Inhibiting adenosine deaminase modulates the systemic inflammatory response syndrome in endotoxemia and sepsis

SIMON ADANIN,1 IGOR V. YALOVETSKIY,1 BETH A. NARDULLI,1 ALBERT D. SAM II,2 ZIVOJN S. JONJEV,1 AND WILLIAM R. LAW1,2

Departments of 1Physiology and Biophysics and 2Surgery, University of Illinois College of Medicine, Chicago, Illinois 60612

Received 3 July 2001; accepted in final form 9 January 2002

Inhibiting adenosine deaminase modulates the systemic inflammatory response syndrome in endotoxemia and sepsis.

Am J Physiol Regulatory Integrative Comp Physiol 282: R1324–R1332, 2002; 10.1152/ajpregu.00373.2001.—By pharmacological manipulation of endogenous adenosine, using chemically distinct methods, we tested the hypothesis that endogenous adenosine tempers proinflammatory cytokine responses and oxyradical-mediated tissue damage during endotoxemia and sepsis. Rats were pretreated with varying doses of pentostatin (PNT; adenosine deaminase inhibitor) or 8-sulfophenyltheophylline (8-SPT; adenosine receptor antagonist) and then received either E. coli endotoxin (lipopolysaccharide; 0.01 or 2.0 mg/kg) or a slurry of cecal matter in 5% dextrose in water (200 mg/kg). Resultant levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-10 were measured in serum and in liver and spleen. Untreated, 2 mg/kg lipopolysaccharide elevated serum TNF-α, IL-1β, and IL-10. PNT dose dependently attenuated, without ablating, the elevation in serum TNF-α and IL-1β and raised liver and spleen IL-10. PNT also attenuated elevation of TNF-α in serum, liver, and spleen at 4 and 24 h after sepsis induction, and 8-SPT resulted in higher proinflammatory cytokines. Modulating endogenous adenosine was also effective in exacerbated (8-SPT) or diminished (PNT) tissue peroxidation. Survival from sepsis was also improved when PNT was used as a posttreatment. These data indicate that endogenous adenosine is an important modulatory component of systemic inflammatory response syndromes. These data also indicate that inhibition of adenosine deaminase may be a novel and viable therapeutic approach to managing the systemic inflammatory response syndrome without ablating important physiological functions.

Our laboratory has demonstrated that endogenous adenosine is involved in maintaining elevated resting hepatosplanchnic (23, 24) and skeletal muscle perfusion in sepsis (24), in part via stimulation of nitric oxide synthase (34, 36). It is not clear whether adenosine’s role as an endogenous modulator of responses to inflammatory processes can be exploited to better manage systemic inflammatory response syndromes (SIRS).

Most of the work describing the immunomodulating abilities of adenosine have been performed in vitro. Adenosine has been reported to inhibit β-galactosidase secretion (30) and chemiluminescence (15) from zymosan particle-stimulated mouse peritoneal macrophages. Adenosine has also been shown to inhibit tumor necrosis factor (TNF)-α produced by monocytes in response to endotoxin [lipopolysaccharide (LPS)] (11) and reduce leukocyte accumulation and TNF-α production after carrageenan stimulation (8). However, these in vitro findings cannot be easily extrapolated to the complex in vivo immune response associated with sepsis. Firestein et al. (12) explored this question by using GP-1–515, an adenosine kinase inhibitor, a proprietary compound that purportedly inhibited adenosine kinase. This compound was able to inhibit LPS-mediated increases in TNF-α and improve survival, effects that were blocked with adenosine receptor antagonism. However, effects were only seen in the presence of GP-1–515, which was structurally similar to adenosine. Thus it is not clear whether endogenous adenosine is a significant signaling molecule for SIRS.

