A_{2A} Adenosine Receptor Activation Improves Survival in Mouse Models of Endotoxemia and Sepsis

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Background. Sepsis is currently treated with antibiotics and various adjunctive therapies that are not very effective.

Methods. Mouse survival (4–5 days) and peritoneal and blood bacteria counts were determined after challenge with intraperitoneal lipopolysaccharide (LPS) or live *Escherichia coli.*

Results. The A_{2A} adenosine receptor (AR) agonist 4-{3-[6-amino-9-(5-ethylcarbamoyl-3, 4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl}-cyclohexanecarboxylic acid methyl ester (ATL146e; 0.05–50 μg/kg) protected mice from challenge with LPS, and protection occurred when treatment was delayed up to 24 h after challenge. Deletion of the A_{2A} AR gene, *Adora2a,* inhibited protection by ATL146e. A putative A_3AR agonist, N^6-3-iodobenzyladenosine-5′-*N*-methyluronamide (IB-MECA; 500 μg/kg but not 5 or 50 μg/kg) protected mice from challenge with LPS. The protective effects of both ATL146e and IB-MECA were counteracted by the A_{2A} AR selective antagonist 4-(2-[7-amino-2-[2-furyl][1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]ethyl)-phenol. In the live *E. coli* model, treatment with ATL146e (50 μg/kg initiated 8 h after infection) increased survival in mice treated with ceftriaxone (5 days) from 40% to 100%. Treatment with ATL146e did not affect peritoneal numbers of live *E. coli* at the time of death or 120 h after infection but did increase numbers of peritoneal neutrophils and decreased the number of live *E. coli* in blood.

Conclusions. AR agonists increase mouse survival in endotoxemia and sepsis via A_{2A} AR–mediated mechanisms and reduce the number of live bacteria in blood.

For the past 20 years, the incidence of sepsis (defined as a systemic inflammatory response to infection) [1] has been rising in the United States. There are ~750,000 new cases of sepsis annually, with a crude mortality of ~30% [2]. Gram-negative organisms account for >50% of these infections, and septic shock develops in half of the gram-negative infections. Mortality in the patients with septic shock is 92,000 deaths/year in the United States [3].

Sepsis initiates a sequence of inflammatory events that activate the immune system but may produce tissue damage to the host. Microbial factors, such as lipopolysaccharide (LPS), that are derived from gram-negative bacteria cause the release of host mediators, including chemokines (e.g., interleukin [IL]–8), pro-inflammatory cytokines (e.g., tumor necrosis factor [TNF]–α, IL-1ß, IL-6, and IL-12), anti-inflammatory cytokines (e.g., IL-1 receptor antagonist and IL-10) [1, 4], eicosinoids, reactive oxygen species, and nitric oxide [1, 5]. Severe sepsis and sepsis syndrome cause activated phagocytes and their products to accumulate in the intravascular space and trigger septic shock, which results in tissue injury and/or death. Another manifestation of severe sepsis is that activated neutrophils accumulate in peripheral uninfected tissues. This can cause acute renal failure, myocardial depression, encephalopathy, liver failure, acute respiratory distress syndrome, and disseminated intravascular coagulopathy [1]. In addition, excessive host release of TNF can result in counterproductive firm adhesion of leuko-

