Adenosine A₂A-Receptor Stimulation Inhibits Lipopolysaccharide-Induced Interleukin-18 Production in Monocytes

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Abstract. Adenosine inhibited interleukin (IL)-18 production in lipopolysaccharide (LPS)-stimulated monocytes. The action of adenosine was antagonized by an adenosine A₂A-receptor (A₂AR) antagonist and was mimicked by an A₂AR agonist, suggesting that the stimulation of A₂AR may be involved in the actions of adenosine. On the other hand, the stimulation of A₁R and A₃R inhibited the actions of A₂AR stimulation, whereas the stimulation of A₂BR had no effect on them. Activation of A₂AR is known to increase cyclic adenosine monophosphate (cAMP) levels and to activate protein kinase A (PKA). A PKA inhibitor prevented the actions of A₂AR stimulation, indicating that the action mechanism of A₂AR stimulation may be via the activation of the cAMP/PKA pathway.

Keywords: adenosine, interleukin-18, monocyte

Lipopolysaccharide (LPS) is a major component of the outer membranes of Gram-negative bacteria, and it induces production of proinflammatory cytokines, leading to development of the sepsis syndrome (1). Recently, we demonstrated the effect of LPS in the concentration range of 1 ng/ml to 1 µg/ml and the kinetic effect of LPS on the production of interleukin (IL)-18, a monocyte-derived cytokine, by monocytes (2). The production of IL-18 was significant at 12 h and reached maximum level at 48 h. LPS at 10 ng/ml, 100 ng/ml, and 1 µg/ml induces the production of IL-18. The cell viabilities of monocytes in the presence of lipopolysaccharide at 1 ng/ml and 1 µg/ml were 88% and 80%, respectively, after 24 h of incubation. Therefore, we chose the following conditions for our experiment: 1 µg/ml as the dose of LPS and 24 h of incubation. IL-18 is located upstream of the secretion of proinflammatory cytokines (3). Recently, we examined whether anti-IL-18 antibody (Ab) inhibited the production of IL-12 by monocytes treated with LPS at 1 µg/ml (2). However, antibodies against IL-12, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α had no effect on the production of IL-18. These results suggested that the production of IL-18 was independent of IL-12, IFN-γ, and TNF-α. In murine models of endotoxemia, IL-18 induces lethal damage and splenocyte apoptosis (4). Therefore, inhibition of IL-18 is thought to reduce death in mice (5). These results indicated that therapeutic strategies aimed at decreasing IL-18 production might be beneficial in the treatment of severe trauma and sepsis.

Adenosine is reported to act as a physiological inhibitor of inflammation via four G-protein-coupled adenosine receptors: A₁, A₂A, A₂B, and A₃ (6). Adenosine levels are elevated during tissue hypoxia and damage associated with sepsis. Adenosine has strong immunosuppressive effects, many of which are mediated by A₂A receptor (A₂AR) expressed on immune cells. Studies have postulated that A₂AR is involved in the inhibitory activity of adenosine against LPS-induced production of TNF-α, a cytokine that exhibits deleterious effects in septic shock (7). In animal models, A₂AR agonists can prevent death from LPS or sepsis (8). Adenosine or A₂A agonists are reported to inhibit the potentially tissue-toxic H₂O₂ production elicited by soluble inflammatory mediators in patients with septic...
shock (9). On the other hand, it is reported that A2AR knockout mice are protected from the lethal effect of sepsis and exhibit improved bacterial clearance compared with wild-type animals (10). Thus, the effect of A2AR stimulation on the immune response in sepsis is still controversial.

In the present study, we examined the effect of adenosine on the production of IL-18 by LPS (1 µg/ml)-treated monocytes. We employed a preparation that was morphologically 95% monocytes, with the cell population of CD14+ monocytes being 85% as determined by flow cytometry with FITC-conjugated anti-CD 14 antibody; the preparation was derived from human peripheral blood mononuclear cells (PBMC) after acquiring institutional review board (IRB) approval (Okayama Univ. IRB No. 106) as previously described (2, 11). Moreover, we investigated the involvement of adenosine receptor subtypes in the actions of adenosine.

As shown in Fig. 1A, the effects of adenosine (Sigma Chemical Company, St. Louis, MO, USA) at concentrations ranging from 0.1 to 100 µM on the production of IL-18 in the incubation media from monocytes at 1 × 10^6 cells/ml were examined in the presence or absence of 1 µg/ml LPS, which was derived from Escherichia coli (L8274, serotype O26:B6, purification more than 97%; Sigma). Pure water produced by MILLIPORE (Millipore Japan, Tokyo) was the solvent solution for LPS. After culturing for 24 h at 37°C in a 5% CO2/air mixture, IL-18 (MBL, Nagoya) was measured by using enzyme-linked immunoadsorbent assay (ELISA) kits. Adenosine had no effect on the cytokine production in the absence of LPS. Adenosine inhibited the LPS-enhanced production of IL-18 with an IC50 value 3.5 µM.

