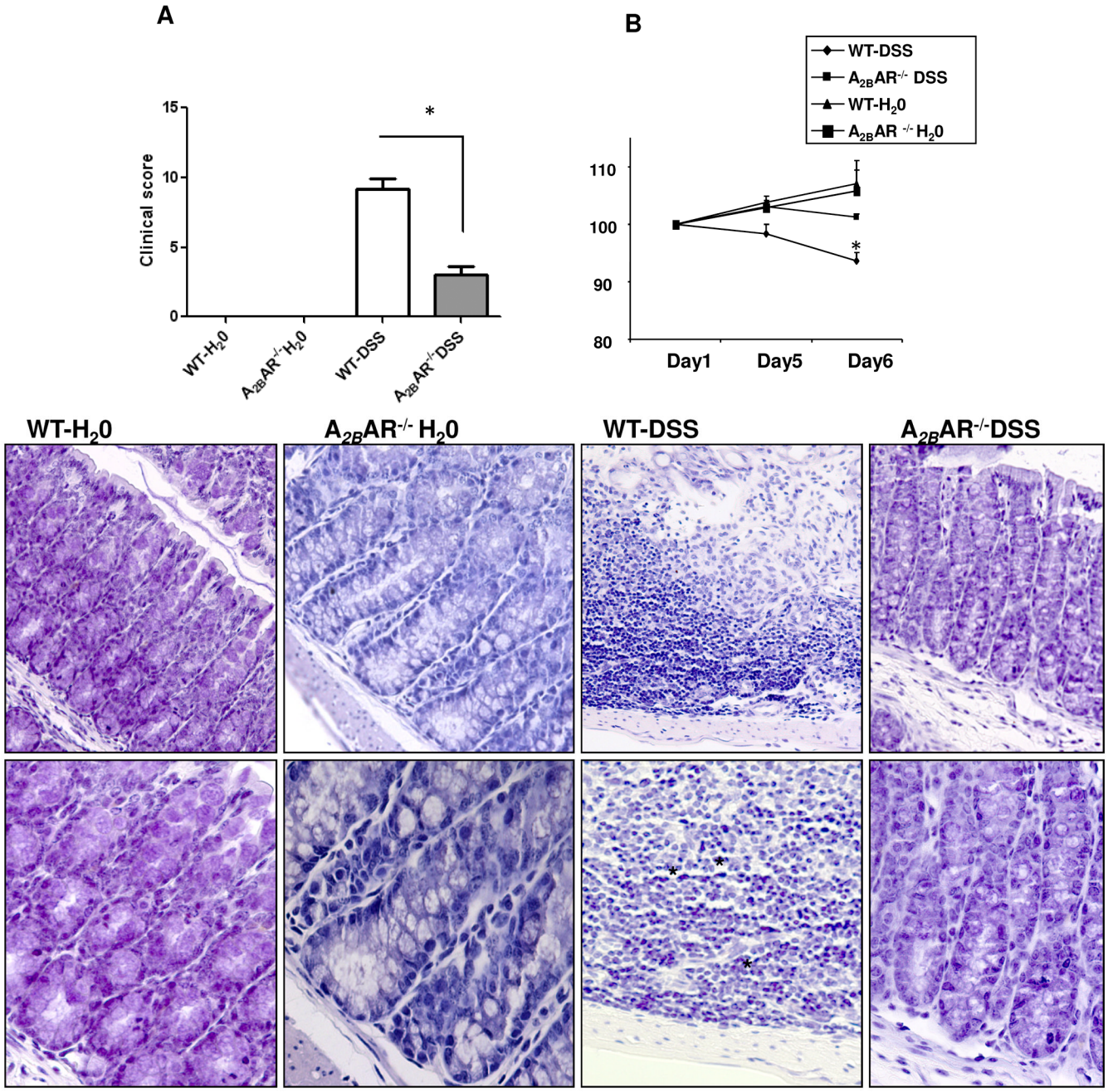


Figure 1. A_{2B} receptor expression is increased during colitis

Six days after DSS or H_2O administration, WT and $A_{2B}AR^{-/-}$ mice were sacrificed and colonic tissues were stained for β-gal (blue) and photographed. Arrows point to staining localized to epithelial cells. Data are representative of 3 independent experiments.