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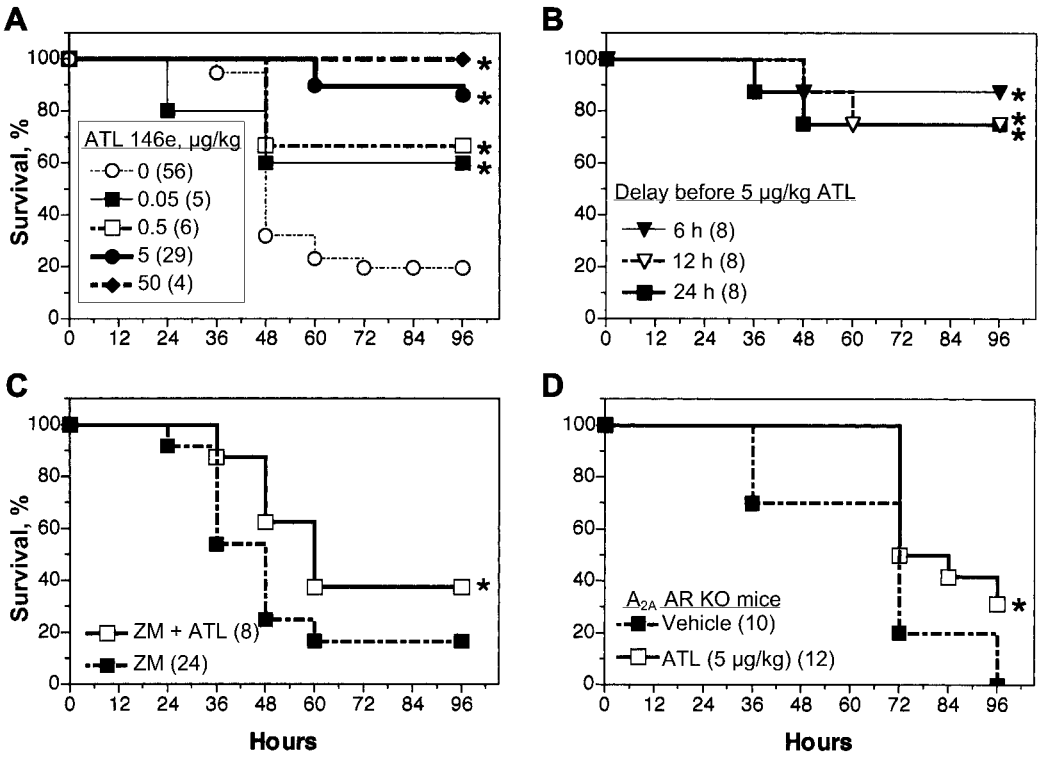

Figure. 1. Treatment with ATL146e (ATL) and decreased lipopolysaccharide (LPS)–induced mouse mortality via A_{2A} adenosine receptor (AR)–mediated mechanisms. Female C57Bl/6 mice (~20 g) were injected intraperitoneally (ip) with LPS from *Escherichia coli* (O26:B6; 12.5 mg/kg). *A,* One hour before challenge with LPS and at 6-h intervals, vehicle or ATL was injected (0.05–50 µg/kg) ip for a total of 8 doses/48 h. ATL protected the mice, compared with LPS-challenged mice in the absence of ATL (*$P < .05$). *B,* Treatment with ATL (5 µg/kg) was delayed 6–24 h after challenge with LPS. ATL protected the mice, compared with LPS-challenged mice in the absence of ATL (*$P < .05$). *C,* Selective A_{2A} AR antagonist ZM241385 (ZM) decreases ATL protection. Mice were treated with ATL (5 µg/kg) as in panel *A* and were also treated ip with ZM (3.4 µg/mL) on the same schedule as ATL dosing. ZM blocked ATL protection, compared with treatment with ATL, in the absence of ZM (*$P < .05$). *D,* ATL did not increase survival after challenge with LPS in mice that did not have the A_{2A} AR gene. The A_{2A} AR knockout (KO) mice were challenged with LPS and treated as described in panel *A,* with or without ATL (5 µg/kg). ATL did not provide as much protection in these mice as it did in wild-type mice (*$P < .05$). Nos. in parentheses are no. of mice per treatment.

cytes (including neutrophils) and decreased neutrophil-directed migration [6], which may limit the host's ability to clear or contain an infection.

Data obtained on selective adenosine receptor (AR) ligands and mice lacking *Adora2a,* the A_{2A} AR gene, have indicated that A_{2A} AR activation produces anti-inflammatory tissue-protective actions during endotoxemia. The activation of A_{2A} ARs by endogenous adenosine alters host release of cytokines in LPS-challenged mice. Serum concentrations of TNF, IL-6, and IL-12p40 are elevated by LPS more in *Adora2a* knockout mice than in wild-type (wt) mice [7]. Selective A_{2A} AR agonists reduce the extravasation of neutrophils into LPS-challenged tissues in animal models of gram-negative bacterial meningitis [8] and septic arthritis [9]. A_{2A} AR agonists decrease the serum concentration of TNF in LPS-challenged mice [10] and inhibit LPS-induced release of IL-12 and TNF from isolated mouse macrophages [11]. In addition, A_{2A} AR agonists counteract the effects of bacterial products to activate phagocyte function. They decrease the release of oxidative and nonoxidative products from activated neutrophils [12–15] and reduce the expression of adhesion molecules on activated neutrophils and on the vascular endothelium [16–18]. A_{2A} agonists decrease neutrophil adhesion to biological surfaces [19, 20] and promote chemotaxis [21, 22]. In sum, pharmacological and genetic data indicate that the activation of A_{2A} ARs inhibits LPS-induced inflammation by actions on many different cell types.

