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Differentiation by LPS and IFN-γ of expression of adenosine receptors in macrophage cell lines RAW264 and J774

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Abstract Lipopolysaccharide (LPS) and interferon-γ (IFN-γ) are external and internal activator of macrophages, respectively. During infection, adenosine has physiological effects at the site of inflammation mainly through four subtypes of adenosine receptors including A1, A2a, A2b, and A3. Moreover, A2a adenosine receptor is a critical part of the physiological negative feedback mechanism for limitation and termination of tissue-specific and systemic inflammatory responses. It was useful and meaningful to gain information about interaction with LPS, which generates the inflammation, and IFN-γ, which is a major activation factor for macrophages, and adenosine receptors, which terminate the inflammation. The aim of this study is to evaluate the abilities of LPS and IFN-γ on the expression of adenosine receptors in mouse macrophage cell lines RAW264 and J774. LPS increased the proliferation in RAW264, but not J774. IFN-γ didn't alter the proliferation in RAW264 nor J774. LPS significantly potentiated the expression of all adenosine receptors in J774, but not in RAW, whereas IFN-γ markedly potentiated the expression of all adenosine receptors in RAW, but not in J774. These results suggest that LPS and IFN-γ may differently affect the expression of adenosine receptors in macrophage cell lines RAW264 and J774.

Key words
Adenosine receptor,
IFN-γ,
LPS,
Macrophages

Introduction

Macrophages are commonly the first cells of the immune system to encounter invaders such as bacteria and fungi, and are ready to leave the circulation and attack the intruder at any place at any time. During infection, lipopolysaccharide (LPS), a predominant glycolipid in the outer membrane of Gram-negative bacteria, stimulates macrophages to produce pro-inflammatory cytokines. LPS induces cellular responses by its complexing with circulating LPS-binding protein and subsequent binding to CD14 that, in turn, facilitates the interaction of LPS with signaling molecules belonging to the Toll-like receptor 4 (TLR4)[1,2]. Interferon-γ (IFN-γ), a major activation factor for macrophages is thought to be produced primarily by activated Th1[3,4] and natural killer (NK)[5,6] cells during the course of an immune response. Moreover, all three classes of IFNs, IFN-α, β and γ have been associated with the control of the rate of monocyte production and maturation[7]. In addition, the IFNs have been shown to be potent 'priming' agents for the induction of a fully activated macrophage. However, this functional maturation step is often dependent upon the presence of a 'second' or 'trigger signal' such as LPS or other bacterial products[8–10].

Adenosine is a purine nucleotide that is released from a variety of cells in response to metabolic stress or from the sympathetic nervous system, and occupies various adenosine receptor subtypes on target cells. Adenosine is known to bind four different types of G-protein coupled cell surface receptors, A1, A2a, A2b and A3 adenosine receptors[11]. In addition, the accumulation of extracellular

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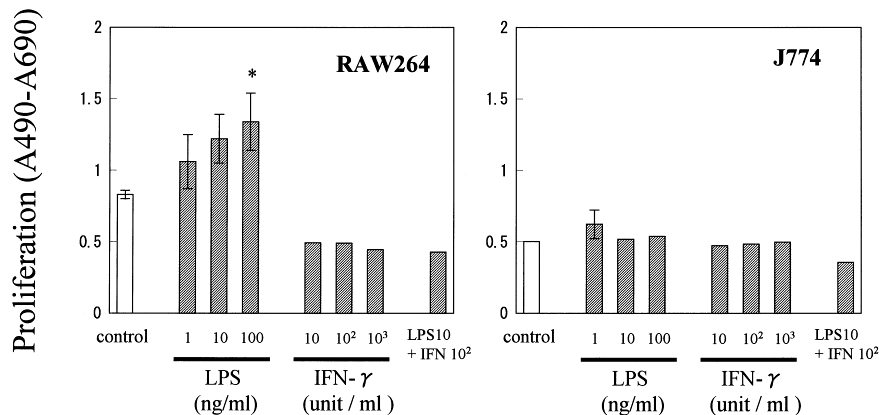

Fig. 1 Effect of LPS and IFN-γ on proliferation in RAW264 and J774 cells

RAW264 and J774 cells were incubated with LPS and/or IFN-γ for 72 h. Cell proliferation was determined by colorimetric MTS assay. The date from three to six different specimens are shown. *$P<0.05$, when compared with untreated cells.