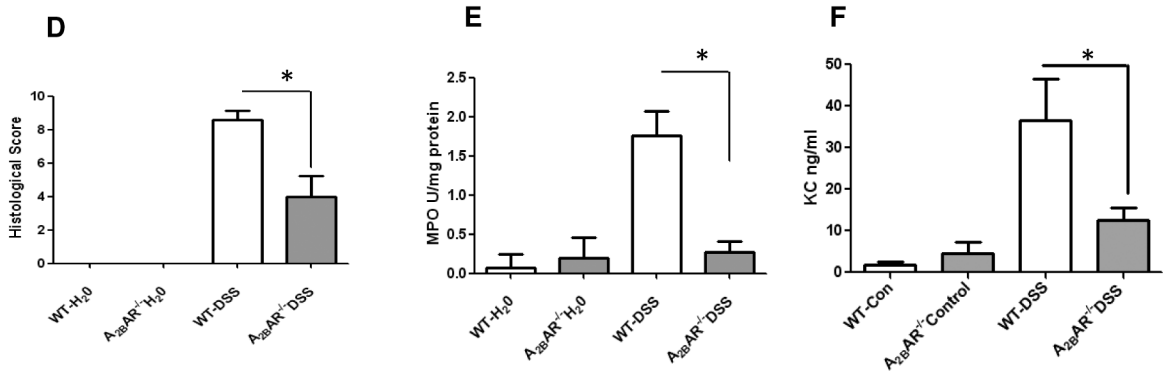


Figure 2. A_{2B}AR mice are resistant to the development of dextran sodium sulfate– induced colitis

Water or DSS treated mice were sacrificed 6 days after treatment. **A**. Disease severity is represented as clinical score. Results are expressed as Mean±SE, n=5 *p<0.002. **B**. Percent change in body weights *p<0.003. **C**. Representative histological sections of colon from each group are shown (n=5). * indicates neutrophil infiltrates. **D**. Histological assessment is represented as histological score. Data expressed as Mean±SE for the group compared (WT +DSS and $A_{2B}AR^{-/-}$ + DSS), *p<0.007 **E**. Myeloperoxidase was measured as an index of neutrophil infiltration into the injured tissue as described in the Methods section. Each bar represents Mean±SE, n=5, *p<0.002. **F**. Colonic tissue culture supernatants were processed for KC secretion. KC levels in WT mice treated with DSS are significantly higher compared to $A_{2B}AR^{-/-}$ treated with DSS (*p<0.01).

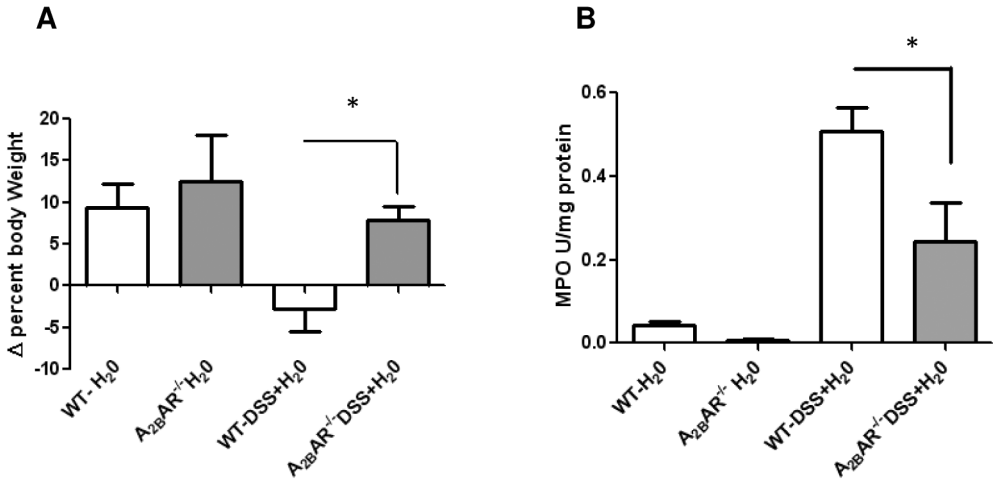


Figure 3. $A_{2B}AR^{-/-}$ mice demonstrated improved recovery after the colitic phase

Mice received water or DSS for seven days followed by water for another 6 days. **A**. Percent change in body weights in WT and $A_{2B}AR^{-/-}$ during recovery phase Mean±SE, n=5, *p<0.01. **B**. Myeloperoxidase activity. Results are expressed as Mean ± SE., n=5, *p<0.049.