The treatment of LPS-challenged mice with a high dose (500 µg/kg) of N^6-3-iodobenzyladenosine-5′-*N*-methyluronamide (IB-MECA) increases mouse survival [23]. Although IB-MECA has greater in vitro binding affinity to A_3 ARs than to other AR subtypes, we have recently shown that its selectivity over G-protein–coupled binding to A_{2A} receptors (5-fold) is less than previously thought. By contrast, ATL146e [24] (4-{3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl}-cyclohexanecarboxylic acid methyl ester) is 225-fold more selective for A_{2A} binding than for binding to A_3 receptors [25]. These data raise the possibility that

some of the effects of IB-MECA that were previously attributed to binding to A_3 ARs may be mediated by A_{2A} ARs.

The goal of the present study was to determine whether A_{2A} AR agonists can increase the survival of mice exposed to LPS or to live *Escherichia coli.* To assess the clinical significance of our findings, in some experiments, the administration of ATL146e, with or without antibiotic treatment, was delayed for 8–24 h. On the basis of pharmacological data and by use of *Adora2a* knockout mice, we show markedly enhanced survival caused by ATL146e and IB-MECA, which is consistent with a primary role for A_{2A} AR activation.

MATERIALS AND METHODS

Materials. Ketamine and xylazine were purchased from Vedco. *E. coli* O26:B6–derived LPS and tryptic soy broth were purchased from Difco Laboratories. ATL146e [24] was supplied by Adenosine Therapeutics. ZM241385 [26] was a gift from Simon Poucher at AstraZeneca Pharmaceuticals. IB-MECA was purchased from Sigma Chemical. Mice lacking *Adora2a* [27] were provided by Jiang-Fan Chen (Harvard University, Boston, MA).

Mouse model of endotoxemia. A marker-assisted breeding strategy was used to move the mutant *Adora2* allele onto a C57BL/6 background (99.3% homozygosity). The A_{2A} AR knockout locus was moved onto a C57Bl/6 background using 5 generations of marker-assisted selection. A_{2A} knockout mice (B6;129P-adora2a^{tm1jfc}) were bred to a C57BL/6 background by use of a speed congenic method. In brief, 5 mice with disrupted *Adora2a(1)* were received from Dr. Jiang-Fan Chen (Harvard University) after several generations of inbreeding from (129-Adora2a^{tm1jfc} X C57BL/6) F1 founder mice [27]. We initiated a new line using a founder with a maximal number of informative loci (B6/129 or 129/129). The new mapping panel consisted of 96 microsatellites covering every chromosome except Y at an average distance of 15 cM. This strategy was used effectively to move the mutant *Adora2a* allele onto the C57BL/6 background. By generation 5, the only detectable 129 contamination surrounded the *Adora2a* locus, which we mapped during this gene transfer onto chromosome 10, in a region of conserved synteny with the human locus. At this point, we could detect the 129 genome only in a region between D10Mit31 and D10Mit42 (8 cM).

Female mice (C57Bl/6; Hilltop Lab Animals) or congenic C57Bl/6 knockout mice, aged 8–10 weeks, were anesthetized once with ketamine (100 mg/kg) and xylazine (5 mg/kg) before being challenged intraperitoneally (ip) with LPS; then they were injected ip with O26:B6 LPS (12.5 mg/kg) derived from *E. coli.* One hour before injection with LPS, and every 6 h thereafter, bolus doses of ATL146e or IB-MECA (0, 0.05, 0.5, 5, 50, or 500 μg/kg) were injected into the peritoneal cavity, for a total of 8 doses spanning 48 h. The control mice received an equal volume (0.5–1 mL) of PBS vehicle or the selective A_{2A} AR antagonist 4-(2-[7-amino-2-[2-furyl][1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]ethyl)-phenol (ZM241385) at a dose (3.4 μg/kg) equimolar to 5 μg/kg ATL146e. Survival was monitored over 4 days. All animal studies were approved by the University of Virginia Institutional Review Board and conducted according to Declaration of Helsinki principles.

