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ADVANCES IN UNDERSTANDING ADENOSINE AS A PLURISYSTEM MODULATOR IN SEPSIS AND THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)

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TABLE OF CONTENTS

1. ABSTRACT

Adenosine is a ubiquitous molecule that influences every physiological system studied thus far. In this review, we consider the influence of this purine nucleoside on some of the physiological systems affected during sepsis and SIRS. In the control of perfusion and cardiac output distribution, endogenous adenosine appears to play an important role in regulating perfusion in various vascular beds. Some of this control is mediated by stimulation of adenylyl cyclase, while part occurs by stimulating the production of nitric oxide. In the heart, adenosine may act as an inhibitory modulator of TNF-alpha expression. With regard to innate immune responses the effects of adenosine vary considerably, and are complex. However, the dominant responses relevant to SIRS indicate attenuation of inflammatory responses. Many of the effects of adenosine may also involve modulating oxyradical-mediated response. This occurs via increased oxyradical production via adenosine degradation (xanthine oxidase pathway), or limiting inflammatory oxyradical generation. Attempts to exploit the beneficial responses to adenosine have met with some success, and are considered here.

2. INTRODUCTION

A purine nucleoside, adenosine is composed of adenine linked by its N9 nitrogen to the C1 carbon of ribose. The earliest clearly identified physiological properties of adenosine was vasodilation, reported by Drury and Szent-Györgi (1). As was not uncommon at that time, these investigators probed multiple physiological systems, and in 1929 first described cardiac chronotropic, dromotropic, and inotropic effects, coronary and systemic vasodilation, and renal vasoconstriction in response to exogenously introduced adenosine. Many decades later, Berne (2) and Gerlach *et al* (3) demonstrated a physiological role for endogenously produced adenosine. Since these humble beginnings, a plethora of roles have been identified for this nucleoside in nearly every cell type, tissue, and organ system. In the pathological manifestations of the systemic inflammatory response syndrome (SIRS), sepsis, and associated multi-organ failure, all of the systems that can be affected can also be influenced by adenosine. The purpose of this review is to consider the evidence demonstrating important roles for adenosine in the responses to SIRS and associated pathologies.

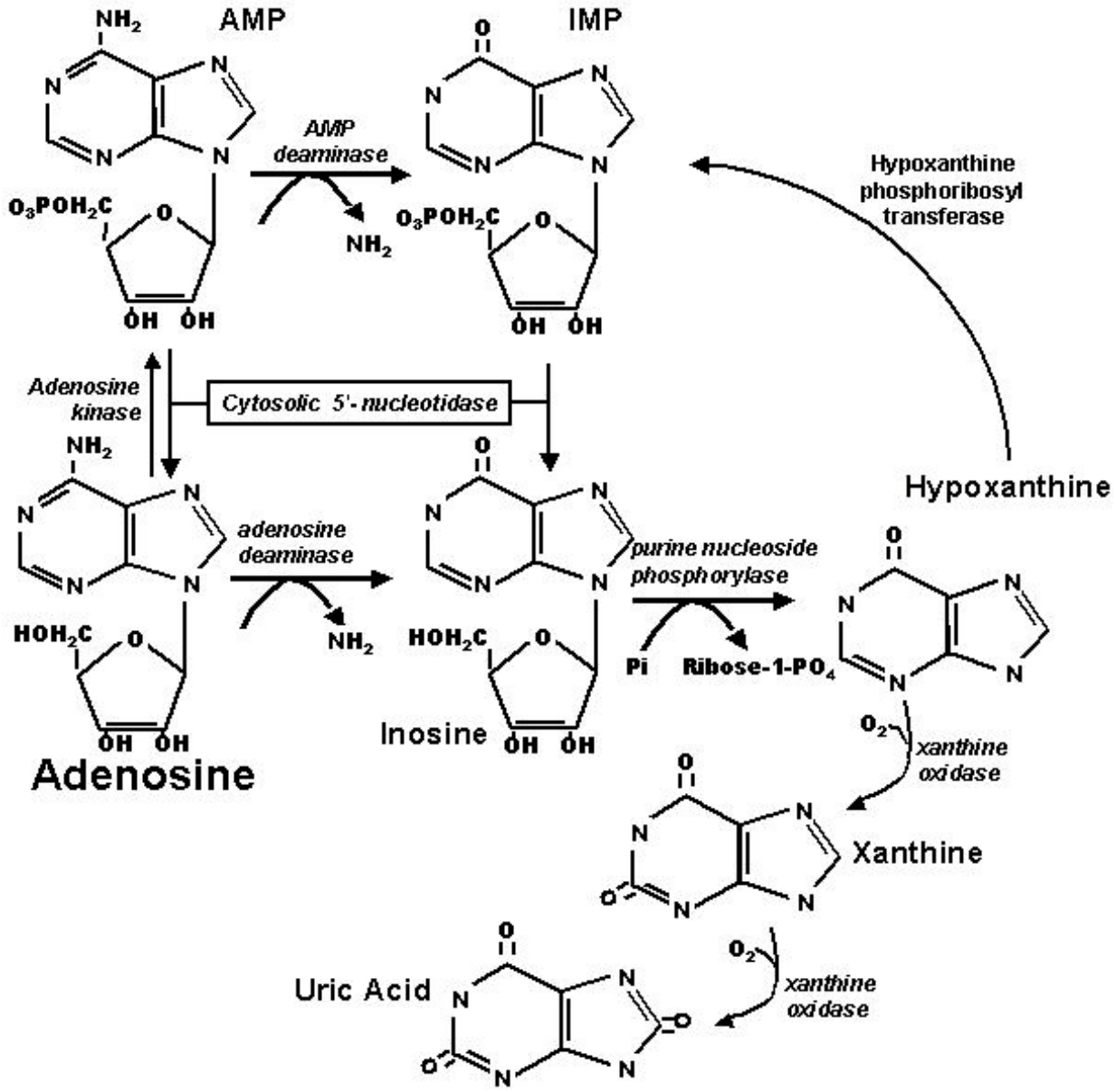

Figure 1. Metabolism of Adenosine: Intracellular. Partial schema of enzyme pathways pertinent to the regulation of intracellular adenosine concentrations. Adenosine monophosphate (AMP) can be directly deaminated to inosine monophosphate (IMP) by AMP deaminase, or acted upon by an endo-5'-nucleotidase to form adenosine. Adenosine can be rephosphorylated to AMP by adenosine kinase, or deaminated to inosine by adenosine deaminase. IMP can also be a source of inosine by the same endo-5'-nucleotidase. Hypoxanthine is formed after removal of ribose from inosine by the actions of purine nucleoside phosphorylase. Hypoxanthine can be salvaged to IMP by hypoxanthine phosphoribosyltransferase, or enter the xanthine oxidase pathway to form xanthine and uric acid sequentially, with the generation of oxyradicals as a byproduct. Intracellular adenosine can be transported into and out of the cell by membrane-associated transporter proteins.

3. BACKGROUND

3.1. Metabolic regulation of adenosine

Intracellular adenosine is produced by the hydrolysis of S-adenosylhomocycysteine (SAH) to form adenosine and L-homocysteine (4;5), or by the actions of 5'-nucleotidase on AMP (6;7). The relative contributions of these pathways to total adenosine production varies, but by most estimates, the hydrolysis if SAH accounts for little physiological adenosine production, and is not affected by altered cellular oxygenation (8). In contrast, the availability of AMP can be varied by numerous processes, most notable relative hypoxia or increased breakdown of cyclic AMP. AMP is preferentially acted upon by AMP deaminases to form inosine monophosphate (IMP; Figure 1). If not salvaged for nucleotide repletion, both AMP and IMP are

available for hydrolysis by 5'nucleotidase to adenosine and inosine, respectively. The adenosine thus produced can be reclaimed into the nucleotide pool by adenosine kinase, be deaminated by adenosine deaminase, or be transported out of the cell by carrier-mediated processes.

In considering adenosine as a molecule involved in autocrine and paracrine communication the extracellular concentration of this nucleoside becomes the focus of investigation. It was long thought that extracellular adenosine arose from intracellular adenosine moving out of the cell. Early work by Schutz *et al* (9) demonstrated that inhibition of adenosine transport in the heart during reduced tissue oxygenation resulted in diminution of interstitial adenosine accumulation, which appeared to confirm the hypothesis that intracellular adenosine was the major source of extracellular adenosine during conditions in which in tissue oxygen supply-demand ratio is severely reduced (10). The ability to measure more physiological concentrations of adenosine, however, revealed that adenosine transport inhibition increases extracellular adenosine accumulation (11).

