Regulation of Macrophage Function by Adenosine

György Haskó, Pál Pacher

Abstract—Following its release into the extracellular space in response to metabolic disturbances, the endogenous nucleoside adenosine exerts a range of immunomodulatory effects and cells of the mononuclear phagocyte system are among its major targets. Adenosine governs mononuclear phagocyte functions via 4 G-protein–coupled cell membrane receptors, which are denoted $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors. Adenosine promotes osteoclast differentiation via $A_1$ receptors and alters monocyte to dendritic cell differentiation through $A_{2B}$ receptors. Adenosine downregulates classical macrophage activation mainly through $A_{2A}$ receptors. In contrast $A_{2B}$ receptor activation upregulates alternative macrophage activation. Adenosine promotes angiogenesis, which is mediated by inducing the production of vascular endothelial growth factor by mononuclear phagocytes through $A_{2A}$, $A_{2B}$, and $A_3$ receptors. By regulating mononuclear phagocyte function adenosine dictates the course of inflammatory and vascular diseases and cancer. (Arterioscler Thromb Vasc Biol. 2012;32:865-869.)

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Monocytes, macrophages, and dendritic cells (DCs) are central components of the mononuclear phagocyte system and arise by passing through the common myeloid precursor, the granulocyte/macrophage precursor, and the macrophage/DC progenitor stages in the bone marrow. Monocytes and macrophages possess remarkable heterogeneity, which is related to their origin, phenotype, tissue localization, and function. Monocytes circulate in the blood and then enter tissues to become macrophages or DCs, where they phagocytose and kill bacteria, scavenge toxic compounds, and present antigens to T lymphocytes. Thus, cells of the mononuclear phagocyte system are major components of the inflammatory response of tissues to infection and injury. Monocytes and macrophages secrete cytokines, free radicals, prostanooids, antimicrobial peptides, proteinases, and angiogenic factors to alarm the tissue to the presence of infectious and injurious stimuli and to orchestrate the ensuing inflammatory response. This inflammatory response is central to ridding the tissue of the infectious or injurious agent and then to laying the groundwork for the restoration of tissue homeostasis. When these processes go awry, tissue homeostasis remains disturbed and chronic inflammatory diseases, such as atherosclerosis, diabetes, and autoimmune diseases, develop. In addition to orchestrating inflammation, macrophages are also important for both the development and normal cellular turnover of organs by virtue of their ability to phagocytose apoptotic cells, cellular debris, and matrix. The goal of this review article is to highlight the role of the adenosine receptor system in regulating mononuclear phagocyte function. We discuss the role of adenosine receptors in governing monocyte differentiation, classical and alternative macrophage activation, and the angiogenic switch.

Adenosine and Its Receptors

Extracellular adenosine generates a constellation of tissue responses that can be generally viewed as organ protective thereby mediating homeostasis. Following its release from the intracellular into the extracellular space, adenosine exerts a range of immunomodulatory effects and cells of the mononuclear phagocyte system are among its major targets. Adenosine is an essential component of intracellular metabolic pathways and can be released into the extracellular space in response to both metabolic disturbances and other types of insults, which include, inflammation, physical damage, and apoptosis. The release of adenosine can occur by 2 mechanisms: via cell membrane embedded adenosine/nucleoside transporters and as constituent of ATP and ADP, which can be released by a number of mechanisms, including membrane damage, through connexin/pannexin and other channels, and via protein or hormone-transporting vesicles. Once ATP and ADP are released, the phosphate groups of extracellular ATP and ADP are sequentially split off, first by nucleoside triphosphate diphosphohydrolases (NT-PDases, including CD39) and then by 5′-ectonucleotidase (Ecto5′Ntase, CD73). In addition to host cells, pathogens such as Staphylococcus aureus, Enteropathogenic Escherichia coli, and Trichomonas vaginalis are equipped with ectonucleotidases, and excessive adenosine generation by these ectonucleotidases represents a means for these pathogens to subvert the

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host immune system thereby aiding their propagation. Adenosine governs cellular functions via 4 G-protein–coupled cell membrane receptors, which have been denoted A₁, A₂A, A₂B, and A₃ receptors.⁶ In general, A₁ and A₃ receptors are coupled to pertussis toxin-inhibited Gₛ-coupled signal transduction proteins and their activation leads to decreased intracellular cAMP levels. A₂A receptors are traditionally appreciated as Gₛ₅- or Gₛ₆₄-linked receptors that stimulate adenylyl cyclase, cAMP accumulation, and protein kinase A activation, but A₂A receptors can also signal independently of G proteins. A₂B receptors can couple to both Gₛ₆ and Gₛ. It is now appreciated that adenosine receptor expression is dynamically altered during both development and activation on the surface of macrophages and DCs. The differential expression of adenosine receptors at various stages of inflammation is important for fine-tuning adenosine receptor responsiveness to maximize their ability to alter cell function in a way that generally leads to the restoration of homeostasis.