The following experiments were designed to test the hypothesis that endogenous adenosine, produced as a consequence of a septic challenge in vivo, serves to directly temper proximal cytokine responses and tissue products of lipid peroxidation, as measured by thiobarbituric acid-reactive substances (TBARS). Specifically, two chemically distinct approaches were used to test the hypothesis. First, the adenosine receptor antagonist 8-sulfophenyltheophylline (8-SPT) was used to determine whether blockade of adenosine receptors would exacerbate early proinflammatory cytokine and TBARS concentrations after a septic challenge. Second, the adenosine deaminase inhibitor 2-deoxycoformycin was used to determine whether reducing the degradation of endogenous adenosine would amplify the tempering influences of adenosine on early proinflammatory cytokine and TBARS concentrations after a septic challenge. Haskó et al. (13) reported that...
adeno sine-stimulated release of the anti-inflammatory cytokine interleukin (IL)-10 is partially responsible for the ability of adenosine to regulate TNF-α release in vitro. Thus we also tested the hypothesis that endogenous adenosine has effects on IL-10 after a septic challenge in vivo similar to those reported in vitro.

METHODS

Materials

8-SPT. 8-SPT (Research Biochemicals International, Natick, MA), a nonselective (A1/A2/A3) but highly specific (no phosphodiesterase inhibition) adenosine receptor antagonist (3, 9, 14), was solubilized in sterile water to obtain an injectate volume of 1 ml/kg at a dose of 20 mg/kg every 8 h. The dose was determined based on pilot studies, which obtained and maintained complete blockade of hemodynamic effects of an exogenously administered adenosine (1 mmol/kg) bolus. 2-Deoxycoformycin. 2-Deoxycoformycin (pentostatin, Supergen, Dublin, CA), a potent inhibitor of adenosine deaminase, was solubilized in sterile water to concentrations ranging from 10⁻⁶ to 1.0 mg/ml at final injection volumes of 1 ml/kg.

Animals

The experiments reported herein were approved by the Animal Care and Use Committee of the University of Illinois and were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, revised in 1996. Male Sprague-Dawley rats (Harlan, IN), weighing 300–350 g, were housed at constant temperature with 10- and 14-h periods of light and dark exposure, respectively. Animals were allowed access to standard rat chow and water ad libitum during an acclimation period of at least 7 days before use in these experiments.

Protocol 1: Pentostatin Dose Response After LPS

To determine the optimal attenuating dose of pentostatin, we used a fixed septic challenge with Escherichia coli LPS (2 mg/kg; serotype 0127:B8, lot 10692.2JA; Difco Labs, Detroit, MI). After weighing, rats received an intraperitoneal (IP) injection of one of five doses of pentostatin or sterile water (treatment control). One hour later, rats were challenged with 2 mg/kg LPS or saline IP (challenge control). Two hours after LPS injection, rats were lightly anesthetized with isoflurane. The chest and abdomen were immediately opened, and blood was withdrawn via cardiac puncture. Blood was allowed to clot, and, after centrifugation, serum was removed and frozen at −80°C for later assay. Our laboratory has used this dose of endotoxin in previous work to create an acute, hypodynamic shock syndrome within 2 h of the endotoxin challenge (10, 17).

Protocol 2: Cecal Soilage Model of Sepsis

Protocol subgroup A. Rats were randomized to treatment (pentostatin, 8-SPT, or sterile water) and challenge groups (septic and nonseptic) before surgery. Rats were weighed and anesthetized with an IP injection of pentobarbital sodium (50 mg/kg; Abbott). Adequate anesthesia was confirmed by the absence of an interdigital pinch reflex. Rats then received either 8-SPT (20 mg/kg) or pentostatin (1 mg/kg) IP. The dose of pentostatin used was based on results from protocol 1 (above). A polyethylene catheter (Intramedic PE-50, Baxter) was inserted into the right carotid artery for blood collection.

The catheter was secured, tunneled subcutaneously to exit in the interscapular region, and flushed with heparinized saline (5 U/ml of 0.9% saline).