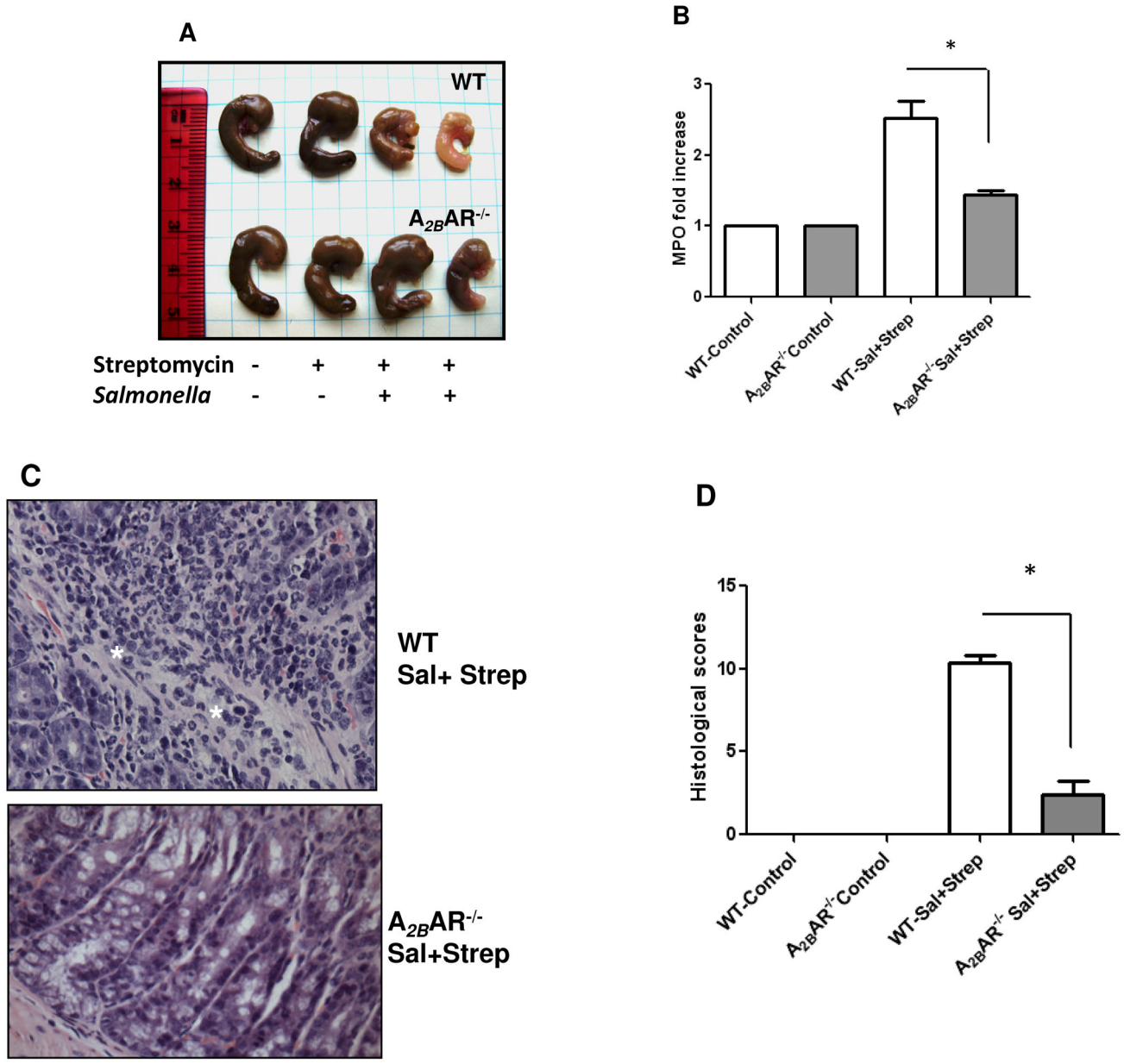