**Adenosine and Monocyte Differentiation**

Adenosine has been shown to regulate the differentiation and maturation of cells of the monocellular phagocyte system. In the presence of adenosine, phorbol myristate acetate-differentiated monocytes develop into multinucleated giant cells via A₁ receptor stimulation, whereas A₂ receptor activation prevents the generation of giant cells.¹¹ Similarly, A₁ receptor activation promotes the formation of multinucleated osteoclasts from monocytes differentiated with colony stimulating factor-1 and receptor activator of NF-κB ligand (RANKL).¹² Consistent with these in vitro results, A₁ receptor inactivation prevents bone loss in a murine model for postmenopausal osteoporosis.¹³

Recent evidence indicates that A₂B receptor stimulation induces aberrant development of DCs from granulocytomacrophage colony-stimulating factor and interleukin (IL)-4–differentiated hematopoietic progenitor cells and monocytes.¹⁴ These “adenosine-differentiated” DCs develop into a cell type that fails to downregulate its surface expression of the monocytic marker CD14 despite upregulating the DC marker CD1a. These aberrant DCs are impaired in their ability to induce T-cell proliferation and are proangiogenic and have been recently shown to contribute to tumor growth in a variety of cancer models.¹⁵,¹⁶

**Adenosine Regulation of Classical Macrophage Activation**

The phenotype and activation state of mature macrophage populations is defined by chemical cues encountered during the differentiation process, which include growth factors, microbial pathogen-associated molecular patterns, tumor products, extracellular matrix, as well as T helper (Th1) or Th2 effector T cells and their secretory products. The well-established “classical activation” or proinflammatory macrophage profile occurs in a Th1 cytokine-rich environment (interferon-γ, tumor necrosis factor [TNF]-α) or on recognition of pathogen-associated molecular patterns (lipopolysaccharide, lipoproteins, dsRNA, lipo-teichoic acid, etc.) and endogenous “danger” signals (heat shock proteins, etc.).¹⁷ As such, the proinflammatory phenotype of these macrophages plays an important role in protection against intracellular pathogens, and under certain conditions also cancer cells. Classically activated macrophages (caMφ or M1) typically produce high levels of TNF-α and IL-12 and low levels of IL-10 and are consequently strong promoters of Th1 immune responses.¹⁸ In addition, these cells exert antiproliferative and cytotoxic activities, resulting partly from their ability to secrete reactive nitrogen and oxygen species (nitric oxide, peroxynitrite, hydrogen peroxide, superoxide) and proinflammatory cytokines (TNF-α, IL-1, IL-6).

Consistent with its generally restorative function in tissues, adenosine receptor activation has been repeatedly shown to have effects that prevent excessive classical macrophage activation thereby resulting in tissue protection (Figure). Although adenosine has been shown to decrease expression of several proinflammatory mediators by macrophages, including IL-12,¹⁹ macrophage inflammatory protein-1α,²⁰ nitric oxide,²¹,²² and superoxide,²³ the best understood of these antiinflammatory effects is the capacity of adenosine to decrease the production of TNF-α. Results of both human and murine studies confirmed that adenosine suppresses monocyte and macrophage TNF-α production.²⁴ The dominant adenosine receptor mediating this suppressive effect is the A₂₅ receptor,²⁵,²⁶ but based on results with both selective A₂A and A₂B receptor ligands and A₂A and A₂B receptor knockout macrophages, it is now apparent that A₂₅ receptors can also contribute, especially in murine systems.²⁵,²⁶,²⁷ These results are also consistent with observations that the expression of A₂₅ receptor predominates over A₂B in human macrophages²⁸ and expression of both A₂₅ and A₂B receptors is highest in murine macrophages.²⁵,²⁶