Despite higher net intracellular production under most circumstances, intracellular adenosine concentrations are typically low owing to very effective salvage mechanisms. Intracellular AMP is readily phosphorylated to ADP by adenylate kinase, or deaminated by AMP deaminase to IMP, which can be further salvaged. If adenosine is formed from AMP by intracellular 5'-nucleotidase, cytosolic rephosphorylation by adenosine kinase or deamination by adenosine deaminase is favored. Further catabolism of inosine by purine nucleoside phosphorylase forms hypoxanthine, which can also be salvaged by hypoxanthine phosphoribosyltransferase (8;12;13). These pathways maintain low intracellular adenosine concentrations, resulting in a physiological adenosine gradient that is extracellular to intracellular.

There are multiple potential sources of signaling-relevant extracellular adenosine (Figure 2). Adenosine can be released directly from some cell types, such as purinergic neurons, but with the possible exception of hypoxic or anoxic conditions, there is mounting evidence that the source of effective extracellular adenosine is enzymatic formation in the extracellular space in many tissues. AMP is released by some cells directly into the interstitial compartment, and is substrate for a specific ecto-5'-nucleotidase isozyme (8). A recent arena of investigation has also demonstrated that extracellular cyclic adenosine monophosphate (cAMP) can also be a significant source of extracellular adenosine (14). Intracellular cAMP, during periods of increased adenylyl cyclase activity, can be transported out of the cell by specific transporter mechanisms. Outside the cell, a specific phosphodiesterase isozyme acts on cAMP to form AMP (15). In contrast to the intracellular compartment, nucleotide salvage enzymes are not prevalent in the extracellular space. Thus, the environment favors adenosine formation. Once in the extracellular compartment, adenosine can be removed by transport into cells, or deamination by extracellular adenosine deaminases. Under conditions when there is an impetus for adenosine production in the extracellular compartment, interfering with the actions of either membrane adenosine transporters or adenosine deaminase results in elevated extracellular adenosine concentrations. These methods have commonly been employed to investigate physiological and pathophysiological roles for adenosine.

Increased adenosine production is associated with decreases in the supply to demand ratio for oxygen (10). The most common example of these conditions is ischemia, wherein the supply of oxygen is diminished. However, imbalances can occur due to alterations in demand as well. In addition to the hyperdynamic state, sepsis is associated with hypermetabolism, and meeting nutritional needs present a unique challenge. Glucose homeostasis becomes deranged and insulin resistance is not uncommon (16). There is considerable mobilization of fat from adipose stores and proteins from muscle. The mobilization of fat can be attenuated by provision of lipid emulsions, but the lean body wasting is often refractory to parenteral and enteral nutritional efforts. Such changes likely contribute to alterations in supply to demand ratio for oxygen. Despite the typical presentation of elevated cardiac output with time commitment oxygen delivery, patients may still demonstrate supply dependent oxygen consumption, although this remains controversial (17). During SIRS, many factors would also contribute to conditions under which adenylyl cyclase activity is elevated. Increased neural sympathetic activity and the actions of multiple circulating mediators are effected, in part, by stimulating adenylyl cyclase in specific cells. In any given tissue, region, or cell group interaction, any or all of these factors must be considered as potential sources of extracellular adenosine during SIRS.

3.2. Adenosine receptors

In 1970 Sattin and Rall (18) showed that adenosine alters cellular functions via specific cell surface receptors. There are at least four subtypes of adenosine receptor that contribute to the ubiquitous, yet varied actions of adenosine. Details of our understanding of the structure, function, and distribution of the adenosine receptors have been extensively reviewed (19;20). One adenosine receptor subtype, or combinations of the four adenosine receptor subtypes, can be expressed on cell surfaces (20). All four adenosine receptor subtypes are hepta-spanning transmembrane G-protein-coupled receptors. Three of the adenosine receptor subtypes (A_1, A_{2A}, and A_{2B}) demonstrate 80–95% sequence homology across a wide evolutionary range of species. In contrast, the A_3 receptors demonstrate significant species variation.

Signal transduction by the adenosine receptors varies, not only amongst the subtypes, but also for a particular subtype between different cell sources (21). A_1 receptors were originally characterized as coupled to pertussis toxin-inhibited Gi-coupled signal transduction, but in some cells, they are directly associated with, and act through, ion channels. The A_2 receptor subtypes (A_{2A} and A_{2B}) are typically coupled to Gs-linked receptors. The prototypic response to adenosine, vasodilation, is effected

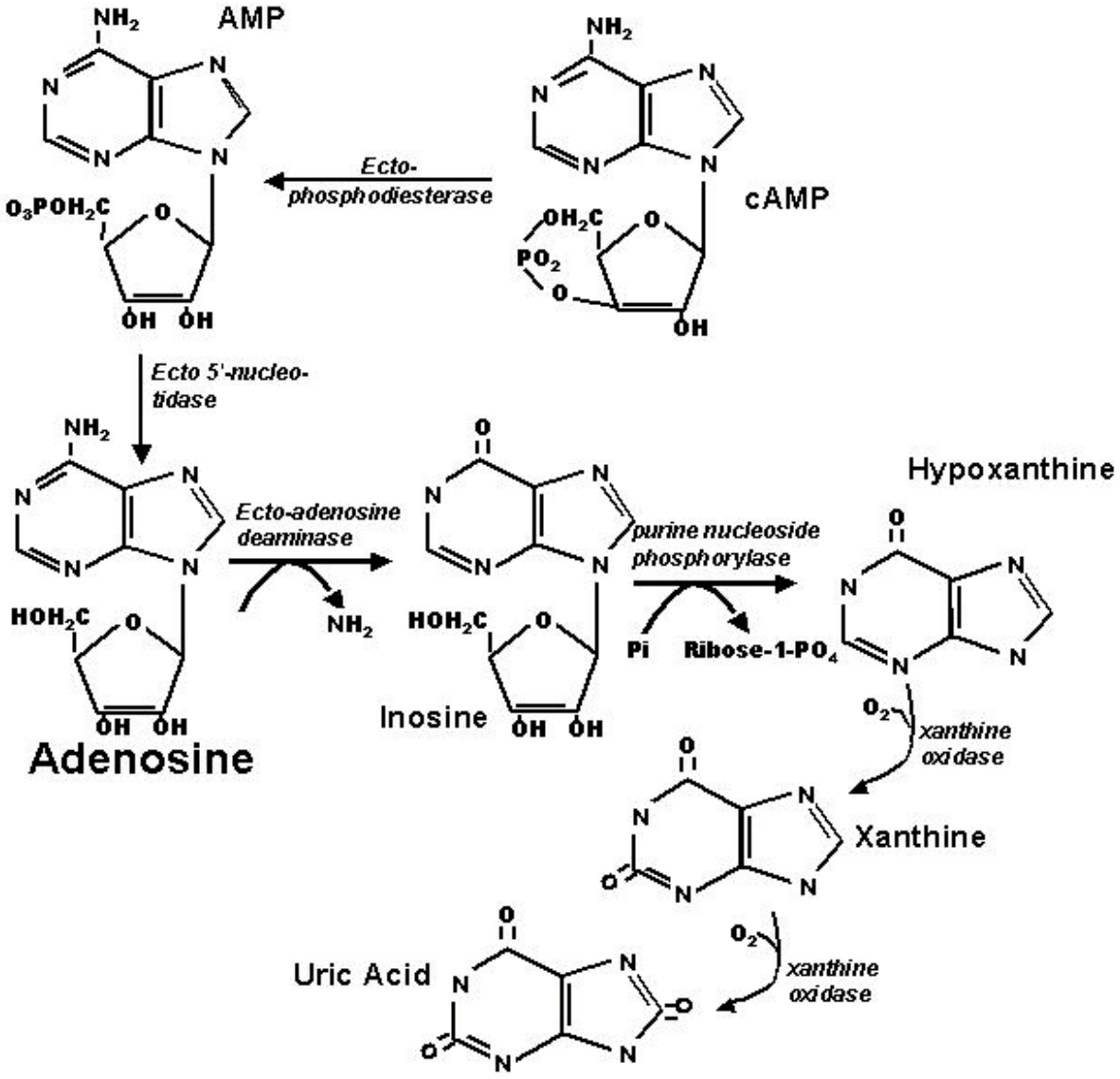

Figure 2. Metabolism of Adenosine: Extracellular. Partial schema of enzyme pathways pertinent to the regulation of extracellular adenosine concentrations. Cyclic adenosine monophosphate (cAMP) can be transported out of cells upon activation of adenylyl cyclase. The actions of an ecto-phosphodiesterase on cAMP results in the formation of AMP. AMP can also be directly released by some cell types. AMP is acted upon by an ecto-5'-nucleotidase to form adenosine. Adenosine can then be transported into the cell, or deaminated to inosine by adenosine deaminase. Hypoxanthine is formed after removal of ribose from inosine by the actions of purine nucleoside phosphorylase. Hypoxanthine enters the xanthine oxidase pathway to form xanthine and uric acid sequentially, with the generation of oxyradicals as a byproduct.

in this way via stimulation of adenylyl cyclase. In some tissues, A_1 adenosine receptor-mediated inhibition and A_{2A} receptor-mediated stimulation of adenylyl cyclase appear to coexist and be counter regulatory (22). Furthermore, A_{2A} receptors that do not stimulate adenylyl cyclase have been identified in heart tissue (23), so alternative signaling mechanisms may be associated with this receptor subtype, as well.