Live* E. coli *sepsis model. *E. coli* O26:B6 (ATCC 12795) were grown overnight (at 37°C) in tryptic soy broth, centrifuged at 3000 *g* for 20 min, washed 3 times, and resuspended in PBS. The concentration of bacteria was adjusted to the McFarland standard 2 by use of a Vitek colorimeter (bioMérieux Vitek) and then further diluted. Mice were anesthetized and inoculated ip with live *E. coli* (O26:B6; 2×10^7 cfu) in sterile PBS (1 mL), to induce sepsis. Eight hours after the inoculation, the mice were given ip a dose of ceftriaxone (25 mg/kg), with or without ATL146e (50 μg/kg), administered every 6 h, for a total of 8 doses. PBS was injected into the groups given only ceftriaxone and into the control mice. All mice were closely observed for 5 days.

Cardiac puncture was used to collect 0.1–0.2 mL of blood only from mice that were identified within 30 min of dying and from all mice that survived for the term of the experiment (5 days). At the end of the study, the mice that had survived were anesthetized with ketamine and xylazine and killed by cervical dislocation. The peritoneum was washed with 2 mL of ice-cold PBS, and the peritoneal fluid was withdrawn. White blood cell (WBC) concentrations in the peritoneal fluid were measured by use of a hemocytometer, after dilution with a unopipette (Becton Dickinson). All samples of blood and peritoneal fluid were cultured. Quantitative cultures were done by serial dilution, and each dilution was streaked onto a blood agar plate and allowed to grow overnight, to determine the number of colony-forming units in the original samples.

Statistical analysis. Statistical comparisons were done by 1-way analysis of variance with Tukey's multiple comparison test. Survival data were plotted, and the survival curves were compared (log-rank test) using PRISM software (GraphPad). Data are displayed as mean ± SE, unless otherwise stated. Differences were considered to be significant at $P < .05$. All experiments were repeated at least twice.

RESULTS

Mouse endotoxemia model. We examined the effect of the A_{2A} AR agonist ATL146e to increase survival in mice exposed to LPS derived from *E. coli* O26:B6. The 96-h survival rate of mice injected ip with 12.5 mg/kg LPS was ~20%. ATL146e (0.05–50 μg/kg, 8 ip doses at 6-h intervals, beginning 1 h before challenge with LPS) increased the survival of LPS-challenged mice. There was >50% survival with 0.050 μg/kg ATL146e and