After catheterization, a 0.25-cm vertical midline abdominal incision was made, and a cecal slurry or 5% dextrose in water (nonseptic control) was injected in a volume of 5 ml/kg under direct vision. The cecal slurry was prepared by mixing cecal contents obtained from donor rats (euthanized with IP pentobarbital sodium; 100 mg/kg) with 5% dextrose in water to yield a concentration of 200 mg cecal material in 5 ml. The slurry was prepared fresh each day, and material from one donor rat was used within 2 h for three to five experimental animals. The incision was closed with interrupted silk sutures, and the abdomen was gently massaged to distribute the injectate. All rats were returned to individual cages with free access to food and water. Blood samples were obtained from conscious, unrestrained rats at 4 and 24 h after sepsis induction. Animals were then euthanized with an overdose of pentobarbital sodium (80 mg/kg iv) and examined for gross pathological changes. After this, the spleen and liver were removed and immediately frozen at −80°C for later assays. Blood was allowed to clot, and, after centrifugation, serum was removed and frozen at −80°C for later assay.

Our laboratory has previously shown that this model produces a hyperdynamic, normotensive, septic state by 24 h (34). In the septic groups, one rat treated with water and two treated with 8-SPT died before 24 h postinduction.

Protocol subgroup B. To determine the efficacy of pentostatin as a postinsult treatment, a more lethal challenge of cecal slurry was required to consistently obtain a significant number of deaths within 24–72 h after the insult. After insertion of jugular catheters under general pentobarbital sodium anesthesia, rats were made septic, as described above in Protocol subgroup A, with the exception that rats received 400 mg/kg cecal slurry IP. Two hours after sepsis induction, when rats begin displaying overt signs of illness (piloerection, lethargy, tachycardia, and leukopenia), each animal received either 1 ml water (vehicle; n = 13) or 1 mg/kg pentostatin (in 1 ml; n = 13) over 5 min intravenously, followed by 50 ml/kg of 0.9% normal saline for resuscitation over 20 min. The number of animals alive at 24–144 h was recorded.

Cytokine Assays

Cytokines were measured from serum or tissue by using commercially available ELISA assays with the use of rat antibodies to each specific cytokine (R&D Systems, Minneapolis, MN). Serum was assayed directly after dilution. Tissue samples were pulverized under liquid nitrogen with homogenizing buffer [10 mM Trizma·HCl, 1 mM EGTA, 350 mM sucrose, 5 mM sodium azide (NaN₃), 10 mM β-mercaptoethanol, 0.02 mM phenylmethylsulfonyl fluoride, 50 mM sodium fluoride (NaF), 1 mg/ml pepstatin, 1 mg/ml leupeptin, pH 7.5, at 4°C]. A total of 10X volume of homogenization buffer was added to the tissue sample and homogenized with an Omni Polytron homogenizer with small size tip for 3 × 10 s bursts while on ice. The homogenate was centrifuged at 100,000 g for 60 min at 4°C, and the supernatant was assayed after dilution.