Adenosine receptor-mediated actions are physiologically widespread, having been demonstrated in nearly every tissue and organ system. The processes affected by adenosine receptors include neurotransmission, numerous cardiac effects (inotropic, chronotropic, dromotropic, and other actions), vasodilation, regulation or modulation of airway tone, and regulation of immune function. Because of the ubiquitous and plurieffective nature of adenosine receptor-mediated actions, these receptors have often been targeted for the development of pharmacologically active agonists and antagonists. However, the various systems upon which adenosine receptors exert influence can also be a hindrance to such

pharmacological approaches, and undesirable side-effects in non-target systems are readily seen.

3.3. Indirect Effects of Adenosine: Oxyradicals

Oxyradicals must be considered amongst the satellite molecules associated with adenosine-mediated actions or metabolism. Excessive oxyradical production can cause deleterious damage through direct oxidative processes, or can serve as a stimulus for cell signaling, as in the case of proinflammatory cytokine expression associated with NF-kB activation (24). Three pathways have been demonstrated to be involved in oxygen free radical production during sepsis: the arachidonic acid pathway (via cyclo-oxygenase), neutrophil activation and degranulation, and from catabolites via xanthine oxidase (25). Spitzer *et al* (26) reported that rat hepatic sequestered neutrophils produce more superoxides after *in vivo* endotoxin infusion, and that the response of hepatic sequestered neutrophils exceeded that of circulating neutrophils. Adenosine is a potent inhibitor of oxyradical burst activity of neutrophils, thus reducing oxyradical production. This will be discussed later. Adenosine can also provide substantial substrate through the xanthine oxidase pathway (27-29), thus serving as a source of oxyradicals for both tissue peroxidation and cellular signaling (30;31). Xu *et al* (29), have clearly demonstrated that the xanthine oxidase inhibitor allopurinol alone protected the bowel from hypoperfusion and increased intestinal permeability caused by endotoxin, and Castillo *et al* (32) demonstrated significantly better survival using allopurinol in their rodent model of cecal ligation and puncture. These findings implicate a significant role for xanthine oxidase-mediated involvement in sepsis and SIRS. Adenosine has the potential to contribute to xanthine oxidase-mediated effects in sepsis and SIRS, but specific involvement has yet to be clarified.

4. ADENOSINE IN SEPSIS AND SIRS

4.1. Regional Perfusion Affected by Adenosine in SIRS and Sepsis

As a physiological vasomediator, adenosine is best known as a reactive molecule. The roles of endogenous adenosine in ischemia, tissue hypoxia, and in active and reactive hyperemia were among the earliest identified responses (2), and have been studied extensively. With a few exceptions, adenosine receptor antagonists have little effect on resting physiological vascular tone. However, their influence becomes substantial during conditions of increased metabolic activity, adenylate cyclase activation, or reduced oxygen supply/demand (as in ischemia). These conditions explain the vasoactive role demonstrated for adenosine during increased work in skeletal muscle (33), post-prandially in the gut (34), and during ischemia or increased adrenergic activity in the heart (35).

The vasoactive role of endogenous adenosine in the perfusion alterations associated with sepsis and SIRS has not been as thoroughly investigated. Much of what is understood of mediators of regional vascular control comes from a variety of animal models of sepsis, septicemia, and septic shock. While each model and species provides for variations on the specific responses, some commonalities exist.

4.1.1. Hepatosplanchnic circulation

There is a hyperdynamic phase early in the development of sepsis or SIRS, characterized by increased cardiac output and low systemic vascular resistance. During the hyperdynamic phase the increased cardiac output is, in large part, diverted to the hepatosplanchnic circulation (16;36-38). Motew *et al* first demonstrated that the elevated hepatosplanchnic blood flow could be reverted to physiologic blood flow levels upon blockade of adenosine receptors during sepsis within 24 hours of a septic insult (36;37), a response later confirmed by Sam *et al* (38;39). These changes appeared to be primarily the result of preventing endogenous adenosine-mediated vasodilation that was maintaining lower vascular resistance in hepatosplanchnic circulations. Calculated vascular resistance in these regions increased in the presence of adenosine receptor blockade with two different receptor antagonists (37;38). There was no evidence of myocardial depression associated with the treatment, indicating that these changes were not secondary to a centralized cardiac insufficiency. Adenosine receptor blockade resulted in increases in left ventricular performance, despite a decrease in cardiac output. In their work, Motew *et al* (37) reported a transient increase in blood pressure immediately after adenosine receptor blockade in septic rats, indicating that the decrease in cardiac output occurred secondary to increased afterload.

Within the hepatosplanchnic circulation, the changes in perfusion to different organs were not uniform (36;39). Increased small intestine blood flow has been observed in the early phases of protracted sepsis (16;40) and accounts for the majority of increased hepatosplanchnic flow that could be attributed to endogenous adenosine (36;39). This may be related to greater responsiveness of this region to adenosine (41). In other regions, including the stomach, cecum, and pancreas, the maintenance of physiological blood flow, rather than elevated flow, appeared to be under some control of endogenous adenosine. Pennanen et a (42) used radiolabelled microspheres to show that adenosine administered to rabbits selectively increased blood flow to the esophageal mucosa, antral mucosa, and small bowel. The use of NECA (A_2 selective agonist) in this study confirmed an A_2 mediated response in these regions. In contrast, the ileum responded only to a low dose of N^6-cyclohexyladenosine (CHA) which is an A_1 selective agonist (42). This may be a result of differential actions of adenosine in the splanchnic circulation due to a variable distribution of receptor subtypes.

The specific cause for the accumulation of vasoactive quantities of adenosine in adenosine-dependent hepatosplanchinc vascular beds during sepsis is not known, but a number of possibilities have been considered. Adenosine has been shown to play a significant role in metabolic vascular regulation of the intestinal microcirculation (43;44).The adenosine receptor antagonist

theophylline was shown to significantly reduced mesenteric flow regulation in fed dogs, with no effects in non-fed animals, and attenuated the reactive hyperemic response in the intestine after a 2 minute arterial occlusion. These responses support a role for adenosine in regulating intestinal perfusion during different metabolic challenges. Alternatively, data from studies during endotoxemia and bacteremia suggest that a critical oxygen supply dependence occurs in the intestine, wherein oxygen extraction fails to increase to maintain consumption as delivery is reduced (45-47). This relative oxygen debt can cause an increase in concentrations of local metabolic by-products that may serve to augment oxygen delivery to the region. Finally, increased sympathetic activity in the gastrointestinal tract (48;49) may contribute to increased adenylyl cyclase activity, as previously noted.

Potential deleterious effects of gastrointestinal adenosine production during sepsis should also be considered, especially regarding its potential role as substrate for oxyradical production via the xanthine oxidase pathway. Xu *et al* (29) examined intestinal blood flow and permeability changes after non-lethal endotoxin administration in rats, and found that the xanthine oxidase inhibitor allopurinol prevented decreases in intestinal flow and increases in intestinal permeability associated with intravenous endotoxin. Deitch *et al* (50) correlated xanthine oxidase activity to endotoxin-induced bacterial translocation in the rat. The proximal small intestine showed increased xanthine oxidase and xanthine dehydrogenase activity compared to the more distal bowel (51). An increase in adenosine production in the proximal small intestine during sepsis would correlate with this augmented enzyme activity. Another potential detrimental action of adenosine was characterized by Granger and Norris (44) who demonstrated that *in vitro,* ileum tissue segments exhibited a significant dose-dependent decrease in mucosal and muscularis oxygen consumption with adenosine superfusion. These studies suggest potential detrimental actions of adenosine on local metabolic processes which might be exacerbated during sepsis.