adenosine in inflamed and damaged tissues[12–16] and the immunosuppressive properties of cAMP-elevating adenosine receptors indicate that signaling by A2a adenosine receptor (A2aR) on immune cells is a possible natural mechanism of inhibition and/or termination inflammation[17–23]. More recently, it has been shown that A2aR is a critical part of the physiological negative feedback mechanism for limitation and termination of tissue-specific and systemic inflammatory responses[24]. Since inappropriate or prolonged inflammation is the main cause of many diseases, it is important to understand the physiological mechanisms that alter inflammatory responses. For example, most of dental disease are caused by inflammation. Release of LPS from the outer membrane of Gram negative bacteria initiates various biological activities including complement activation, cytotoxicity, and bone resorption. Moreover, many investigators have suggested that IFN-γ is associated with progressive periodontal lesions[25]. To gain information, we sought to evaluate the ability of LPS and IFN-γ on the expression of adenosine receptors in macrophage cell lines RAW264 and J774.

Materials and methods

Materials

LPS was provided by Sigma (St. Louis, MO, USA). Goat polyclonal antibodies against four kinds of adenosine receptors were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). IFN-γ and a fluorescence-conjugated anti-goat antibody were obtained from Wako Purity Chemical Industries (Osaka, Japan).

Cell culture

Mouse macrophage cell lines RAW264 cells and J774 cells (Riken Gene Bank, Tokyo, Japan) were respectively maintained in DMEM and RPMI 1640 supplemented with 10% FBS, 100 units/m*l* of penicillin and 100 μg/m*l* of streptomycin.

Proliferation assay

Proliferation assay was performed by CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega Corporation; Madison, WI, USA) as previously described[26] with minor modifications[27]. Briefly, cells (6×10^3) were incubated with LPS and/or IFN-γ in 96-well culture plates for 72 h. After incubation, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] and phenazine ethosulfate were added to culture plates. The absorbance at a wavelength of 490 nm was measured and reference absorbance is 690 nm.

Assessment of receptor expression

Assessment of receptor expression was performed as previously described[28] with minor modifications[29].