TBARS Assay

TBARS were determined according to the methods of Ohkawa et al. (26) by using malondialdehyde to generate a standard curve. Samples of liver and spleen were homogenized on ice in 1.10 wt/vol of 1.15% KCl buffer containing 0.01% butylated hydroxytoluene. Samples were centrifuged at 21,000 g for 10 min at 4°C. Sample was added to 0.8%
rats 1 h before LPS with 10
after intraperitoneal (IP) LPS (2 mg/kg) administration. Pretreating
ing doses of pentostatin. Serum TNF-
polysaccharide (LPS)-challenged rats pretreated with water or vary-
played signs of acute endotoxemia, including piloerec-
attenuated, without ablating, the LPS-induced rise in serum TNF-
attenuated, without ablating, the LPS-induced rise in serum IL-1
were in parentheses. †Significantly different relative to LPS alone,
P < 0.05.
aqueous solution of thiobarbituric acid with 8.1% sodium
dodecyl sulfate and 20% acetic acid and heated at 90°C for 60
After cooling to room temperature with tap water, the
reaction mixture was centrifuged for 10 min at 1,500 g, and
absorbance of the supernatant was read at 532 nm. Protein
concentrations were measured by the bicinchoninic acid
method (Pierce, Rockford, IL).
Statistics
Data were assumed to be distributed normally. After we
tested for and ensured homogeneity of variances by Bartlett’s
test, data were analyzed by two-way ANOVA (treatments vs.
groups) followed by Student-Newman-Keuls test to identify
differences. Data are expressed as means ± SE. The α was
set at 0.05 and β at 0.2 to reject the null hypothesis.
RESULTS
Two hours after endotoxin administration, rats dis-
attenuate tumor necrosis factor-
were significantly elevated 2 h
after intraperitoneal (IP) LPS (2 mg/kg) administration. Pretreating
rats 1 h before LPS with 10-6 to 1.0 mg/kg pentostatin significantly attenuated, without ablating, the LPS-induced rise in serum TNF-
was signiﬁcantly elevated at 2 h post-LPS (Fig. 1). Pretreatment with pentostatin attenuated TNF-α concentrations in a dose-dependent manner, reaching a maximum attenuating effect at 0.1–0.5 mg/kg. This is 5- to 10-fold lower than a single-infusion pentostatin dose when used as an antineoplastic agent (4). It is important to note that pentostatin attenuated the concentrations of this proximal cytokine but was unable to ablate the response. LPS administration also resulted in significant elevation of serum IL-1β (Table 1). Pentostatin was able to attenuate IL-1 concentrations, similar to its effects on TNF-α. Both TNF-α and IL-1β were not detectable in serum from saline-challenged (non-LPS) rats, regardless of the presence or absence of pentostatin. Low concentrations of IL-10 were found in saline-challenged rats (82 ± 31 pg/ml). Significantly higher concentrations of IL-10 were measured 2 h after LPS administration (Table 1). Pentostatin had no effect on the IL-10 response to LPS at the doses that caused maximal attenuation of TNF-α and IL-1β, suggesting that the suppression of proinflammatory cyto-
kines by pentostatin was not secondary to stimulation of this anti-inflammatory cytokine. However, examination of IL-10 concentrations in the liver and spleen revealed some further elevation in LPS-induced IL-10 after treatment with pentostatin. Gross appearance in pentostatin-treated LPS rats was indistinguishable from that of saline-challenged rats. Serum TNF-α and IL-1β were below detection of the assays in saline-challenged rats.
The effects of pentostatin on serum TNF-α in re-
sponse to a much lower dose of LPS were investigated as well. The results are shown in Fig. 2. Administration of 0.01 mg/kg ip LPS resulted in elevated serum TNF-α, but this was significantly lower than that seen in response to 2 mg/kg LPS. Neither 0.5 nor 1.0 mg/kg pentostatin administration reduced serum TNF any

Table 1. Serum IL-1β and IL-10 and tissue IL-10 measured in endotoxic rats pretreated with water or pentostatin (0.1, 0.5, or 1.0 mg/kg)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Pentostatin Dose, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (water)</td>
</tr>
<tr>
<td>Serum IL-1β, pg/ml</td>
<td>1,059 ± 128* (7)</td>
</tr>
<tr>
<td>Serum IL-10, pg/ml</td>
<td>278 ± 41* (5)</td>
</tr>
<tr>
<td>Liver IL-10, pg/g tissue</td>
<td>486 ± 137* (5)</td>
</tr>
<tr>
<td>Spleen IL-10, pg/g tissue</td>
<td>479 ± 143* (5)</td>
</tr>
</tbody>
</table>

Values are means ± SE; sample sizes are in parentheses. Both interleukin (IL)-10 and IL-1β were significantly elevated 2 h after intraperitoneal lipopolysaccharide (LPS) (2 mg/kg) administration. Pretreating rats 1 h before LPS with 0.1 to 1.0 mg/kg pentostatin significantly attenuated, without ablating, the LPS-induced rise in serum IL-1β, reaching maximal attenuation at similar doses used to attenuate tumor necrosis factor-α. There were no significant effects of pentostatin on serum IL-10, but IL-10 in the liver and spleen was elevated by pentostatin. ND, not done. P ≤ 0.05 relative to * no LPS control and † LPS alone.
lower than it had when the higher dose of endotoxin was used ($P = 0.94$ and $0.901$, respectively), suggesting a lower limit to the ability of pentostatin to attenuate this TNF response. Relative to untreated endotoxic rats, pentostatin resulted in a diminished response that did not achieve $\alpha$-criteria for significance ($P = 0.21$) but had a power equal to $0.14$, suggesting some true diminution of a much smaller magnitude.