The intracellular mechanisms of suppression, however, remain incompletely understood. In murine alveolar macrophages stimulated with hyaluronan fragments, adenosine receptor activation decreased TNF-α mRNA levels via exchange protein activated by cAMP (Epac)-1, but independently of PKA.²⁷ In contrast, in murine peritoneal macrophages stimulated with lipopolysaccharide, adenosine receptor stimulation suppressed TNF-α mRNA transcription independently of both Epac and
PKA. Surprisingly, the adenosine suppression of TNF-α production was reversed by okadaic acid indicating involvement of phosphatases. Finally, a recent line of investigation uncovered an important role for heme oxygenase-1 and its product carbon monoxide in contributing to the adenosine-mediated suppression of TNF-α production by macrophages. Studies by Haschemi et al.33 and Weis et al.34 have revealed that heme oxygenase-1 and carbon monoxide upregulate the expression of A2A receptors on macrophages and these increased levels of A2A receptors, in turn, sensitize macrophages to the TNF-α-suppressing effect of adenosine.

In contrast to the suppressive effect of adenosine on the production of proinflammatory mediators, adenosine augments production of the antiinflammatory cytokine IL-10.3 We recently demonstrated a pivotal role for A2A receptors, as adenosine failed to upregulate E. coli-induced IL-10 secretion by murine peritoneal macrophages lacking A2A receptors but not WT macrophages.35 This upregulation of IL-10 production was achieved through a CCAAT-enhancer-binding protein β-mediated transcriptional process. In the RAW264.7 macrophage cell line, which expresses low basal levels of A2A receptors, adenosine augments IL-10 production via an A2B receptor-mediated posttranscriptional mechanism.36

In an inflammatory environment, ATP supply by anaerobic glycolysis is an essential source of energy for macrophages. In classically activated macrophages, adenosine increases glycolytic flux by upregulating expression of the pfkb3 isozyme of 6-phosphofructo-1-kinase.37 Both A2A and A2B receptors are involved in mediating the effect of adenosine and the transcription factor Specificity Protein 1 appears to play a central role in the adenosine induction of pfkb3 expression. Thus, in addition to deactivating classically activated macrophages, A2A and A2B receptors support macrophage metabolism, which may enable the macrophages to persist in the tissue and switch their phenotype to alternatively activated macrophages to participate in tissue restoration.

Adenosine Regulation of Alternative Macrophage Activation

In contrast to a Th1 milieu, a Th2 cytokine-rich environment, which arises during parasitic disease and wound healing imparts immunomodulatory and antiinflammatory rather than proinflammatory properties on macrophages. These macrophages generated in the presence of the Th2 cytokines IL-4 and/or IL-13 are termed alternatively activated or M2a.17,38 Exposure to Th2 cytokines, such as IL-4 and IL-13 triggers a specific gene expression pattern in macrophages, and the most prominent gene that is induced is arginase-1.39 Arginase-1 metabolizes arginine to urea and ornithine, and ornithine can then be used for proline and collagen synthesis resulting in extracellular matrix deposition and fibrosis.40 Importantly, arginase-1 can outcompete the other major arginine-utilizing enzyme, inducible nitric oxide synthase, for the substrate in alternatively activated macrophages, and, thus, decrease nitric oxide production. Other genes that are often induced are genes whose products are involved in matrix remodeling such as tissue inhibitor of metalloproteinases-1 (TIMP-1).41 TIMP-1 regulates tissue remodeling by inhibiting extracellular matrix-degrading matrix metalloproteinases. In addition, TIMP-1 has complex effects on cell growth and mutations in the matrix metalloproteinases-inhibitory domain of TIMP-1 fail to abrogate the effects of TIMP-1 on cell growth and survival. Finally, macrophage galactose-type C-type lectin (mgl)-1,42 Ym1 (chitinase-like-3), and found in inflammatory zone (Fizz)1 are also signature markers of alternatively activated macrophages.43

The effect of adenosine on alternative macrophage activation is less broad than the wide-ranging inhibitory effects of adenosine on classical macrophage activation, as adenosine increased the expression of arginase-1, TIMP-1, and mgl-1, but not that of Ym1 and Fizz1. The stimulatory effects of adenosine on alternative macrophage activation are mediated chiefly by A2B receptors, which contrasts with the predominant role of A2A receptors in regulating classical macrophage activation.41 Of the transcription factors known to mediate alternative macrophage activation, CCAAT-enhancer-binding protein β was required, whereas cAMP response element-binding protein and signal transducer and activator of transcription 6 were dispensable in mediating the stimulatory effect of adenosine.44