4.1.2. Other Regional Perfusion changes attributable to adenosine.

Even though the weight-normalized resting blood flow to skeletal muscle is low, the large portion of total body mass represented by muscle means that muscle perfusion can represent a significant percentage of the body's distribution of cardiac output. Thus, small changes in weight-normalized flow can represent large changes in redistribution of the cardiac output. Skeletal muscle perfusion can be normal or elevated in sepsis and SIRS, and heterogeneous at the microvascular level (37;38;40;52). These changes may be related to metabolic changes in skeletal muscle associated with SIRS and sepsis (16;52-56). It may not be surprising, then, that skeletal muscle vascular resistance is increased with adenosine receptor blockade in septic rats (37;38). Although such perfusion of skeletal muscle is sometimes referred to as luxury perfusion, it may also reflect microvascular coagulopathies, some of which may be prevented by adenosine, as well (57).

In addition to carbon dioxide in the brain, adenosine can act as an autoregulatory mediator. This is often considered in the context of a cerebral compensatory response to conditions that might result in reduced carotid arterial perfusion pressures. However, decreased cerebral blood flow in the face of normotensive conditions was found to be associated with adenosine receptor antagonism during hyperdynamic sepsis (37), implicating endogenous adenosine in maintaining normal cerebral blood flow sans major perfusion pressure alterations. This response was seen only with 8-phenyltheophylline, an adenosine receptor antagonist capable of crossing the blood brain barrier. Later studies using 8-sulfophenyltheophylline (8-SPT), an antagonist that does not cross the blood brain barrier (58), had no effect on cerebral blood flow (38). As in the instances above, this raises questions regarding the source of the adenosine. In addition to serving as a metabolic vasoregulator, adenosine is also a ubiquitous neurotransmitter, and sepsis is often associated with lethargy and mentation alterations, both of which can be caused by increased purinergic neural activity.

4.1.3. Adenosine and Nitric Oxide (NO)

Some of the adenosine-mediated regional perfusion redistribution could be attributed to adenosine-stimulated NO production. In two series of experiments, Sam *et al* demonstrated that partial perfusion reductions in some regions after blockade of adenosine receptors with 8-SPT prevented any flow reduction that would otherwise result from inhibition of nitric oxide synthase (NOS) with NG-nitrio-L-arginine methyl ester (L-NAME) (38;39). When the order of inhibition was reversed, and regional vascular resistance increased with L-NAME, no significant effect of adenosine receptor blockade could be elicited. The evidence clearly demonstrated that the vascular responses had not reached a maximal limit, and indicated an interdependence of adenosine and NO in mediating some of the perfusion changes associated with SIRS in this model. Nearly complete interdependence was demonstrated in the hepatosplanchnic circulation, while partial responses were found in skeletal muscle. There, a NO-independent action of endogenous adenosine was also unmasked with adenosine receptor blockade. In contrast, there was no interdependent effects in adipose and renal circulations, which responded to L-NAME and not adenosine receptor blockade.

The induction of calcium-independent NO synthase (iNOS) occurs in response to TNF-alpha and other proinflammatory stimuli. This process also plays a role in the evolution of cardiovascular changes associated with sepsis. In this regard, two of the recognized effects of adenosine, its ability to inhibit TNF-alpha production and release (see below), and its ability to stimulate NOS, may be involved in these responses. Adenosine may also attenuate the expression of iNOS during sepsis via inhibition of TNF-alpha expression and release (59;60). Again, metabolomic and temporal considerations need to be better understood to determine the net effective actions of adenosine in this regard during different phases of SIRS. Studies indicating that adenosine can stimulate NOS have been conducted in the absence of known inducers of, or

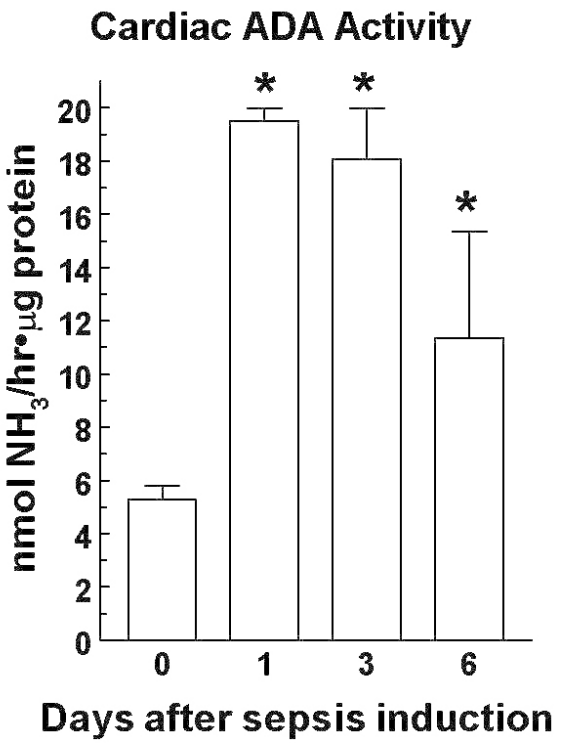

Figure 3. Cardiac Adenosine Deaminase Activity in SIRS. Adenosine deaminase activity is elevated in rat hearts during SIRS associated with peritonitis. Diffuse peritonitis with fluid resuscitation was induced by peritoneal introduction of a slurry of cecal material (400 mg/kg in D5W) as previously described (30;104). Hearts were obtained at 1, 3, and 6 days after the septic insult, and adenosine deaminase (ADA) measured in whole heart homogenates according to previously described methods (70).

conditions associated with, increased iNOS expression (61-63), so it is likely that the responses observed were mediated via a constitutive isoform of NOS. In sepsis, wherein iNOS expression can be stimulated by TNF-alpha, the importance of adenosine's ability to stimulate NO production in specific regions may also depend upon both its ability to reduce iNOS expression via inhibition of TNF-alpha, and any stimulatory action it may have on eNOS. While this hypothesis has support in many cell types, including smooth muscle, cardiac myocytes, and hepatocytes, it is not invariate. In macrophages, wherein TNF-mediated induction of iNOS is documented, adenosine has only been shown to increase the production of NO metabolites. Whether this represents low eNOS expression, as can occur in human macrophages, or stimulation of iNOS, how it applies to cardiovascular responses, and the effective balance of these during sepsis are not clear, and is worthy of more investigation.

4.2. Cardiac Cytokine Expression

The first published study that described the effects of adenosine focused on cardiac mechanical and vascular responses (1). The list of cardiac physiological actions has since grown considerably and more detailed in the ensuing decades. Many reviews have been written, often focusing on one particular aspect of one of the myriad roles played by adenosine in the heart. Here, we will briefly consider the effect of adenosine on cytokine regulation in the heart, which has particular relevance to SIRS, but also to a variety of other pathological conditions.

The characteristic elevated cardiac output of the hyperdynamic state associated with sepsis and SIRS in patients belies the myocardial depression that is typically present. While many causative factors have been implicated, recent findings also suggests that pro-inflammatory cytokines, such as TNF-alpha, contribute to this cardiac depression. Elevated TNF-alpha in the heart contributes to calcium dyshomeostasis and contractile dysfunction in two phases. These two phases have been characterized as the immediate, NO independent phase, and the late, NO dependent phase (64). In the immediate phase, cardiac TNF-alpha acts through sphingosine to impair ryanodine-sensitive calcium release channels in the cardiac sarcoplasmic reticulum. This decreases the magnitude of calcium transients, resulting in contractile depression. In the late phase, contractile dysfunction is the result of NO induced myofilament desensitization to calcium. These are interrelated in that TNF-alpha in the heart upregulates iNOS expression resulting in increased NO production and subsequent cardiac depression (64).

The expression of cardiac TNF-alpha during sepsis is modulated by adenosine signaling and metabolism in the heart. Adenosine is an inhibitor of cardiac TNF-alpha production while at the same time, its metabolism via the xanthine oxidase pathway leads to production of reactive oxygen which is a potent stimulus for increased TNF-alpha expression.