**Chronic Sepsis**

We used a model of sepsis previously employed by our laboratory to study the role of endogenous adenosine in modulating the cytokine response to a more clinically relevant challenge. This model results in a hyperdynamic state within 24 h of sepsis induction (34, 36) and a progressive sepsis beyond day 3, with progressive leukocytosis and lactacidemia through day 7 (25). As shown in Table 2, serum TNF-α was elevated as early as 30 min after sepsis induction and remained elevated up to 72 h after sepsis induction. In liver and spleen, soluble TNF-α was also elevated at 24 h after sepsis induction ($84.2 \pm 10.8$ and $63.8 \pm 21.2$ ng/g tissue, respectively). The surgical procedure (nonseptic controls) used to induce sepsis also resulted in a transient elevation of TNF-α in both liver ($18.4 \pm 5.3$ ng/g) and spleen ($9.3 \pm 3.8$ ng/g) at 24 h, but these were significantly lower than that in the septic rats.

The influence of endogenous adenosine on the 4- and 24-h serum TNF-α response to the septic challenge was determined in response to either pentostatin or 8-SPT. In the water-treated septic group, serum TNF-α was elevated at 4 and 24 h (Fig. 3), similar to that seen in Table 2. Pentostatin attenuated this response at both 4 and 24 h after sepsis induction. 8-SPT treatment resulted in significantly higher serum TNF-α at 24 h; at 4 h, the power of the comparison was too low to definitively state a difference, or lack thereof, but the direction of change was consistent with the 24-h effect. Similar results were found in liver and spleen total TNF-α at 24 h after sepsis induction (Fig. 4). These results indicate that preventing endogenous adenosine degradation with pentostatin diminishes the in vivo TNF-α response to sepsis, whereas blockade of adenosine receptors alone amplifies this response. These data are consistent with the hypothesis that endogenous adenosine is an important endogenous modulator of the proximal cytokine response to a septic challenge.

As a consequence of these effects of adenosine, we also postulated a modulation of oxyradical-mediated damage. Samples of liver and spleen were tested for evidence of TBARS resulting from the peritonitis. Liver and spleen TBARS in each group are shown in

Table 2. Serum tumor necrosis factor-α (pg/ml) at various times after induction of septic peritonitis using cecal slurry

<table>
<thead>
<tr>
<th>Hours After Sepsis Induction</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>4.0</th>
<th>24</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham ($n=4$)</td>
<td>83 ± 257</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sepsis ($n=6$)</td>
<td>506 ± 173</td>
<td>1,927 ± 611</td>
<td>2,788 ± 406</td>
<td>1,369 ± 280</td>
<td>1,920 ± 342</td>
<td>650 ± 45</td>
</tr>
</tbody>
</table>

Values are means ± SE in pg/ml. ND, below the ability to detect.
Fig. 5. In septic rats treated with 8-SPT, the concentration of tissue TBARS was increased in the spleen relative to that in the water-treated septic rat group. Liver values were also consistently elevated, albeit not to a statistically significant level. Pretreatment with pentostatin significantly reduced the tissue concentrations of TBARS relative to that in the water control-treated septic rats and 8-SPT-treated septic rats. These data indicate that endogenous adenosine is also an important modulator of oxyradical damage after a septic challenge.