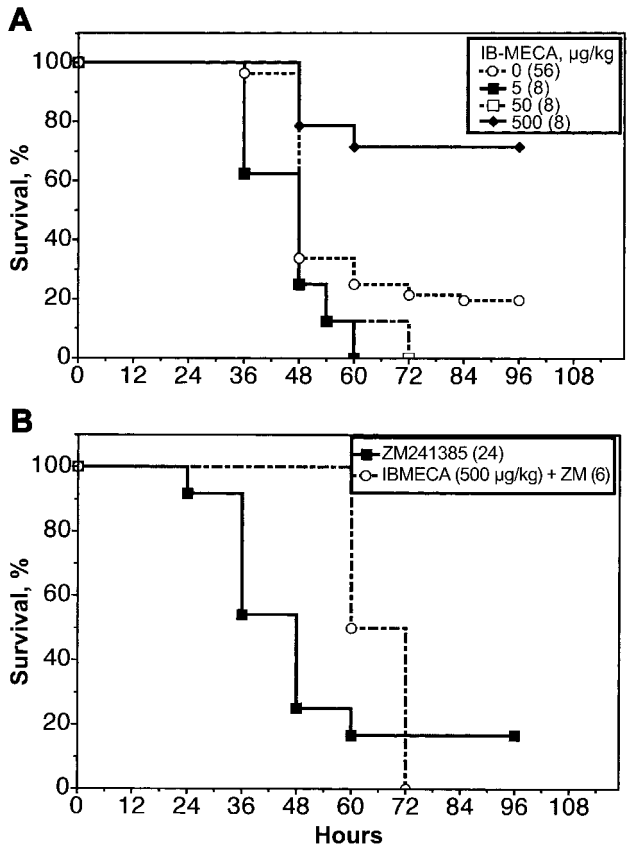

Figure. 2. Treatment with N^6-3-iodobenzyladenosine-5′-*N*-methyluronamide (IB-MECA) and decreased lipopolysaccharide (LPS)–induced mouse mortality via A_{2A} adenosine receptor (AR)–mediated mechanisms. Female C57Bl/6 mice (~20 g) were injected intraperitoneally (ip) with LPS from *Escherichia coli* (026:B6; 12.5 mg/kg). *A,* One hour before challenge with LPS and at 6-h intervals, vehicle or IB-MECA was injected (5–500 μg/kg) ip for a total of 8 doses/48 h. IB-MECA (500 μg/mL) protected mice, compared with LPS-challenged mice in the absence of IB-MECA (*$P <$ .05). *B,* Selective A_{2A} AR antagonist ZM241385 (ZM) decreased protection provided by IB-MECA. Mice were treated with IB-MECA (500 μg/kg) as in panel *A* and were also treated ip with ZM (3.4 μg/mL) on the same schedule as IB-MECA dosing. ZM blocked IB-MECA protection, compared with treatment with IB-MECA in the absence of ZM ($P <$ .05). Nos. in parentheses are no. of mice per treatment.

up to 100% survival with 50 μg/kg ATL146e (figure 1*A*). Treatment with 5 μg/kg ATL146e after challenge with LPS could be delayed for 6 h with no change in protection and for 12 or 24 h with little change in the degree of protection (figure 1*B*). The injection of a selective A_{2A} AR antagonist, ZM241385 (3.4 μg/kg, the molar equivalent of 5 μg/kg ATL146e), did not significantly increase mouse mortality in the absence of an AR agonist, but it greatly attenuated the protective effect of ATL146e at the 5 μg/kg dose ($P = .0008$; figure 1*C*).

As has been reported elsewhere [23], a high dose of IB-MECA (500 μg/kg) increases the survival of mice after challenge with LPS (71% survival after 96 h; $P = .0005$) (figure 2*A*). However, in contrast to treatment with ATL146e, lower doses of IB-MECA (5 or 50 μg/kg) were not protective—that is, no mice survived after 60 and 72 h, respectively (figure 2*A*). The A_{2A} AR antagonist, ZM241385 (3.4 μg/kg) counteracted the protective effect of IB-MECA (500 μg/kg; $P = .04$; figure 2*B*). In the presence of ZM241385 and IB-MECA, all of the mice exposed to LPS died within 72 h. This is consistent with a lack of protective effect of IB-MECA mediated by A_3 ARs.

We next examined the effects of ATL146e on mice lacking *Adora2a.* Although these mice had sensitivity to LPS comparable to that of wt C57Bl/6 mice, ATL146e had only a very modest effect to protect A_{2A} AR knockout mice from challenge with LPS (figure 1*D*).

Mouse* E. coli *sepsis model. Having established that ATL146e protects mice from LPS predominantly by an A_{2A} AR–dependent mechanism, we next sought to determine whether mice infected with live bacteria could also be protected. Mice were injected ip with 20 million live *E. coli* (n = 8–25/group). Subsets of mice were treated with ATL146e alone, the antibiotic ceftriaxone alone, or a combination of ceftriaxone and ATL146e. Ceftriaxone was administered at a single dose of 25 mg/kg 8 h after the infection of mice with *E. coli.* ATL146e was dosed ip, starting 8 h after *E. coli* injection, 8 times at 6-h intervals with 50 μg/kg. This is the same dose that produced 100% survival in the endotoxemia model (figure 1*A*). Figure 3 shows that the survival of mice treated with the combination of ceftriaxone and ATL146e was 100%, compared with 8% of mice that received the vehicle control and 37.5% of mice that