4.2.1. Direct and indirect effects of adenosine on cardiac TNF-alpha production

Recent evidence from both human patient cardiac tissue and animal models has demonstrated a role for adenosine in inhibiting exaggerated production of TNF-alpha in the heart after septic and ischemic insults (65-69). Adenosine attenuated the LPS-induced increase in TNF-alpha mRNA transcription in isolated rat neonatal cardiac myocytes (67). Adenosine also was effective in inhibiting LPS-stimulated production of TNF-alpha in human trabeculae from patients with end-stage cardiomyopathy (69). In both of these studies, similar effectiveness with an A_2 adenosine receptor specific agonist implicated the involvement of the A_2 adenosine receptor in inhibiting TNF-alpha mRNA transcription.

Reactive oxygen generated from adenosine degradation may also play a significant role in modulating cardiac TNF-alpha production. Adenosine deaminase is responsible for the first step in degrading endogenously produced adenosine in the heart. Further metabolism (see figure 1) results in the production of reactive oxygen. Sepsis results in elevated plasma adenosine deaminase enzyme activity (70), and elevated adenosine deaminase activity has been measured in hearts from septic rats (Figure 3). This would rapidly shuttle cardiac adenosine

into the xanthine oxidase pathway with subsequent generation of oxyradicals. Hydrogen peroxide has been used to increase cardiac TNF-alpha production. This appears to involve a p38 MAPK-dependent mechanism in the isolated rat heart (66). Reactive oxygen species have also been linked to cardiac TNF-alpha production via the transcription factor NF-kB. Thus, adenosine may play an indirect role in the control of cardiac TNF-alpha production in the myocardium via its own metabolism through the xanthine oxidase pathway.

4.2.2. Transcriptional regulation of cardiac TNF-alpha by adenosine and reactive oxygen

NF-kB is a ubiquitous transcription factor that is associated with induction of TNF-alpha expression in the heart (71). Adenosine has been shown to inhibit cardiac TNF-alpha mRNA production at the level of transcription by preventing the translocation of NF-kB, while concomitantly enhancing activator protein 1 (AP-1) binding (72). In contrast, reactive oxygen species can stimulate TNF-alpha mRNA transcription via NF-kB activation (73). Two dimers of NF-kB have been implicated in the transcriptional regulation of TNF-alpha mRNA. A p65/p50 heterodimer promotes TNF-alpha mRNA transcription (74), while a p50/p50 homodimer does not actively inhibit TNF-alpha transcription but competes for binding sites on the TNF-alpha promoter effectively reducing TNF-alpha transcription by the heterodimer (75). Heterodimers of c-fos, c-jun and ATF-2 gene products have also been implicated in LPS-induced TNF-alpha mRNA transcription via AP-1 binding site activity (74). As such, these two transcriptional mechanisms may work in tandem to regulate adenosine's actions on cardiac TNF-alpha production directly via adenosine receptors or indirectly through the generation of oxyradicals.

4.3. Adenosine in Modulating Immune Function

The immune system exhibits a bi-phasic response to SIRS. Initially there is an exaggerated immune response followed by a period of immune cell hyporesponsiveness. Uncontrolled inflammatory responses that characterize sepsis and SIRS contribute to collateral tissue damage and eventually multi-organ system failure. Cytokines and other cytotoxic molecules aid in the destruction of pathogens by activated immune cells. However, the actions of many proinflammatory molecules and cytotoxic cells can have deleterious effects on the host. This has spurred interest in identifying some of the downstream molecules involved in the modulation of inflammation. Adenosine has emerged as one of these immunomodulatory molecules.

The development of mice deficient in specific adenosine receptors has furthered our understanding of the importance of adenosine receptor signaling in the modulation of inflammation *in vivo*. Recent studies by Ohta and Sitkovsky (76)have showed a critical role for the A_{2A} receptor in the down-regulation of acute inflammation. Inflammation in A_{2A} receptor deficient mice (*Adora2a*$^{-/-}$) (77) was tested in models of acute liver inflammation and endotoxin-induced septic shock. A_{2A} deficient mice showed significantly increased local tissue damage and elevated levels of TNF-alphalpha, IFN-gamma, and IL-12 for prolonged periods of time as compared to wild-type controls (76;78). Interesting effects in the A_3 adenosine receptor deficient mouse also suggest a role for this receptor, and implicate multi-receptor interactions as well (79). In addition, pharmacological approaches have implicated all four major adenosine receptor subtypes under various conditions. We shall consider the effects of adenosine in three major cell types involved in sepsis and SIRS.

4.3.1. Monocytes and Macrophages

As critical components of the innate immune response, monocytes and macrophages play a pivotal role in the development of acute and chronic inflammation. Infiltration of the body by gram-negative organisms initiates a sequence of events that has been well documented (80). The LPS of the gram-negative bacterial cell wall is one of the primary molecules responsible for the initiation of the inflammatory response. LPS is one of the best characterized activators of macrophage function. LPS binds LPS-binding protein in the plasma. This complex then interacts with the membrane bound CD-14 molecule on macrophage, which presents to the TLR4 receptor. Activation of the LPS signal transduction receptor, TLR4, results in macrophage activation and cytokine release. This signal becomes amplified as proinflammatory cytokines attracts other macrophages and monocytes and the cycle repeats. Although the sequence of events with gram-positive organisms is different and not as well understood, the end result of macrophage activation and cytokine release is also seen.

Results in monocytes, macrophages, or related derived cell lines, must be interpreted cautiously. Among the changes that have been shown to occur as monocytes differentiate into macrophages are adenosine receptor subtype profile changes (81). Further complicating this is the distinct differentiation related to the final tissue residence of the macrophages. Isozyme profiles of PKC, one of the signaling pathways used by adenosine receptor signaling, has been shown to vary between alveolar and peritoneal macrophages (82). Still, consideration of commonalities may provide insights into the role of adenosine in these cells.

Numerous studies on the effects of adenosine on immune cell function have been done *in vitro* using LPS. If has be shown to simulate the production of many proinflammatory cytokines such as TNF-alpha, IL-1, IL-6, IL-8 and IL-12. Macrophages also secrete NO and oxygen free radicals in response to LPS stimulation and infection. TNF-alpha is a central, and temporally proximal mediator in the development of the inflammatory response and sepsis. TNF-alpha is involved in the recruitment and activation of macrophages, neutrophils and lymphocytes, as well as in stimulating the release of other cytokines and proinflammatory proteins. Elevated levels of TNF-alpha are found in patients with sepsis, and the degree of elevation has been correlated with outcome and severity of infection (83-85). In addition, the administration of TNF-alpha to animals and human volunteers mimics several of the characteristics of sepsis (86-88).

Adenosine has been shown to inhibit TNF-α and other proinflammatory cytokines produced in response to LPS. Using LPS, Eigler *et al* (59) stimulated human peripheral blood mononuclear cell production of TNF-alpha. The addition of adenosine deaminase (to remove endogenous adenosine), or an adenosine A2 receptor antagonist, amplified LPS-stimulated TNF-alpha production, while an adenosine A_1 receptor antagonist had no effect. These results suggested that endogenous adenosine production after stimulation with LPS served to limit the TNF-alpha response of the monocyte by an A2R-mediated action. Studies done in A_{2A} receptor-deficient mice showed a suppression of TNF-alpha levels only with concentrations of adenosine greater than 1 mM. While not ablated, there is a decrease in the extent of TNF-alpha inhibition in A_{2A} receptor-deficient mice suggesting that multiple adenosine receptors are involved. These data support the hypothesis that activation of A_{2A} receptors at low levels is supplemented by the activation of the A_3 receptor at higher concentrations of adenosine to further increase the extent of TNF-alpha suppression. Further support for this can be found in the work of Salvatore *et al* (79), who demonstrated that an A_3 receptor agonist, 2-Cl-IB-MECA, injected intravenously prior to LPS injection can prevent an elevation in serum TNF-alpha levels in wild type but not $A_3AR^{-/-}$ mouse. Interestingly, they found that the A_{2A} agonist CGS21680 inhibited LPS-stimulated TNF-alpha levels in both the wild type and A_3 deficient mice, again supporting the concept of multi-subtype adenosine receptor involvement.