Whereas the previous protocol was designed to assess effects of pentostatin on blood and tissue parameters while minimizing the confounding influence of examining only survivors, a more lethal challenge was used to study mortality per se. The survival data presented in Table 3 demonstrate the effectiveness of pentostatin in reducing mortality, even when it is administered 2 h after a more lethal septic challenge. One and six days after sepsis induction, 4 of 13 and 7 of 13 rats, respectively, had died in the vehicle-treated group. In contrast, only 1 of 13 rats in the pentostatin-treated group died (significantly different by Fishers exact test: \( P = 0.03 \) at 6 days).

DISCUSSION

The data from these experiments compliment previous work from our laboratory (23, 24, 34, 36) and demonstrate a significant role for endogenous adenosine as a modulating component in SIRS. The results demonstrate that prevention of adenosine degradation attenuates proinflammatory cytokine responses after either LPS or a septic challenge, but a robust response remains intact. Blockade of adenosine receptors had the opposite effect, amplifying the elevation in TNF-\( \alpha \). As such, this modulation of proinflammatory cytokines can be directly associated with adenosine receptor-mediated actions and indicates that endogenously produced adenosine is modulating the response intermediate to either 8-SPT or pentostatin that was seen in the untreated LPS or septic rats. Importantly, beneficial effects of pentostatin use were not limited to pretreatment. Septic rats treated with 1 mg/kg pentostatin 2 h after sepsis induction were very well protected up to 6 days after the insult. These data indicate that endogenous adenosine is an important modulator of the responses to inflammatory processes. Furthermore, these data suggest that endogenous adenosine should not be considered an anticytokine molecule. Pentostatin had no significant effect on the TNF-\( \alpha \) response to a milder LPS challenge of 0.01 mg/kg, and it appeared that there was a lower limit to which manipulation of endogenous adenosine could be used to influence TNF-\( \alpha \). This may make this a novel therapeutic approach in that a significant proinflammatory response is left intact.

Earlier attempts to explore this question left unresolved problems. Firestein et al. (12) reported the ability of GP-1–515 to inhibit the TNF-\( \alpha \) response to LPS and that this could be blocked by adenosine receptor

---

Fig. 4. Concentrations of TNF-\( \alpha \) are elevated in liver and spleen 24 h after induction of sepsis (\( n = 7 \)). Consistent with the response seen in serum TNF-\( \alpha \), blockade of adenosine receptors (8-SPT) amplified this response (\( n = 6 \)), whereas inhibiting adenosine deaminase with pentostatin attenuated serum TNF-\( \alpha \) concentrations in both liver and spleen (\( n = 8 \)). †Significant difference from untreated septic rats, \( P < 0.05 \).

Fig. 5. Concentrations of thiobarbituric acid-reactive substance (TBARS) in spleen and liver samples 24 h after induction of sepsis. Values are presented as malondialdehyde equivalents in pmol/mg protein. Blockade of adenosine receptors (8-SPT) amplified this response, whereas inhibiting adenosine deaminase with pentostatin attenuated tissue TBARS concentrations. Significant difference from *untreated septic rats and †8-SPT group, \( P < 0.05 \).
antagonism. However, the adenosine receptor antagonist had no effect on the LPS response in the absence of GP-1–515, which seemed to suggest that naturally evolved endogenous adenosine was playing no role. However, some important differences between those studies and ours should be pointed out. First, GP-1–515 is structurally similar to adenosine, leaving open the possibility of direct receptor-mediated influences of the compound. Their data also differ from ours in that adenosine receptor antagonism, in the absence or presence of GP-1–515, had no effect on IL-1β responses to LPS. Our data extended beyond LPS responses into a clinically relevant model of sepsis. In this setting, our data clearly demonstrate the capability to amplify or diminish influences of endogenous adenosine on multiple responses to a septic challenge. We also demonstrate a lower limit to the influences of endogenous adenosine, which may also explain the mixed results seen by Firestein et al. (12). In a recent report, Martin et al. (20) demonstrated that plasma adenosine concentrations were elevated in patients with sepsis and that the increased concentrations correlated with the severity of the patients’ condition. Earlier work from our laboratory demonstrated that endogenous adenosine is also a significant modulator of resting vascular tone in sepsis. Combined with the findings from the experiments reported herein, the evidence indicates that adenosine is a modulator of multiple physiological responses to inflammatory processes, strongly influencing, but not mediating, the clinical picture of SIRS. A summary hypothesis diagram of the proposed multiple modulatory functions of endogenous adenosine in SIRS is shown in Fig. 6.