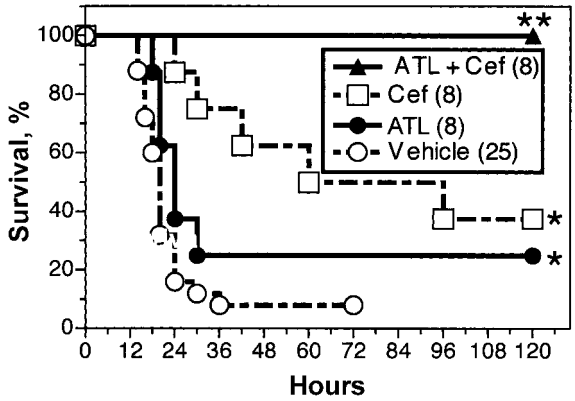

Figure. 3. Treatment with ATL146e (ATL) in mice challenged with live *Escherichia coli.* Mice were injected intraperitoneally (ip) with 20 million live *E. coli.* Mice were treated with ATL, the antibiotic ceftriaxone (cef), or a combination of cef and ATL. Cef was administered at a single dose of 25 mg/kg 8 h after the inoculation of mice with *E. coli.* ATL was dosed ip 8 h after the *E. coli* injection, 8 times at 6-h intervals with 50 μg/kg. ATL or cef alone protected the mice, compared with untreated mice (*$P <$ .05). Treatment with ATL plus cef protected mice better than treatment with cef alone (**$P <$ .05). Nos. in parentheses are no. of mice per treatment.

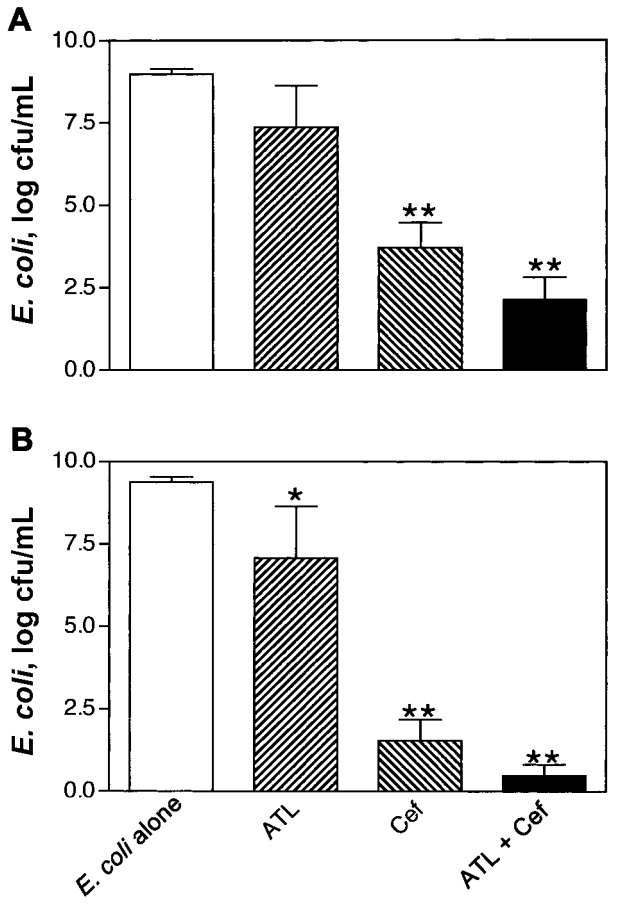

Figure. 4. Treatment with ATL146e (ATL) and bacterial survival in the peritoneum vs. in the blood. Live *E. coli* organisms in a peritoneal wash *(A)* and blood *(B)* were done within 30 min of death or 120 h after infection and were counted by use of serial dilution and plate-count methods, in the mice from figure 3. ATL (50 μg/mL) decreased the no. of live bacteria in blood (*$P < .05$). Treatment with ceftriaxone (cef) and treatment with cef plus ATL decreased the no. of live bacteria in the peritoneum and blood (**$P < .001$; $n = 8$–23 mice/treatment).

received ceftriaxone alone ($P < .0001$ vs. control and $P < .009$ vs. ceftriaxone).

In mice infected with *E. coli,* treatment with ceftriaxone markedly reduced the live bacteria concentration in the peritoneal wash measured 120 h after the initial infection or within 30 min of the time of death ($P < .001$; figure 4*A*). ATL146e did not significantly influence bacteria concentrations in the peritoneum, but treatment with ATL146e did reduce the number of live bacteria in blood, with or without concomitant treatment with ceftriaxone (figure 4*B*). These data indicate that treatment with ATL146e does not interfere with bacterial killing by the immune system and does promote the containment of infection. In mice that received the combination of ceftriaxone and ATL146e, the number of WBCs (>95% neutrophils according to the results of hemocytometer examination) in the peritoneal fluid was significantly higher than that in untreated animals ($P < .01$; figure 5).