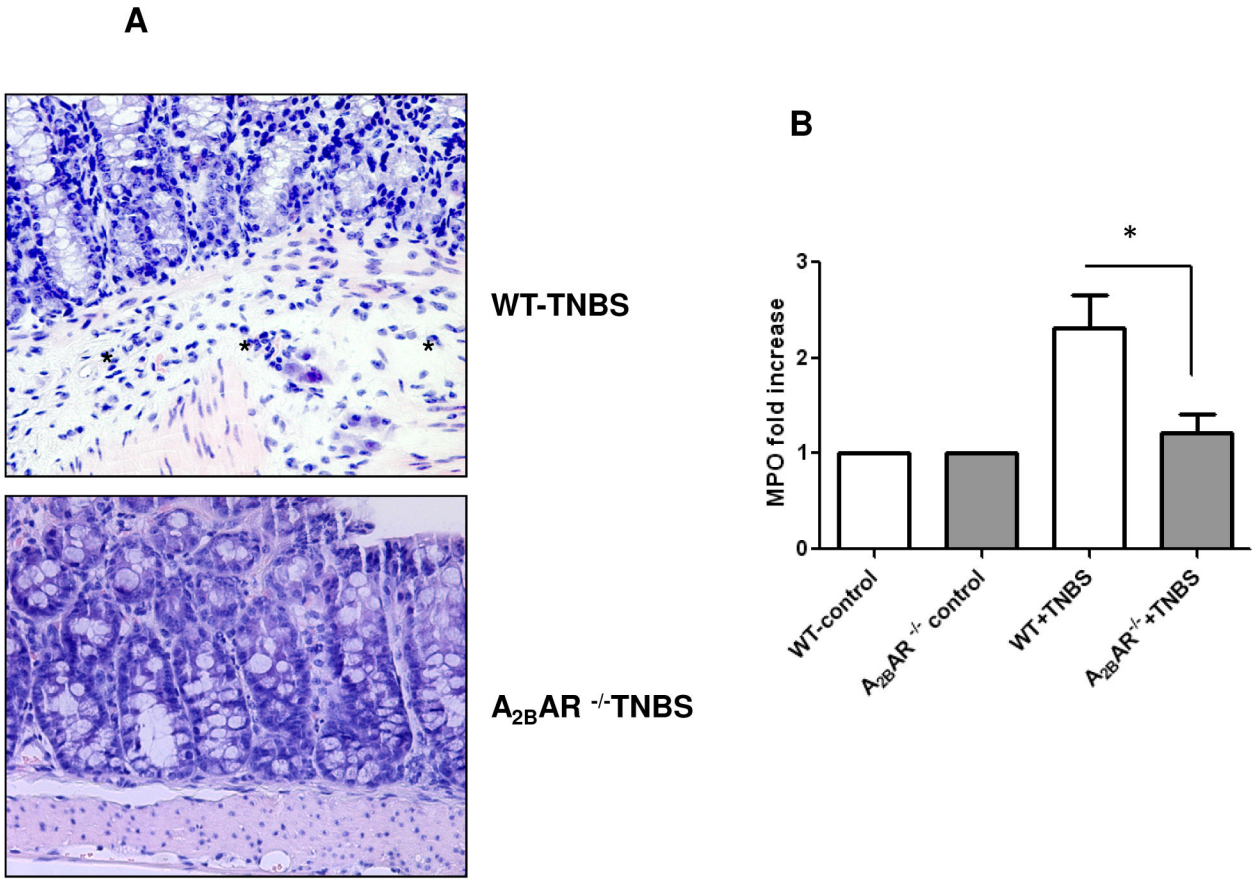


