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.
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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1. Zelcer, N., and Tontonoz, P. 2005. Liver X receptor; A


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 A2A adenosine receptors (A2ARs) 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 A2B adenosine receptor (A2B AR) in the mouse, suggesting that activation of the A2BAR can also have antiinflammatory effects (see the related article beginning on page 1913). Nevertheless, the role of the A2BAR remains enigmatic since its activation can either stimulate or inhibit the release of proinflammatory cytokines in different cells and tissues.

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 A2A adenosine receptor (A2AR), 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 A2AR mRNA in macrophages and T lymphocytes in response to inflammatory or ischemic stimuli (2, 3, 6, 7). The A2B adenosine receptor (A2B AR) also appears to mediate antiinflammatory effects in macrophages by inhibiting the production of TNF-α and IL-1β, stimulating IL-10 and inhibiting macrophage proliferation (8–11) (Figure 1A). Macrophage A2B AR signaling increases during

Nonstandard abbreviations used: A2AAR, A2A adenosine receptor; A2B AR, A2B adenosine receptor.

Conflict of interest: The author owns stock in Adenosine Therapeutics, LLC.

inflammation, as the macrophage-activating cytokine IFN-γ causes induction of macrophage A2BAR mRNA (12). However, the A2BAR is somewhat unusual in that it is dually coupled to the generally antiinflammatory G protein Gi and the generally proinflammatory G protein Gq (13); in addition, numerous proinflammatory effects mediated by activation of the A2BAR have also been described (Table 1).

### Inflamed phenotype of the A2BAR knockout mouse

Given the opposing cellular effects mediated by A2BAR activation, the phenotype that would result from deletion of the gene encoding A2BAR 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 A2BAR transcript, noting high levels in monocytes/macrophages and the vasculature (14). Interestingly, they report a moderately inflamed phenotype in the A2BAR 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 A2BAR knockout is surprising since the A2BAR 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 A2BAR. 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 A2BAR 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 A2BAR 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 A2BAR 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 A1AR. 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 A2BAR

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**Table 1**

<table>
<thead>
<tr>
<th>Cell</th>
<th>Response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cell</td>
<td>Degranulation, IL-8 release</td>
<td>22–24</td>
</tr>
<tr>
<td>Bronchial smooth muscle</td>
<td>IL-6 and MCP-1 release</td>
<td>25</td>
</tr>
<tr>
<td>Intestinal epithelial cell</td>
<td>IL-6 release</td>
<td>26</td>
</tr>
<tr>
<td>Astrogloma</td>
<td>IL-6 release</td>
<td>27</td>
</tr>
<tr>
<td>Astrocytoma; astrocyte</td>
<td>IL-6 release</td>
<td>27–29</td>
</tr>
<tr>
<td>Osteoblast</td>
<td>IL-6 release</td>
<td>30</td>
</tr>
<tr>
<td>Lung fibroblast</td>
<td>IL-6 release</td>
<td>31</td>
</tr>
<tr>
<td>Pituitary folliculostellate cell</td>
<td>IL-6 release</td>
<td>32</td>
</tr>
<tr>
<td>Airway epithelial cell</td>
<td>Prostenoïd release</td>
<td>33</td>
</tr>
</tbody>
</table>

MCP-1, monocyte chemoattractant protein 1.
knockout mouse in the setting of hypoxia or ischemia, which elicit the accumulation of large levels of adenosine. A selective A2B AR antagonist blocks myocardial preconditioning when applied to the isolated rabbit heart after ischemia (20). This could occur either because A2B ARs on the heart mediate cardioprotection or, as discussed above, because A2B AR activation facilitates the release and/or metabolism of adenine 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 A2B 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 A2B AR activation can be proinflammatory (Table 1), it will be of interest to use the newly available A2B AR knockout mouse in order to determine whether A2B AR activation on different cells can elicit both pro- and antiinflammatory responses.

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