DISCUSSION

Endogenous adenosine or synthetic AR agonists have been shown to decrease morbidity and mortality in animal models of endotoxemia and sepsis [28–31]. Of the 4 adenosine receptor subtypes, protection has been attributed primarily to the activation of either A_{2A} [10, 32] or A_3 [23] ARs. The results of the present study (figures 1 and 2) implicate agonist protection via A_{2A}, and not A_3, AR–mediated mechanisms. This evidence includes the following: (1) the A_3 AR–selective agonist IB-MECA is protective only at the relatively high dose of 500 μg/kg; (2) in contrast, the highly selective A_{2A} AR agonist ATL146e causes a dose-dependent increase in survival at much lower doses (0.05–50 μg/kg); (3) the benefits of treatment with both IB-MECA and ATL146e are blocked by the highly selective A_{2A} AR antagonist ZM241385; and (4) the protective effects of ATL146e are greatly attenuated in mice that lack the A_{2A} AR gene.

A_{2A} AR agonists have effects on multiple cell types that are involved in inflammation and coagulation. On platelets, agonist binding to A_{2A} ARs inhibits aggregation [33], which has the potential of preventing sepsis-induced disseminated intravascular coagulation [34]. In neutrophils, adenosine inhibits adhesion and oxidative activity [15, 35], and, on monocytes and macrophages, it decreases the release of proinflammatory cytokines [36–39]. The A_{2A} AR antagonist ZM241385 counteracts the effects of the agonists 2-[*p*-(carboxyethyl)-phenyl-ethyl-

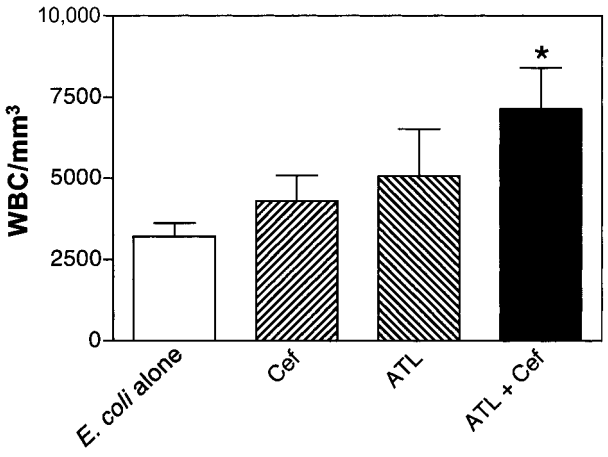

Figure 5. Treatment with ATL146e (ATL; 50 μg/kg) and ceftriaxone (Cef; 25 mg/kg) increased white blood cell (WBC) migration into the peritoneum. Nos. of WBCs were determined by hemocytometer count in the peritoneal wash within 30 min of death or 120 h after infection, in the mice from figure 3. Treatment with ATL (50 μg/mL) plus cef increased the no. of WBCs in the peritoneum, compared with untreated, *Escherichia coli*–infected mice (*$P < .05$; $n = 8$–23 mice/treatment).

amino]-5′-N-ethylcarboxamidoadenosine (CGS21680) and IB-MECA to inhibit LPS-stimulated release of TNF from human monocytes [38] and also reverses the protective effects of the adenosine uptake inhibitor 3-[1-(6,7-diethoxy-2-morpholinoquinazolin-4-yl)piperidin-4-yl]-1,6-dimethyl-2,4(1*H*,3*H*)-quinazolinedione hydrochloride (KF24345) in vivo [32].

Adenosine and rapidly metabolized adenine nucleotides are released from injured tissues [40, 41]. In addition, factors that are elevated during endotoxemia and sepsis, including LPS and cytokines (e.g., TNF, interferon-γ, and IL-1), trigger the induction of mRNA and protein for A_{2A} ARs (but not A_3 ARs) [42, 43]. This raises the possibility that the induction of A_{2A} AR in monocytes and macrophages increases their susceptibility to A_{2A} AR–mediated negative-feedback control after exposure to LPS or live bacteria.