Manipulation of adenosine’s metabolic pathways has been a therapeutic approach in the treatment of diseases such as myocardial ischemia and hairy cell leukemia (4). With the findings from our experiments and others, there is considerable evidence that manipula-

Fig. 6. Our hypothesis is that endogenous adenosine plays a major role in modulating the responses of a variety of mediators of the septic response. Our evidence indicates that endogenous adenosine plays a significant role in maintaining resting perfusion to select regions, both directly and via simulation of nitric oxide synthase (NOS). Endogenous adenosine also modulates the cytokine response to a septic challenge, acting as any significant inhibitor of TNF-α and interleukin (IL)-1β release. Immunomodulation may occur via the known effects of adenosine on macrophages, as well as its ability to inhibit oxygen free radical production by neutrophils. Increased production of oxygen free radicals, however, can be a direct result of the degradation of adenosine through the xanthine oxidase pathway. Inhibition of adenosine deaminase may be uniquely suited to exploit the modulatory roles of endogenous adenosine. Inhibition of adenosine deaminase would slow the disappearance of endogenously produced adenosine, thus potentiating its vasodilatory, cytokine modulating, and neutrophil inhibiting functions (without ablating these functions), while also preventing the production of free radicals via the degradation of adenosine (15a). iNOS, inducible NOS; cNOS, constitutive NOS.
tion of adenosine metabolism could be beneficial in sepsis. Our manipulation of adenosine activity to affect the pathophysiology of septic insults reveals a self-limiting modulatory effect. The inability to suppress serum TNF-α below a limit, regardless of the severity of the challenge (Fig. 2), suggests that endogenous adenosine becomes an important modulator only when stimulation of the inflammatory response exceeds a minimum level. These responses are similar to in vitro effects. Adenosine is capable of suppressing macrophage activation and limiting cytokine release (11, 21, 32, 33), which is a likely source of the effects we are reporting herein. Adenosine also attenuates neutrophil adherence and production of reactive oxygen radicals moieties by neutrophils (7, 8).

One of the most intriguing potentials in manipulating the adenosine pathway is that it would only dampen these responses, rather than resulting in total blockade or inhibition of these immune responses. This would allow for a more tempered response, which appears to be critical for survival (29). This is reminiscent of endogenous adenosine’s role as a negative feedback inhibitor of cardiac inotropic responses to stimulators of adenylate cyclase (16). Adenosine is also a potent vasodilator, active only at sites of its production, where decreases in the oxygen supply-to-demand ratio prevail or where excessive adenylate cyclase activity occurs. Even in this regard, manipulation of the adenosine metabolic pathway would only affect regions wherein endogenous adenosine is being produced in significant quantities and would have no effect in other regions. Such an approach is worth investigating for the treatment and management of sepsis. The fundamental premise for such an approach is that there are sufficient increases in endogenous adenosine production in relevant physiological systems during sepsis to render manipulation of adenosine’s metabolism effective for the treatment of clinical sepsis. The results presented herein attest to the validity of that premise.

We focused our attention primarily on changes in tissue and plasma TNF-α concentrations as a marker of the proximal cytokine response. TNF-α is the most thoroughly studied cytokine with regard to modulation by adenosine (2, 8, 11–13, 19, 27, 32). In addition, TNF-α is a proximal cytokine, initiating inflammatory responses to infection. However, the results of our studies in LPS-challenged rats indicate that endogenous adenosine can also play a role as modulator of other proximal proinflammatory cytokines, such as IL-1β. Pentostatin treatment also resulted in elevated liver and spleen IL-10 after LPS in vivo, which supports a role for this anti-inflammatory cytokine in these responses as well. Hasko et al. (13) reported that adenosine-mediated attenuation of macrophage proinflammatory responses could be explained, in part, by adenosine-mediated stimulation of IL-10. Such a cytokine interaction may be at work in the setting of SIRS. Further work is needed to determine how manipulation of endogenous adenosine pathways affects the mechanisms underlying inflammatory processes and cytokine interactions in SIRS responses.

