

New insights into the regulation of inflammation by adenosine

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Commentary

Adenosine, long known as a regulator of cardiovascular function, has recently been identified as a significant paracrine inhibitor of inflammation that acts primarily by activation of A_{2A} adenosine receptors ($A_{2A}ARs$) on lymphoid or myeloid cells. In this issue of the *JCI*, Yang et al. describe a proinflammatory phenotype resulting from deletion of the gene encoding the A_{2B} adenosine receptor ($A_{2B}AR$) in the mouse, suggesting that activation of the $A_{2B}AR$ can also have antiinflammatory effects (see the related article beginning on page 1913). Nevertheless, the role of the $A_{2B}AR$ remains enigmatic since its activation can either stimulate or inhibit the release of proinflammatory cytokines in different cells and tissues.

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(9). Other genes are regulated selectively by either LXR (e.g., ABCA1) or SREBP-1c (e.g., FAS). Dietary polyunsaturated fatty acids suppress both LXR and SREBP-1c but are also crucial to StAR expression (6). It is notable that the proteolytic activation of SREBP-1c, unlike that of other SREBPs, is regulated by insulin and probably not suppressed by cholesterol (10).

Little is known about the physiological ligands for LXR. However, there is evidence that mitochondrial cholesterol metabolism may be a source of these activators. StAR may therefore also participate as an activator of LXR activity. The steroid intermediate 22R-hydroxycholesterol, which is formed by CYP11A1, is a potent LXR agonist but is unlikely to be released from the mitochondrial cholesterol-cleavage process. However, StAR can also mediate cholesterol transfer to mitochondrial cholesterol hydroxylases that may generate LXR agonists. Other members of the StAR family — StARD4 and StARD5 — exhibit cholesterol transfer activity in steroidogenic cells and macrophages, respectively (11). Interestingly, elevation of these StAR relatives (and perhaps also of StAR) causes LXR activation, possibly by forming a hydroxysterol agonist. Hydroxysterols also stimulate StAR

expression in steroidogenic cells (12), possibly through this new LXR mechanism.

This connection between LXR and StAR, introduced in this issue of the *JCI* by Cummins et al. (5), provides a new avenue for regulation of steroid synthesis. This may extend to other steroidogenic processes, including testosterone synthesis in the Leydig cells of the testis, estrogens in the ovary, and even neurosteroids produced in glial cells of the brain, each of which utilize StAR. It should be noted that cholesterol homeostasis in human adrenals is primarily mediated by LDL rather than HDL (13). It remains to be determined whether the LXR gateway to StAR and steroid synthesis remains open in tissues where cholesterol fluxes are less than in the adrenal or when the LDL pathway partially bypasses the lipid droplets (see Figure 1).

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New insights into the regulation of inflammation by adenosine

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Adenosine, long known as a regulator of cardiovascular function, has recently been identified as a significant paracrine inhibitor of inflammation that acts primarily by activation of A_{2A} adenosine receptors (A_{2A}ARs) on lymphoid or myeloid cells. In this issue of the *JCI*, Yang et al. describe a proinflammatory phenotype resulting from deletion of the gene encoding the A_{2B} adenosine receptor (A_{2B}AR) in the mouse, suggesting that activation of the A_{2B}AR can also have antiinflammatory effects (see the related article beginning on page 1913). Nevertheless, the role of the A_{2B}AR remains enigmatic since its activation can either stimulate or inhibit the release of proinflammatory cytokines in different cells and tissues.

Nonstandard abbreviations used: A_{2A}AR, A_{2A} adenosine receptor; A_{2B}AR, A_{2B} adenosine receptor.