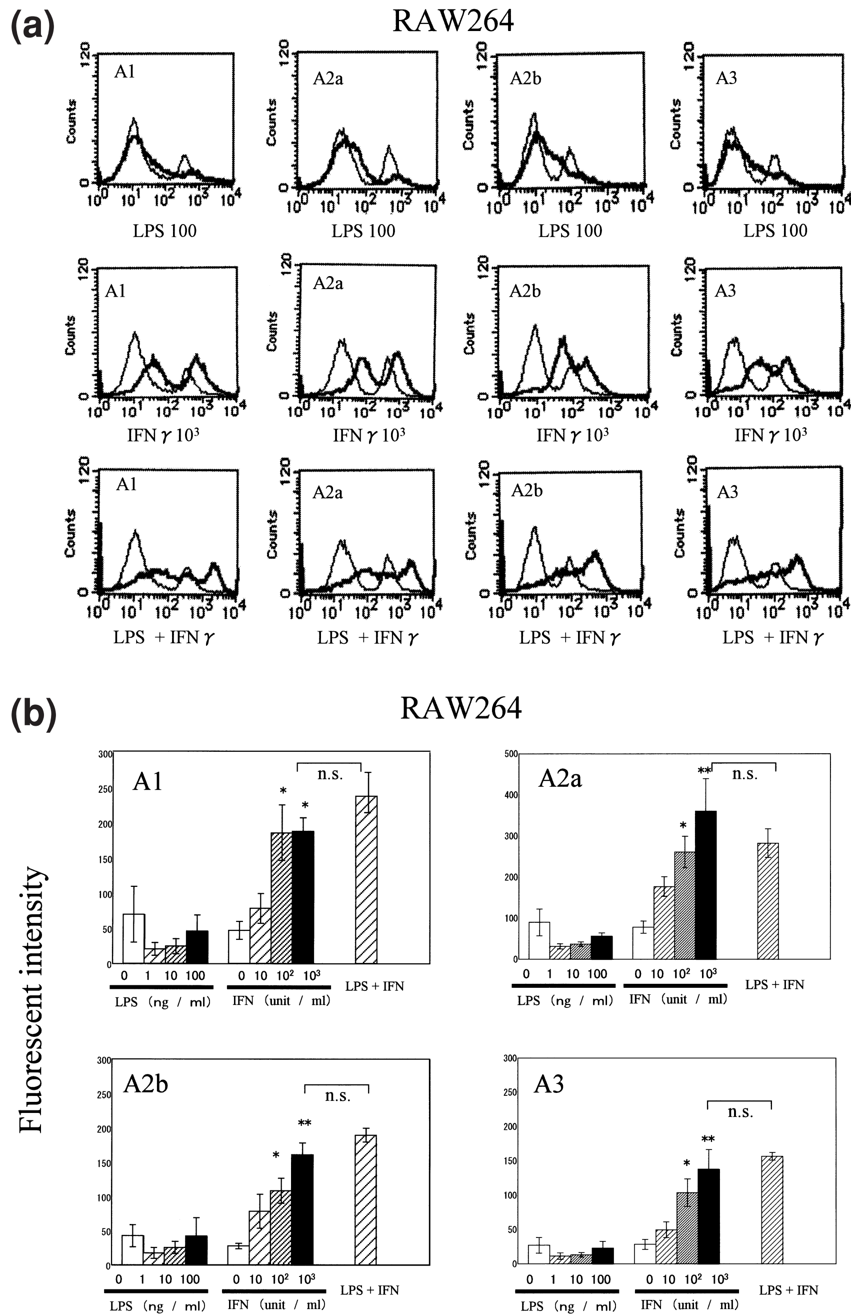

Fig. 2 Effects of LPS and IFN-γ on the expression of A1, A2a, A2b and A3 adenosine receptors in RAW264 cells
RAW264 cells were incubated with LPS and/or IFN-γ for 72 h, followed by staining with anti-A1, A2a, A2b or A3 adenosine receptors antibody. Representative histograms of RAW264 treated with each drugs at a highest concentration examined as compared with untreated cells are shown in Fig. 2 (a). The thin line shows untreated cells, and the thick line shows cells treated with each drug. Mean fluorescent intensity is provided in Fig. 2 (b) where values are the Geo Mean ± SE from three to five different specimens. *$P<0.05$, **<0.01, when compared with untreated cells.

Cells (3×10^5) were incubated with LPS and/or IFN-γ for 72 h. After washed with PBS, cells were incubated with goat polyclonal antibodies against A1, A2a, A2b, or A3 receptors for 30 min at 4°C. Cells were then washed with PBS, followed by incubation with a fluorescence-conjugated anti-goat antibody for 30 min at 4°C in the dark. After cells were washed with PBS, stained cells were analyzed on a flow cytometry (Becton Dickinson, Mountain View, CA, USA). Data are expressed as mean