The term alternative macrophage activation is sometimes more widely used and describes various anti-inflammatory macrophage phenotypes induced by immune complexes (M2b), as well as heterogeneous deactivating stimuli such as apoptotic cells, glucocorticoids, and IL-10 (M2c).38 In macrophages engulfing apoptotic cells, endogenously released adenosine activates A2A receptors and suppresses the production of the proinflammatory chemokines macrophage inflammatory protein-2 and cytokine-induced neutrophil-attracting chemokine (KC).44 In addition, glucocorticoids induce survival of anti-inflammatory monocytes via upregulation and autocrine stimulation of A1 receptors.45 Thus, in macrophages exposed to apoptotic cells and glucocorticoids, adenosine shifts macrophage phenotype from proinflammatory to antiinflammatory.

Adenosine and the Angiogenic Switch

Vascular endothelial growth factor (VEGF) is a potent inducer of angiogenesis and vascular permeability and is vital for endothelial cell differentiation during vasculogenesis, and for the sprouting of new capillaries from preexisting blood vessels.46 VEGF is thus an important component of tissue repair and is critical for the resolution of injury through the process of wound healing. Macrophages are primary producers of VEGF during wound healing, as well as in chronic inflammation and cancer.47

There is an increasing line of evidence that adenosine can accelerate angiogenesis, in part, by augmenting VEGF production by macrophages.48-50 Because adenosine boosts VEGF production and decreases proinflammatory cytokine production by toll-like receptor-activated murine macrophages, thereby shifting macrophages away from a proinflammatory to an angiogenic phenotype, we termed this process angiogenic switch.50 The synergistic stimulatory effect of adenosine and...
TLR activation on VEGF production is A2A receptor-dependent in murine macrophages. The stimulatory effect of adenosine on VEGF production involves transcriptional induction of the VEGF promoter by the transcription factor hypoxia-inducible factor-1α through hypoxia response element. Unlike hypoxic stabilization of hypoxia-inducible factor-1α protein, adenosine increases the activation of hypoxia-inducible factor-1α by up-regulating its mRNA levels through a mechanism that is mostly post-transcriptional and is controlled by upstream activation of PKC and phosphoinositide 3-kinase.

Adenosine can induce VEGF mRNA level upregulation in and VEGF secretion by human monocytes and macrophages and pathogen-associated molecular patterns and adenosine additively or synergistically increase VEGF production by human macrophages. The adenosine-mediated increase in VEGF secretion by human monocytes appears to be mediated by A2A, A2B, and A3 receptors and appears to be secondary to increased hypoxia-inducible factor-1α expression.

Finally, it is noteworthy that VEGF induction is likely the most important but not the only mechanism by which adenosine promotes angiogenesis. For example, adenosine has recently been shown to inhibit the macrophage production of sFlt, which is the antiangiogenic, soluble form of the VEGF receptor.

Conclusions
As indicated above, adenosine receptors modulate disease activity in a wide range of pathophysiological processes that involve mononuclear phagocytes, such as tumors, bone disease, and wound healing. There is also emerging evidence that cells of the mononuclear phagocyte system are important components of the protective effects of adenosine receptor agonists against vascular disease. For example, recent evidence shows that A2A receptor stimulation reduces inflammation and neointimal growth in a murine carotid ligation model and that protection is associated with decreased macrophage recruitment and function. Similarly, A2B receptors prevented neointima formation following femoral artery injury, and the protective action of A2B receptors was paralleled by attenuated levels of macrophage-derived proinflammatory cytokines. In contrast to these adenosine receptor-mediated protective effects, A3 receptors were found to contribute to the formation of atherosclerotic lesions in apolipoprotein E-deficient mice, which was explained by an A2A receptor-induced decrease in macrophage (foam cell) apoptosis.

A better understanding of how mononuclear phagocyte function is regulated by adenosine receptors will assist in the design of novel therapeutic modalities to treat vascular and inflammatory diseases as well as cancer.

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Disclosures
None.

References
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