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There is growing interest in elucidating the mechanisms by which adenosine inhibits the immune system, since these inhibitory adenosine receptors and their downstream signaling pathways are promising targets for new antiinflammatory

therapies. By signaling through the A_{2A} adenosine receptor (A_{2A}AR), adenosine suppresses the immune system, primarily by inhibiting lymphoid or myeloid cells including neutrophils (1), macrophages (2), lymphocytes (3, 4), and platelets (5). These responses are amplified by rapid induction of A_{2A}AR mRNA in macrophages and T lymphocytes in response to inflammatory or ischemic stimuli (2, 3, 6, 7). The A_{2B} adenosine receptor (A_{2B}AR) also appears to mediate antiinflammatory effects in macrophages by inhibiting the production of TNF- α and IL-1 β , stimulating IL-10 and inhibiting macrophage proliferation (8–11) (Figure 1A). Macrophage A_{2B}AR signaling increases during



Table 1
Proinflammatory effects of activating $A_{2B}R$

Cell	Response	References
Mast cell	Degranulation, IL-8 release	22–24
Bronchial smooth muscle	IL-6 and MCP-1 release	25
Intestinal epithelial cell	IL-6 release	26
Astroglioma	IL-6 release	27
Astrocytoma; astrocyte	IL-6 release	27–29
Osteoblast	IL-6 release	30
Lung fibroblast	IL-6 release	31
Pituitary folliculostellate cell	IL-6 release	32
Airway epithelial cell	Prostenoind release	33

MCP-1, monocyte chemoattractant protein 1.

inflammation, as the macrophage-activating cytokine $IFN-\gamma$ causes induction of macrophage $A_{2B}AR$ mRNA (12). However, the $A_{2B}AR$ is somewhat unusual in that it is dually coupled to the generally antiinflammatory G protein G_s and the generally proinflammatory G protein G_q (13); in addition, numerous proinflammatory effects mediated by activation of the $A_{2B}AR$ have also been described (Table 1).

Inflamed phenotype of the $A_{2B}AR$ knockout mouse

Given the opposing cellular effects mediated by $A_{2B}AR$ activation, the phenotype that would result from deletion of the gene encoding $A_{2B}AR$ was uncertain. In this issue of the *JCI*, Yang and coworkers use a receptor knockout/reporter gene

knock-in approach to confirm the expected expression pattern of the $A_{2B}AR$ transcript, noting high levels in monocytes/macrophages and the vasculature (14). Interestingly, they report a moderately inflamed phenotype in the $A_{2B}AR$ knockout mouse at baseline, including elevated adhesion molecule expression on vascular endothelial cells and elevated cytokine production. The inflammatory phenotype is exaggerated in mice given endotoxin. The baseline response to $A_{2B}AR$ knockout is surprising since the $A_{2B}AR$ is recognized to have a low affinity for adenosine, yet the data imply that baseline adenosine levels are high enough to produce some activation of the $A_{2B}AR$. A well-recognized limitation of the global knockout approach is that the resulting phenotype

may reflect a developmental adaptation to the gene knockout and not an acute direct consequence of deleting the gene product. In order to address this possibility, Yang et al. examined the effect of deleting the gene that codes for $A_{2B}AR$ on mRNA levels of the other adenosine receptor subtypes and found no changes. However, other possible adaptations have yet to be examined. It has recently been demonstrated that hypoxia triggers coordinate upregulation on endothelial cells of mRNA for the $A_{2B}AR$ and ectoenzymes involved in the conversion of extracellular adenine nucleotides to adenosine (15) (Figure 1B). In addition, the release of adenine nucleotides from various cells, including neutrophils and endothelial cells, is a regulated process (16). It will be of interest in future studies to determine whether deletion of the gene encoding $A_{2B}AR$ influences either nucleotide release or extracellular nucleotide metabolism. Such effects could indirectly influence inflammation by altering the availability of adenosine to other adenosine receptor subtypes, particularly the $A_{2A}AR$. Deletion of either the ecto-apyrase CD39 (also known as NTPDase1; ref. 17) or the ecto-5'-nucleotidase (also known as CD73; refs. 18, 19) results in a proinflammatory phenotype.