The cellular mechanisms by which adenosine decreases TNF-alpha production by macrophages are not clear. Nuclear binding of NFκB p50/p65 heterodimers and p50/p50 homodimers regulate transcription of multiple proinflammatory cytokines (89). Lukashev *et al* (78) has presented evidence that greater NFκB binding was seen in macrophage nuclear extracts from A_{2A} receptor deficient mice. The phosphorylation of IκB, inhibitor of NFκB activity, was also higher as compared to wild type mice. In contrast, Nemeth *et al* (90) explored the ability of adenosine and its analogs to modulate NFκB-mediated expression of TNF-alpha after LPS in the RAW 264.7 macrophage cell line. Despite demonstrating significant adenosine-mediated reduction in TNF-alpha levels, they reported no evidence of decreased NFkB binding in nuclear extracts, alterations in relative amounts of p65 and p50, nor any change in the decrease in IkB protein by adenosine or any of its receptor agonists. Further, no change in TNF-alpha mRNA was found, suggesting a post-translational mechanism of adenosine (91). This perspective is supported by evidence of adenosine-mediated decreases in TNF mRNA stability (92). Many factors may underlie the divergent results that have been published thus far, including differences between primary cells and cell lines, species differences, and unexpected or unrecognized alternate responses to individual gene deletions. As such, further work is required before we have a better picture of the molecular mechanisms behind adenosine's ability to modulate macrophage responses.

In addition to it's effects on cytokine expression adenosine also regulates other aspects of macrophage function including chemotaxis (93), phagocytosis (94), superoxide production (31), and NO production (95). Itoh *et al* (96) reported that both adenosine and 1-methyladenosine inhibited chemiluminescence by zymosan-stimulated mouse peritoneal macrophages *in vitro*. Riches *et al* (97) reported that adenosine inhibited β-galactosidase secretion from zymosan particle-stimulated mouse peritoneal macrophages. The adenosine nucleotides ATP, ADP, and AMP were also effective inhibitors only after hydrolysis to adenosine. The authors also found that the inhibitory effect of adenosine could be increased with erythro-9- (2-hydroxy-3-nonyl) adenine (EHNA), a potent inhibitor of adenosine deaminase. By thus inhibiting adenosine breakdown to inosine and hyopxanthine the inhibitory effects of adenosine were prolonged. IL-12 is involved in the induction of cell-mediated immunity and the Th1 response in sepsis, and administration of IL-12 to humans has been shown to induce SIRS (98). The induction of IFN-gamma by IL-12 is thought to play a key role in the development of sepsis through it's stimulation of phagocytic cell activation and inflammation. IL-12 also favors Th1 cell proliferation and differentiation (99). Adenosine was reported to decrease LPS-stimulated macrophageγγ12 production in A_{2A} receptor dependent and independent mechanisms (100). Adenosine may also modulate IL-6 and cytokine receptor expression (57). An extracellular source for the endogenous adenosine was implicated when Eigler *et al* (59) demonstrated that TNF-alpha production by LPS-stimulated monocytes could be attenuated by dipyridamole, an agent that prevents cellular adenosine reuptake a major pathway for adenosine removal by monocytes (101).

Adenosine also inhibits the immune response by increasing the expression of anti-inflammatory cytokines. IL-10 is an anti-inflammatory cytokine that inhibits the release of TNF-alpha, IL-1beta and IL-6 in LPS stimulated macrophages (102). Treatment of mice with adenosine receptor agonists prior to IP injection of LPS has been shown to augment IL-10 plasma levels, primarily through A2 and A_3 receptor mediated mechanism (103). Similarly, inhibition of adenosine deaminase has been shown to modestly increase IL-10 in monocytic cell-rich tissues (104).

4.3.2. Neutrophils

The most abundant of the circulating lymphocytes is the neutrophil. It is one of the first innate immune cells to respond at sites of bacterial invasion or acute injury. The vascular endothelial cells within the site of infection help recruit neutrophils from the circulation to the tissue through a multi-step process. Neutrophils use the adhesion molecule L-selectin to resist the flow of blood and roll along vascular walls. Once stimulated by chemoattractants, such as cytokines, bacterial products, or activated complement components, neutrophils "stick" to the vascular wall by the β2 integrins CD11b and CD18. The differential expression of adhesion molecules by neutrophils allows them to be recruited accurately to inflamed or injured sites by transmigration across the vascular wall (105). One of the major roles of the neutrophil in the innate immune response is to ingest

foreign organisms and cellular debris through the formation of phagolysosomes. The generation of superoxide anions and other toxic metabolites of oxygen as byproducts of phagocytosis, and often damage surrounding healthy tissue. This is exacerbated during SIRS as the infiltration of neutrophils begins to occur distal to the site of injury or specific inflammatory stimuli as the abundance of proinflammatory mediators upregulates adhesion molecules. This leads to the damage of non-infected tissues and endothelial cells throughout the vasculature (106). Thus, while being a central and vital component of the innate immune response, it is essential to have mechanisms for limiting neutrophil accumulation and activation to the site of inflammation.

Neutrophils and endothelial cells have been suggested to be major sources of adenosine during times of infection, inflammation and metabolic distress (107). In a healthy individual extracellular adenosine levels are below 1 mM. In instances of inflammation and ischemia, it has been estimated that local adenosine concentrations may be as high as 100 mM . In patients with sepsis, extracellular adenosine levels reach between 4-10 mM (108). During these times of uncontrolled inflammation or immune activation it may be beneficial to limit neutrophil activation and infiltration. At low adenosine concentrations the A_1 receptor is activated on neutrophils and can promote adhesion, increase chemotaxis, and stimulate phagocytosis (107;109-111). Some of these responses can be mimiced with N6-phenylisopropyladenosine, an A_1 receptor agonist (112). However, adenosine may be more effective in reducing neutrophil migration to sites of injury. The A2 receptor, which is activated at higher concentrations of adenosine, decreases stimulated neutrophil adhesion and injury to the vascular endothelium (112;113). Adenosine, acting through the A2 receptor, modulates activated neutrophil rolling by interfering with L-selectin-dependent neutrophil adhesion (113;114) and inhibiting other adhesion proteins on the surface that are normally upregulated during neutrophil activation (115;116). The inhibition of adhesion proteins on activated neutrophils as well as vascular endothelial cells by adenosine results in a reduction of neutrophil migration and accumulation into an inflamed site (117;118).

Adenosine may also directly reduce neutrophil-mediated tissue damage. Cronstein *et al* (112) demonstrated that both adenosine, and the adenosine A2 receptor agonist, NECA, inhibited neutrophil H_2O_2 production. Acting through the A_{2A} receptor, adenosine produced by activated neutrophils acts as an endogenous inhibitor of superoxide generation (119;120). These data suggested a primary role for A2 receptor mediation in biological systems, because the natural nucleoside adenosine has consistently been shown to be an inhibitory modulator of other neutrophil functions, including TNF-stimulated lactoferrin secretion (121), degranulation (122), phagocytosis (22), and cytokine stimulated respiratory burst (123).

4.3.3. T-cells

Macrophage-T cell interactions are central to the development of the immune response. Each cell type influences the function of the other. T lymphocytes are important mediators of cell-mediated and humoral immunity. The type of response mounted depends on the differentiation of helper T cells into Th1 or Th2 cells which is directed by a variety of factors including local cytokine concentrations and antigen load and mode of presentation (124). The Th1 response is more efficient at eradicating infections agents, but a prolonged or exaggerated Th1 response often results in host damage. The development of a Th2 response may act as an inhibitory mechanism to limit the harmful effects of an inappropriate and/or exaggerated Th1 response (125). T cells are classified by their cytokine profiles. Th2 cells preferentially produce IL-4, IL-5, and IL-13 as opposed to IFN-gamma and IL-12 which are typical of the Th1-type response. A dominant Th2 response is seen in both human and rodent models of sepsis (126;127).

Macrophage IL-12 production is a driving force behind the development of the Th1 response. Therefore a decrease in macrophage IL-12 production, as is caused by adenosine (100), may contribute to the inhibition of the Th1 type response. In addition IL-10, which is enhanced by adenosine (103), favors the development of a Th2 type response. A shift from Th1 to Th2 cell development will cause a decrease in IFN-gamma production, which may result in further suppression of the immune response due to the loss of IFN-gamma stimulation of macrophages. Adenosine may also indirectly affect T cell function through it's inhibition of MHC-II expression on macrophages (128). T cell activation could be effected in this manner by sub-optimal antigen presentation by the macrophage.