ZM241385 is a selective A_{2A} AR antagonist (the inhibition binding constants [K_i] for A_{2A}, A_{2B}, A_1, and A_3 ARs are 1.9, 33, 250, and 774 nmol/L, respectively [15]). At doses up to 3 μg/kg, the cardiovascular effects of ZM241385 are entirely mediated by A_{2A} AR blockage [44]. ZM241385 has been widely used and is devoid of any intrinsic toxicity in mouse models [45]. In the present experimental model, wt mice challenged with LPS had mortality rates that were comparable to those for challenged mice treated with the selective A_{2A} AR antagonist ZM241385 (figure 1*C*) and mice that lacked the A_{2A} AR gene (figure 1*D*). These data suggest that the increased levels of endogenous adenosine generated by endotoxemia are not always effective at enhancing survival. Also, the administration of exogenous adenosine (100 mg/kg ip adenosine) does not improve survival in mice challenged with LPS [28], most likely because of the short half-life of adenosine in blood [46]. Because levels of adenosine increase within stressed tissues, several strategies have been tried to protect animals from endotoxemia by inhibiting the metabolism of endogenous adenosine. These include blocking the uptake of adenosine [32] and inhibiting adenosine deaminase [29–31]. Both of these strategies improve survival and/or decrease morbidity in endotoxemia [32] or sepsis models, and the effects of increasing endogenous adenosine on survival are reversed when LPS-challenged animals were given AR antagonists [30], including ZM241385. Hence, although endogenous adenosine is not consistently sufficient to protect animals from challenge with LPS, increasing the amount of endogenous adenosine available to A_{2A} AR by inhibiting its uptake or metabolism is protective.

The results of past clinical trials, as well as current and future strategies for treating sepsis, have recently been reviewed by Hotchkiss and Karl [47] and by Riedemann et al. [48]. Attempts to increase survival by neutralizing LPS have been unsuccessful. Other strategies may have failed clinically because they had only small time windows for effectiveness. For example, treatments that neutralize host proinflammatory cytokines need to be administered early after infection, and they appear not to be clinically practical. These treatments may even be counterproductive during later stages of sepsis associated with immunosuppression. By comparison, treatment with A_{2A} AR agonists may be particularly effective because these receptors are found on many cell types (including neutrophils, monocytes, macrophages, platelets, and vascular endothelial cells), and they are induced on cells after exposure to LPS. This may account for the unusual effectiveness of these agonists that is seen even when treatment is delayed for up to 24 h after endotoxemia (figure 1*B*) and 8 h after infection with live *E. coli* (figure 3).

At high concentrations, A_{2A} AR agonists have been found to inhibit in vitro neutrophil bactericidal function [49]. Thus, in sepsis, A_{2A} AR agonists may have both beneficial and harmful immunosuppressive activities. Our finding that treatment with ATL146e is very protective in the live *E. coli* sepsis model (figure 3) indicates that, in this model, the beneficial effects of an A_{2A} AR agonist outweigh its potentially harmful effects to inhibit the immune system. In fact, we observed a decrease in the number of *E. coli* colony-forming units in blood with treatment with ATL146e (figure 4). Concomitant with the decrease the live concentration of bacteria in blood in mice given ATL146e and ceftriaxone, we observed an increase in numbers of neutrophils in the peritoneum (the site of initial infection; figure 5). This apparent paradox of A_{2A} AR agonists decreasing neutrophil extravasation at sites of challenge with purified LPS [8, 9] but increasing neutrophil migration into bacterially infected tissues (figure 5) can be explained by the effects of A_{2A} AR agonists on neutrophil adhesion. When neutrophils are moderately activated, A_{2A} AR activation can reduce extravasation by reducing adherence. On the other hand, when neutrophils are firmly adhered, A_{2A} AR activation may increase extravasation by counteracting firm adherence. Thus, depending on the degree of neutrophil activation, A_{2A} AR agonists can either enhance or inhibit extravasation. In addition to LPS, gram-negative bacteria release potent peptide chemoattractants that chemically attract neutrophils. Although A_{2A} AR agonists inhibit neutrophil recruitment into LPS-induced inflamed tissues [8, 9], an increase in directed neutrophil infiltration to sites of bacterial infection (figure 5) may arise from the actions of A_{2A} AR agonists to promote neutrophil chemotaxis [21, 22]. Enhanced neutrophil migration to the initial infection has the potential to prevent the spread of infection to the blood and other organs.

The anti-inflammatory effects of A_{2A} agonists may be particularly useful for treatment in bacterial diseases when antibiotics have been administered. In these instances, antibiotics can rapidly kill large numbers of bacteria, augmenting inflammation through the rupture and dissemination of bacterial cell-wall products. We speculate that the protective mechanism(s) of adenosine and A_{2A} AR agonists in endotoxemia and sepsis arise from A_{2A} AR activation and may be particularly effective

because they produce anti-inflammatory actions that reduce tissue destruction while promoting neutrophil migration to sites of infection.

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