Future questions

It will also be of interest in future studies to examine the response of the $A_{2B}AR$

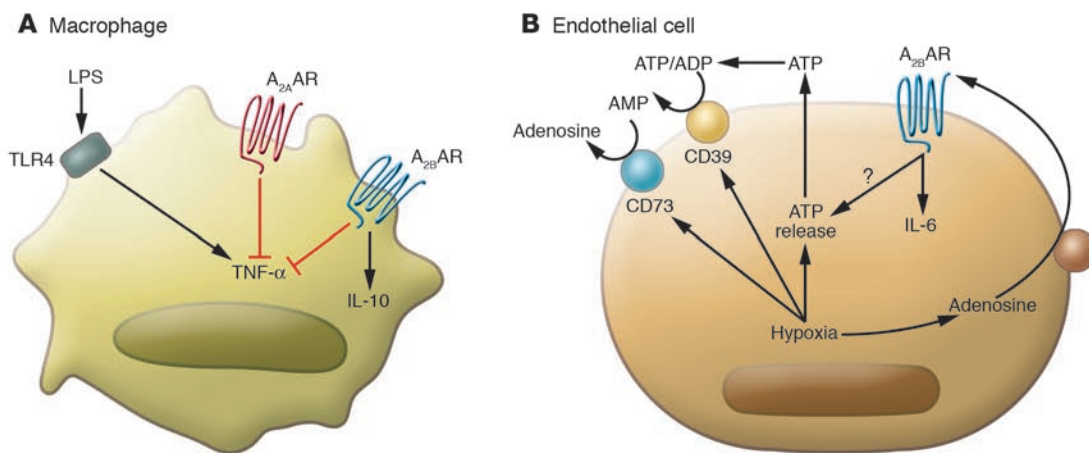


Figure 1

Anti- and proinflammatory effects mediated by activation of the $A_{2B}AR$. (A) In macrophages, the release of $TNF-\alpha$ and other proinflammatory cytokines is inhibited as a result of activating either the $A_{2A}AR$ or the $A_{2B}AR$. These effects are amplified if $TNF-\alpha$ production is stimulated by LPS, which signals through TLR4 and other Toll-like receptors. Activation of the $A_{2B}AR$ also stimulates production of the antiinflammatory cytokine IL-10. (B) In endothelial cells and other cells noted in Table 1, activation of the $A_{2B}AR$ stimulates IL-6 and other proinflammatory cytokines. Hypoxia increases the intracellular production of adenosine, which is transported outside the cell by nucleoside transport proteins. Hypoxia and possibly activation of the $A_{2B}AR$ stimulate the release of ATP and the production of the $A_{2B}AR$ and ectoenzymes (CD39 and CD73) that convert ATP to adenosine. Vasodilation in response to $A_{2B}AR$ activation may increase shear stress to stimulate ATP release.



knockout mouse in the setting of hypoxia or ischemia, which elicit the accumulation of large levels of adenosine. A selective A_{2B}AR antagonist blocks myocardial preconditioning when applied to the isolated rabbit heart after ischemia (20). This could occur either because A_{2B}ARs on the heart mediate cardioprotection or, as discussed above, because A_{2B}AR activation facilitates the release and/or metabolism of adenosine nucleotides to indirectly enhance adenosine production. The latter scheme is consistent with the observation that myocardial preconditioning has a remote adenosine-mediated effect on platelet function (21).

In conclusion, the study by Yang et al. (14) bolsters the conclusion that activation of A_{2B}ARs on certain cells, particularly macrophages, inhibits inflammation. A proinflammatory phenotype noted at rest is somewhat unexpected, and the mechanism underlying this inflammation is not yet known. In view of a number of previous reports indicating that A_{2B}AR activation can be proinflammatory (Table 1), it will be of interest to use the newly available A_{2B}AR knockout mouse in order to determine whether A_{2B}AR activation on different cells can elicit both pro- and antiinflammatory responses.

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