Adenosine has also been shown to inhibit a variety of neutrophil functions, including adherence (7), TNF-stimulated lactoferrin secretion (31), and, importantly, H₂O₂ production (7). Oxyradical injury can also be a result of adenosine accumulation (1, 5, 6, 18, 22, 28, 37–39). Via either of these pathways, manipulation of endogenous adenosine pathways can influence net oxyradical-mediated damage. Our results support this, but the data cannot be used to determine the relative contribution of the pathways involved. The blockade of adenosine receptors could exacerbate oxyradical-mediated damage by preventing adenosine-mediated inhibition of neutrophil activity (7) or by reducing perfusion (23, 24, 34, 36). Reduction of oxyradical damage by preventing the degradation of endogenous adenosine could occur via increased inhibition of neutrophil activity and by preventing adenosine’s entry into the xanthine oxidase pathway. More work is needed to identify the relative contributions of each pathway that could be involved in these responses. Still, the data indicate that inhibition of the adenosine deaminase enzymes is also beneficial in the setting of sepsis in decreasing lipid peroxidation.

Therapeutic implications of these results are being explored. Endogenous adenosine’s immunomodulating actions behave as a physiological negative feedback system. As such, manipulation of adenosine pathways and receptor-mediated actions act via amplification or attenuation of complex physiological effector systems rather than the more conventional approaches that served to intervene directly on specific effector mediators. Thus physiological regulatory systems remain intact and active; inhibition of adenosine deaminase enzymes still allows for a robust immune response. Because sepsis is associated with an exaggerated immune response, tissue perfusion maldistribution, oxyradical-mediated tissue damage, and manipulation of adenosine deaminase hold therapeutic promise via modulation of all of these pathways in which endogenous adenosine serves as a physiological feedback mechanism.

**Perspectives**

Significant advances in our understanding of SIRS reveal a complex, multisystem pathology. SIRS have eluded significant advances in treatment, in part, as a result of its influences on diverse, yet integrated, phys-

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Days After Sepsis (Sham) Induction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Vehicle</td>
<td>9/13 (69%)</td>
</tr>
<tr>
<td>Pentostatin</td>
<td>12/13 (92%)</td>
</tr>
<tr>
<td>Sham (nonseptic)</td>
<td>6/6 (100%)</td>
</tr>
</tbody>
</table>

Survival at 1 and 6 days after sepsis (or sham) induction indicated by no. of animals alive/total and percent surviving (in parentheses). Pentostatin (1 mg/kg) was administered intravenously 2 h after sepsis induction. *Significantly greater survival compared with vehicle-treated septic rats (P = 0.03).
iological systems. The approach to managing SIRS reported herein, manipulating endogenous adenosine, is novel from two perspectives. First, the goal is to amplify normal physiological responses, those that occur via endogenously produced adenosine, to regain homeostasis. This differs from past approaches that target a single inflammatory molecule or second-messenger system in an attempt to directly influence outcome. Second, the approach influences diverse, yet integrated, physiological systems. Specifically, endogenous adenosine modulates local tissue perfusion, responses to inflammatory agents and inflammatory molecule interactions, oxyradical production via multiple pathways, and neurohumoral function, to name a few. These functions are accomplished through the various signaling pathways coupled to adenosine receptors in each cell type.

The authors thank Drs. James L. Ferguson and H. Bruce Bosmann for discussion of results and review of this manuscript. This work was funded by the University of Illinois at Chicago College of Medicine and Supergen. Pentostatatin was a generous gift from Supergen.

REFERENCES


28. Riches DWH, Watkins JL, Hensen PM, and Stanworth DR. Regulation of macrophage lysosomal secretion by adenosine,