In addition to the possible indirect immunomodulatory actions of adenosine on T cell activation and function, a variety of direct effects have been demonstrated. Activation of the A_{2A} receptor on T cells results in a series of anti-inflammatory effects including: inhibition of TCR-triggered proliferation, pro-inflammatory cytokine expression, granule exocytosis, and FasL expression (129;130;130). The A2 adenosine receptor CGS21680 inhibits T-cell proliferation through suppression of TCR-triggered IL-12 receptor upregulation (130). It has also been postulated that adenosine signaling is responsible for the T-cell depletion seen in mice lacking the adenosine deaminase gene (131). Because of the critical role that A_{2A} receptors play in the down-regulation of inflammation and T cell function it is important that its expression be tightly regulated. Recent studies suggest that there is no adenosine receptor reserve in T-lymphocytes as evidenced by the 50% decrease of total receptor expression seen in A_{2A} deficient mice as compared to wild type. In addition T lymphocytes from A_{2A} deficient mice showed only 50% of the maximal functional response (132).

Because of the critical role that the A_{2A} receptor plays in the downregulation of inflammation and T cell function it has been suggested that transcriptional and translational regulation of the A_{2A} receptor may play an important role in determining how T-cells respond to adenosine (132). T-cells have been shown to predominantly

express the A_{2A} receptor and respond to adenosine treatment by accumulating cAMP (132;133). When treated with adenosine or CGS21680, an A_{2A} receptor agonist, T cells harvested from $A_{2A}R^{+/-}$ mice responded with significantly less cAMP accumulation as compared to $A_{2A}R^{+/+}$ mice. In addition, CGS21680 was only half as effective in triggering apoptosis in thymocytes from $A_{2A}^{+/-}$ mice. These data suggest a lack of reserve of A_{2A} receptors in thymocytes or mature T cells and that changes in the number of active genes directly correlates to the number of functional A_{2A} receptors (132).

5. THEREUTIC PARADIGMS

The potential methods that have been employed to exploit the many actions of adenosine are numerous. Generally, these fall into one of three categories: the use of adenosine itself or a precursor, receptor agonists and antagonists, and manipulation of adenosine metabolism. Any one of these, or some combination, may prove useful in the variety of clinical presentations that fall within the broad entity that is SIRS. All are being actively investigated.

5.1. Adenosine or Adenosine Precursors

While adenosine itself has been explored as a pharmacological strategy to manage inflammatory insults, it has a very short half-life owing to its cellular uptake and phosphorylation, or rapid extracellular deamination. This limits the usefulness of the nucleoside itself to rapidly resolved conditions, such a supraventricular tachycardia. Thus, the beneficial effects have been relatively moderate and unimpressive (134-137). Acadesine (5-aminoimidizole-4-carboxamide), a cell permeable nucleoside compound, has been used as an adenosine precursor to provide longer-lasting effects attributable to adenosine. Acadesine is rapidly metabolized to acadesine monophosphate by adenosine kinase (Fig 1). The nucleotide then enters the purine *de novo* synthesis pathway to form, IMP, and thereafter is distributed throughout the systemic nucleotide pool. Under conditions wherein adenosine formation is favored, it thus increases available substrate to form adenosine. Most of the beneficial actions of acadesine have been shown to be adenosine-mediated, but increased adenine nucleotide concentrations have also been implicated via purinergic receptors and activation of AMP-dependent kinase (138). The use of acadesine has been explored most thoroughly for use in treating ischemia-reperfusion-associated injury, especially in the heart, but Proctor and colleagues have seen some success with its use in endotoxin shock and sepsis (139;140).

5.2. Agonists and Antagonists

Employment of adenosine agonists and antagonists is a useful means of targeting specific adenosine receptor subtypes with the goal of achieving selected effects. However, the outcomes are not always clear. Hasko *et al* (103) reported similar effects of A_1, A2 or A_3 agonists in countermodulating TNF-alpha and IL-10 responses in a murine endotoxemia model. In contrast, Yao *et al* (141) found that an $_{A1}$ receptor-selective antagonist improved renal function during endotoxemia in pigs. Improved survival after LPS was demonstrated with an A_3 receptor agonist, and the effectiveness of the A_3 agonist to attenuate LPS-induced increases in IL-12, interferon-gamma, and plasma nitrate and nitrite concentrations could be blocked with an A_3-specific antagonist (142;143). Such similarities may simply represent cross-over activation of untargeted receptor subtypes, or may indicate redundancy of actions between the receptors. Some indications of both ideas have been found. Mice with genetic deletion of either A_1 (144) A_{2A} (145), or A_3 (79) receptors have been shown to demonstrate increased mortality to LPS, indicating all three receptors influence the response to inflammatory insults. Receptor cross-over has also been suggested in some of these knock-out models.

The drawback to this approach is the ubiquitous distribution and widely varied actions of each of the adenosine receptors that can produce undesirable side-effects. Even though some selectivity can be achieved, crossover activation of receptors not targeted, and the inability to effectively metabolize or rapidly eliminate some agonists or antagonists, can undermine the desired actions. For example, intravenous administration of phenylisopropyladenosine, which preferentially activates adenosine A_1 receptors, can produce hypotension normally attributed to A2 receptor activation (146).

5.3. Manipulating Adenosine Metabolism

With few exceptions, extracellularly active quantities of endogenous adenosine are not produced in most tissues and organs under physiological conditions. As a result, manipulating adenosine concentrations by interfering with aspects of its catabolism or salvage can provide insight into the physiological functions in which endogenous adenosine becomes important when homeostasis is disturbed by pathological events, as in sepsis and SIRS. The underlying premise is that modifying the metabolism or salvage of adenosine effectively modifies its regional concentration commensurate with the net balance toward adenosine production at the site. Therefore, only regions producing relevant quantities of adenosine would be affected.

5.3.1. Adenosine Kinase

One approach has used the inhibition of adenosine kinase to prevent the phosphorylation of adenosine, slowing its reentry into the nucleotide pool (Fig 1). Cronstein *et al* (60) examined the effect of inhibiting adenosine kinase on leukocyte accumulation and TNF-alpha production induced by carrageenan. Pre-treatment of rats with oral GP-1-515, an adenosine kinase inhibitor reduced leukocyte accumulation and TNF-alpha production in skin pouches injected with carrageenan. The direct involvement of adenosine in this response was demonstrated by reversal with either excess exogenous adenosine deaminase (to remove endogenous adenosine) or with an adenosine A2 receptor antagonist. Inhibition of adenosine kinase has also proved effective in treating aspects of the inflammatory response during sepsis. Administration of GP-1-515 in LPS-challenged mice reduced circulating TNF-alpha levels and attenuated pulmonary neutrophil accumulation (113). These effects

were reversed by an adenosine antagonist. These authors also reported that GP-1-515 reduced mortality in a model of bacterial peritonitis. Similar to what is seen adenosine treatment *in vitro*, plasma levels of TNF-alpha were significantly lower compared to control mice (118).

While the inhibition of adenosine kinase can be used to increase interstitial adenosine concentrations, this approach allows the increased endogenous adenosine to enter the xanthine oxidase pathway, which may increase oxyradical formation and tissue damage, It also prevents adenosine salvage into nucleotides by this most direct pathway. Inhibition of adenosine kinase may also obviate its' combined use with the adenosine precursor, acadesine, owing to the necessary function of adenosine kinase.

5.3.2. Adenosine Deaminase

Adenosine deaminase is often considered a secondary pathway for removal of adenosine concentrations, because nucleoside salvage is extremely efficient. Even under conditions of ischemia or hypoxia, vascular endothelial cells and erythrocytes effectively limit the appearance of adenosine in plasma (147;148). However, the recognition that the inability to express adenosine deaminase, or express certain mutant versions of adenosine deaminase, is the second most common cause of severe combined immunodeficiency syndrome (SCIDS) underscored the importance of this enzyme (149).

Inhibition of adenosine metabolism by pentostatin *in vivo* has been shown to increase survival from septic insults. Use of 2-deoxycoformycin (dCF) in mice to inhibit adenosine deaminase concomitant with cecal ligation and puncture significantly improved survival (57). When dCF was administered after signs of significant illness (leukopenia, tachycardia, piloerection, lethargy) significant improvement in 6-day survival during SIRS associated with septic peritonitis (from approximately 40% to 90%) was observed (30;104). Nearly identical improvement in survival was achieved when dCF was administered as much as 24 hours prior to a septic insult. This suggests that inhibition of adenosine deaminase may be a viable means of reducing the incidence of SIRS in predisposing conditions.