Figure 4. $A_{2B}AR^{-/-}$ mice are protected from oral *Salmonella Typhimurium*–induced colitis

Mice were pretreated with streptomycin (Strep) before the administration of *S typhimurium*, (Sal) and were sacrificed 48 hours after the administration of *S .typhimurium*. **A**. Cecum was dissected out from these animals and photographed. **B**. Cecum samples were processed for myeloperoxidase assay. Results are expressed as Mean±SE, n=6,*p<0.02. **C**. Representative histological sections of cecum from each group are shown. A predominant neutrophilic infiltrate is shown (*). **D**. Histological score. Bar graphs represent Mean±SE, *p< 0.001, n=6.



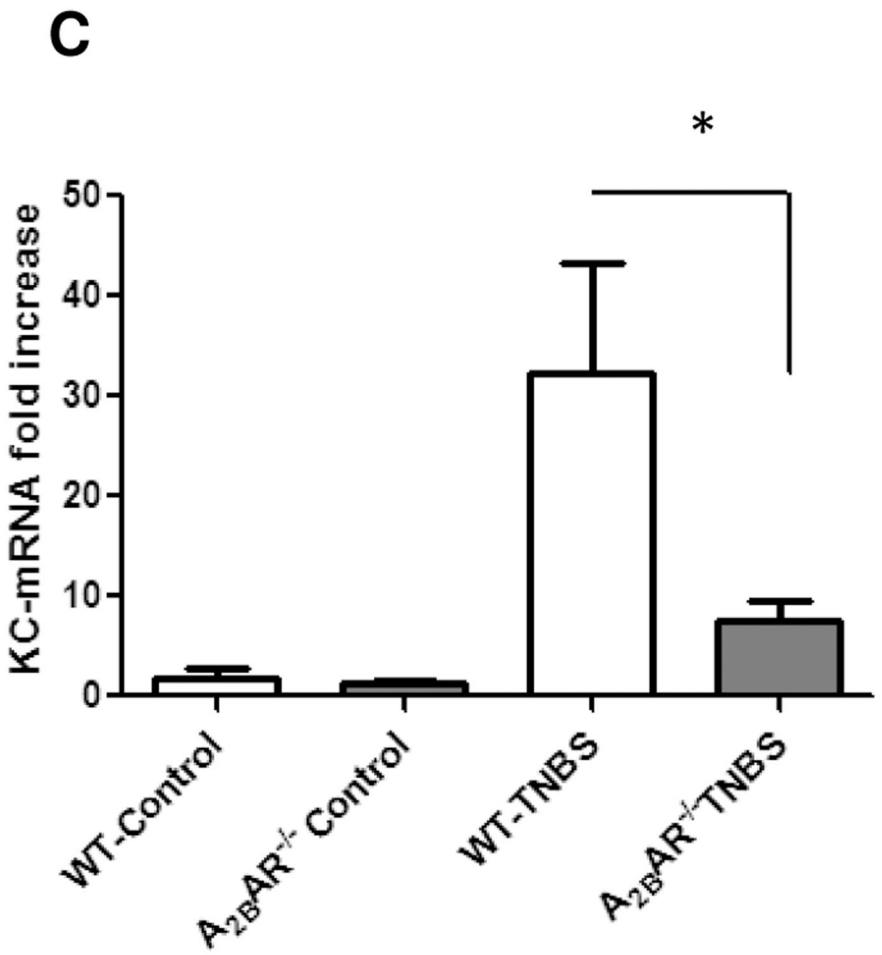


Figure 5. A_{2B}AR receptor$^{-/-}$ mice are resistant to the development of TNBS–induced colitis

Both WT and $A_{2B}AR^{-/-}$ mice were administered ethanol or TNBS (150 mg/kg body weight) intrarectally. **A**. Representative histological sections of colon from each group are shown (n=5), * indicates neutrophil infiltrates. **B**. Myeloperoxidase activity is represented as fold increase over control. **C**. mRNA levels of KC were determined by real time RT-PCR. Data is represented as Mean±SE, n= 5.