To investigate the involvement of A1R, A2AR, A2BR, and A3R in the actions of adenosine, we examined the effects of selective A1R, A2AR, A2BR, and A3R antagonists: DPCPX (8-cyclopentyl-1,3-dipropylxanthine), ZM-241385 {4-[2-(7-amino-2-furyl)[1,3,5]triazin-5-ylamino]ethyl phenol}, alloxazine {benzo[g]pteridine-2,4(1H,3H)-dione}, and MRS1220 {N-(9-chloro-2-furan-2-yl)[1,2,4]triazolo[1,5-c]quinazolin-5-yl]-2-phenyl-acetamide} (Sigma), respectively, on the production of IL-18 in the presence of LPS (Fig. 1B). The A2AR antagonist reversed both the adenosine-induced inhibition of IL-18 production induced by LPS and that in the absence of LPS, but the A2BR antagonist had no effect. On the other hand, the A1R and A3R antagonists each enhanced the actions of adenosine. Moreover, the effects of the selective A1R, A2AR, A2BR, and A3R agonists: CPA [2-chloro-N(6)-cyclopentyladenosine], CGS-21680 {4-[[6-amino-9-(N-ethyl-b-d-ribofuranosamidinosy)]-9H-purin-2-yl]amino][ethyl]benzenepropanoic acid hydrochloride}, NECA (5’-N-ethylcarboxamidoadenosine), and IB-MECA {1-deoxy-1-{6-[(3-iodophenyl)methyl] amino]-9H-purin-9-yl}-N-methyl-b-d-ribofuranosamide} (Sigma), respectively, on the IL-18 production in the presence or absence of LPS were determined (Fig. 2A). In the presence of LPS, the A2AR agonist inhibited the cytokine production. However, the A1R, A2BR, and A3R agonists had no effect in the presence or absence of
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LPS (data not shown). Furthermore, we investigated the effects of the A1R, A2AR, A2BR, and A3R antagonists on A2AR agonist-initiated inhibition of IL-18 production in the presence of LPS (Fig. 2B). The A2AR antagonist, but not the A1R, A2BR, or A3R antagonist, reversed the action of the A2AR agonist. As shown in Fig. 2C, we examined the effects of the A1R, A2BR, and A3R agonists individually on A2AR agonist-initiated inhibition of IL-18 production in the presence of LPS. The A1R and A3R agonists, but not the A2BR agonist, reversed the action of the A2AR agonist. A2AR and A2BRs are both coupled to Gs proteins and stimulate adenyl cyclase activity. However, the activation of A2BRs is suggested to facilitate the release of allergic and pro-inflammatory mediators (12).

Stimulation of A1R or A3R inhibits adenylate cyclase via Gi protein, suggesting that the inhibition of adenylate cyclase might reverse the stimulatory action on A2AR. Our study using primary monocytes suggests that stimulation of A2AR, rather than A2BR, plays a significant role in the inhibition of the LPS-enhanced IL-18 production.

Stimulation of A2AR results in elevation of cAMP and the activation of protein kinase A (PKA) in human monocytes (13). The Gs-coupled high-affinity A2AR mediates many anti-inflammatory actions of adenosine in monocytes (14). An analog of cAMP, dibutyryl cAMP (dbcAMP), inhibited the LPS-elicited production of IL-18 (15). The PKA inhibitor H89 \{(N-[2-[(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide-HCl} (Sigma), reversed the inhibitory effect of the A2AR agonist CGS-21680 on the LPS-elicited produc-

Fig. 2. The effect of A2AR agonist CGS-21680 on LPS-induced IL-18 productions in monocytes. A: The effect of the A2AR agonist CGS-21680 in the presence (filled circles) or absence (open circles) of 1 µg/ml LPS on the production of IL-18 in the conditioned media was determined. The results are expressed as the means ± S.E.M. of triplicate findings from five donors. *P<0.05, **P<0.01, compared with the values for LPS alone. B: The effects of the A1R, A2AR, A2BR, and A3R antagonists on A2AR agonist-initiated inhibition of IL-18 production in the presence of LPS. Monocytes treated with 100 µM CGS-21680 were incubated with selective A1R, A2AR, A2BR, and A3R antagonists, DPCPX (filled circles), ZM-241385 (filled squares), alloxazine (open squares), and MRS1220 (open circles), at concentrations ranging from 0.1 to 100 µM in the presence of 1 µg/ml LPS for 24 h. **P<0.01, compared with the value for LPS and adenosine. C: The effects of the A1R, A2BR, and A3R agonists on A2AR agonist-initiated inhibition of IL-18 production in the presence of LPS. Monocytes treated with 100 µM CGS-21680 were incubated with selective A1R, A2BR, and A3R agonists, CPA (filled circles), NECA (open squares), and IB-MECA (open circles), at concentrations ranging from 0.1 to 100 µM in the presence of 1 µg/ml LPS for 24 h. *P<0.05, **P<0.01, compared with the value for LPS and CGS-21680. D: The involvement of PKA in the production of IL-18. The effect of PKA inhibitor H89 at concentrations ranging from 0.1 to 100 µM in the presence of 1 µg/ml LPS with 100 µM CGS-21680 on IL-18 concentrations was determined. **P<0.01, compared with the values for LPS and CGS-21680. The detection limit of ELISA for IL-18 was 10 pg/ml.
tion of IL-18 (Fig. 2D). H89 had no effect on the cytokine production in the absence of CGS-21680 (data not shown). Therefore, cAMP and PKA are likely to be involved in the action of adenosine.

In conclusion, adenosine inhibited interleukin IL-18 production in LPS-stimulated monocytes. While the stimulation of A2AR is involved in the actions of adenosine, the stimulation of A1R and A3R inhibited the actions of A2AR stimulation.

References