Inhibition of adenosine deaminase reduced proinflammatory cytokines, including TNF-alpha and IL-1beta, in multiple models of sepsis and SIRS (30;57;104). These effects could not be explained exclusively by an adenosine-mediated increase in IL-10. Blockade of adenosine receptors did not only prevent the effects of adenosine deaminase inhibition; the septic sequelae were exacerbated by non-selective adenosine receptor blockade with 8-SPT (30). With 8-SPT, amplification of proinflammatory cytokine responses, tissue peroxidation, and gross pathological signs of peritonitis were evident, providing evidence that endogenously produced adenosine was providing some natural protection.

Leukocytosis, a common indicator of the SIRS response, was not present in dCF-treated rats after the induction of sepsis (104). Cohen *et al* (57) found that adenosine deaminase inhibition with dCF strongly abrogated the sepsis-induced increase in leukocyte rolling and adhesion distal to a septic focus. In a different model of SIRS secondary to diffuse peritonitis the infiltration of leukocytes to the tissue was reported to be widespread and occurred at sites distal to the septic insult. This was markedly reduced with inhibition of adenosine deaminase (104). Such effects on leukocyte activation and migration may be responsible for the reported dCF-elicited reductions in tissue peroxidation, extravasation of fluid, alveolar disruption, and mesenteric hemorrhage associated with SIRS in these models (30;57;104). Lymphocyte apoptosis is a common component of septic shock and is usually associated with a poor outcome (150). In stark contrast to the extensive apoptosis that was reported in septic rats, no evidence of lymphocyte apoptosis was observed in septic rats treated with adenosine deaminase inhibition (104).

Cardiovascular benefits of adenosine deaminase inhibition during SIRS have also been reported. Overt signs of mesenteric vascular damage included phagocytosis of erythrocytes in mesenteric lymph nodes in SIRS associated with peritonitis (104), and distant vascular damage indicated by increased FITC-albumin leakage in cremaster vessels (57). Inhibition of adenosine deaminase at the same time as the insult (57) or after waiting until the appearance of clinical signs (104) was able to abrogate these changes. Cardiac-specific increases in TNF-alpha expression associated with sepsis or direct LPS challenge to the heart have recently been found to be attenuated with dCF, as was deteriorating cardiac function (Figure 4).

Initial results with inhibition of adenosine deaminase to manage septic sequelae have been extremely encouraging. Still, caution has been expressed owing to the known pathological consequences of adenosine deaminase deletion or mutation in humans and mice. Some of this pathology has been attributed to increased adenosine concentrations, and some have suggested that adenosine may contribute to immune hyporesponsiveness late in sepsis (151). However, used judiciously, the pharmacological inhibition of adenosine deaminase is transient (104;152) and has not been associated with any indication of immunosuppression (30;104). This approach also has the added advantage of limiting adenosine entry into the xanthine oxiadase pathway and the resulting generation of oxyradicals.

6. PERSPECTIVE

Adenosine has been shown to play important roles in nearly every organ system and tissue type. Metabolism of adenosine , and its influences on cardiac and vascular control, and innate immune function, influence all physiological systems, making them integral parts of the pathophysiological responses to septic insults, and subsequent SIRS (Figure 4). The therapeutic usefulness of adenosine receptor-mediated actions are beginning to be explored, but the complex, multimechanistic, redundant nature of this purine nucleoside and its actions will require consideration of any therapeutic modality from the vantage of the integrated organism, as well as specific response

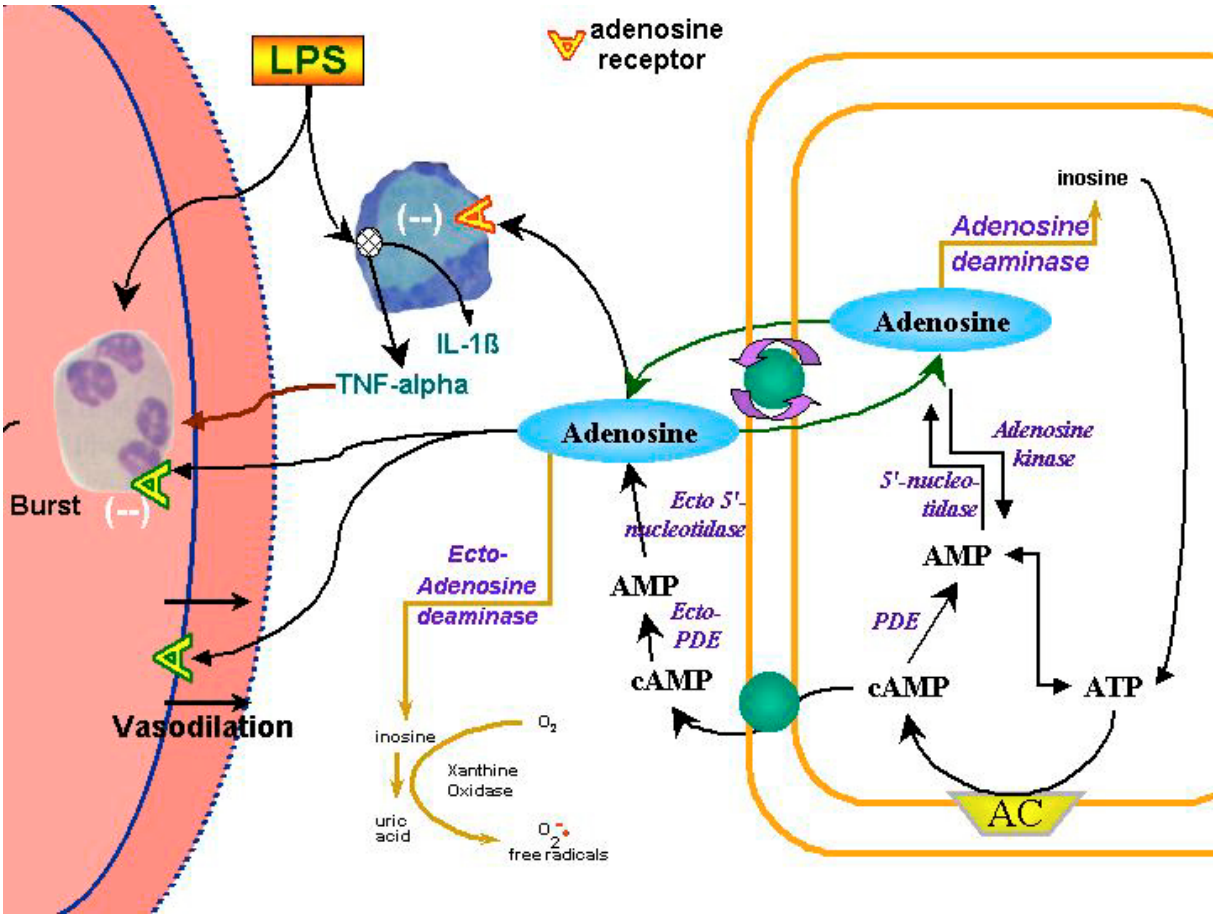

Figure 4. Adenosine is a Plurisystem Modulator. Adenosine is a plurisystem mediator/modulator, influencing responses in various cell and tissue types, and via numerous receptor and cell signaling pathways. This figure presents a partial summary of interrelated system effects of adenosine that exemplify the potential roles during conditions associated with systemic inflammatory response syndrome (SIRS). Adenosine can be generated by intracellular and extracellular enzyme pathways depending upon the specific and unique conditions giving rise to elevated extracellular concentrations. Both equilibrative and concentrative adenosine transport proteins can move adenosine across cellular membranes, influencing extracellular adenosine concentrations. the enzymes involved in adenosine metabolism in and out of the cell are covered in Figures 1&2. LPS and other inflammatory stimuli activate innate immune cells, including macrophages and neutrophils. Paracrine and autocrine communication through cytokines can lead to an exaggerated inflammatory response that moves beyond the confines of the locale wherein the inflammatory stimulus originated. By direct actions on innate immune cells, and interruption intracellular signaling molecules, the inflammatory process is generally abated, but not ablated by adenosine. In the regions where it is produced in sufficient quantities, adenosine can also cause regional vasodilation by direct actions on smooth muscle, of by influencing NO synthase. The latter is influenced both by adenosine stimulation of NOS activity, and altering expression of the iNOS protein.

systems. However, there is considerable promise in the approaches that have been explored to date, and one of these, some combination thereof, or new methods of exploiting this versatile molecule, are likely to provide some measure of protection from sepsis and SIRS in the future.

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