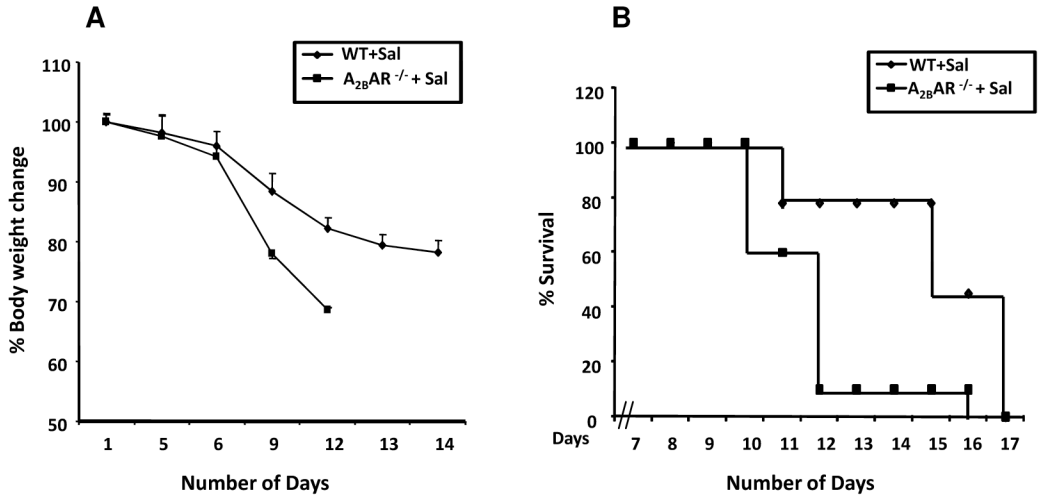


Figure 6. $A_{2B}AR$ is not required for systemic *S. typhimurium* sepsis

WT and $A_{2B}AR^{-/-}$ mice were administered *S. typhimurium* (SL3201; 10^4 colony-forming units per mouse, gavage). Mice were weighed daily and were observed for clinical signs of sepsis and for mortality, n=10.

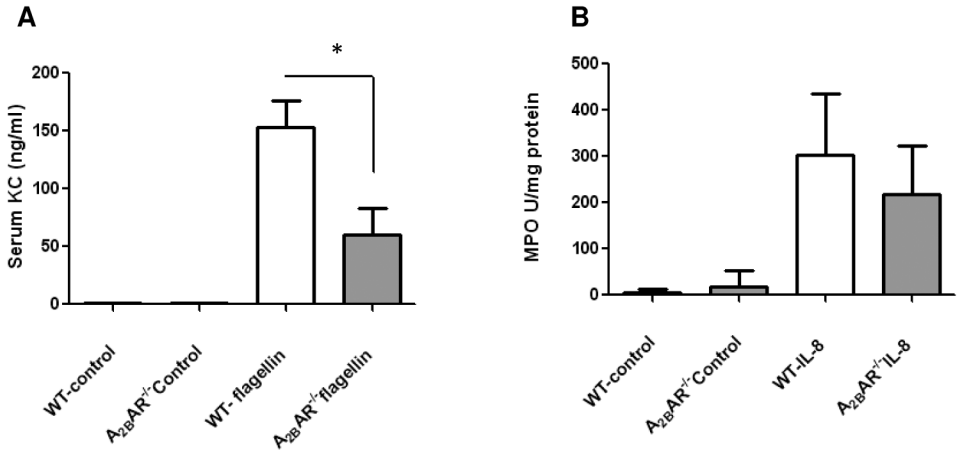


Figure 7. Flagellin-induced KC levels were attenuated in $A_{2B}AR^{-/-}$ mice

WT and $A_{2B}AR^{-/-}$ mice were injected with vehicle or flagellin intraperitoneally. Serum samples were collected 2 hours after administration. **A**. KC Mean±SE. **B. Intraperitoneal administration of IL-8 rescued neutrophil migration in $A_{2B}AR^{-/-}$ mice:** WT and $A_{2B}AR^{-/-}$ mice received vehicle or IL-8 (1μg/mouse) intraperitoneally. Peritoneal fluid was collected 4 hours after administration and processed for MPO. Data represents Mean±SE, n=6, P<0.63.