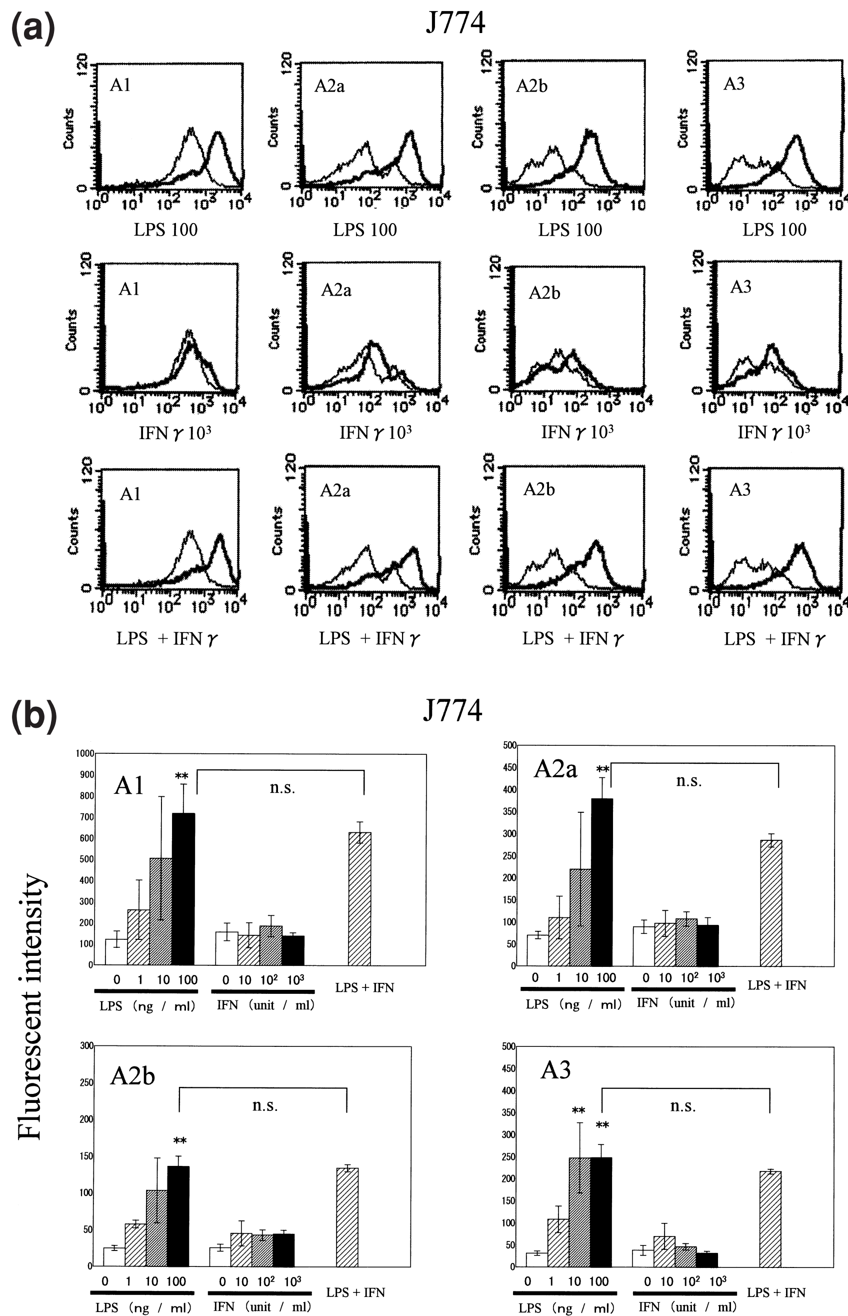

Fig. 3 Effects of LPS and IFN-γ on the expression of A1, A2a, A2b and A3 adenosine receptors in J774 cells

J774 cells were incubated with LPS and/or IFN-γ for 72 h, followed by staining with anti-A1, A2a, A2b or A3 adenosine receptors antibody. Representative histograms of J774 treated with each drugs at a highest concentration examined as compared with untreated cells are shown in Fig. 3 (a). The thin line shows untreated cells, and the thick line shows cells treated with each drug. Mean fluorescent intensity is provided in Fig. 3 (b) where values are the Geo Mean ± SE from three to five different specimens. **$P<0.01$, when compared with untreated cells.

fluorescent intensity for each sample as calculated by the CellQuest® software (Becton Dickinson).

Assessment of morphological changes

Cells (3×10^5) were incubated with LPS and/or IFN-γ for 72 h. The photographs were obtained using by the microscope (OLYMPUS IX70) with OLYMPUS digital camera, and the pictures were taken at $\times 300$ magnifications, and modified by Inter video win DVR and Photoshop software.

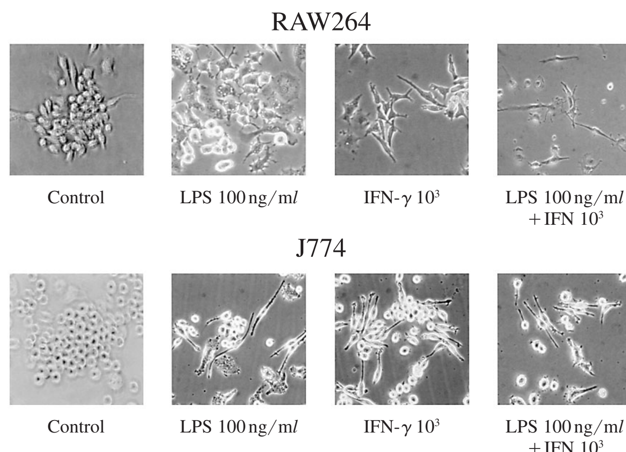

Fig. 4 LPS and IFN-γ induced morphological changes in RAW264 and J774 cells
RAW264 and J774 cells were incubated with LPS and/or IFN-γ for 72 h. Cells were observed under microscope with a digital camera and pictures taken at $\times$300 magnifications.

Statistical analysis

Results were expressed as the mean ± SE. Statistical analysis was determined by one-way ANOVA for non-repeated to detect differences between multiple groups. Differences between groups were determined by Dunnett' test or Student-Newman-Keuls (SNK) test. Differences were considered to be significant when the P value was <0.05.

Results

Effects of LPS and IFN-γ on proliferation

We first assessed effects of LPS and IFN-γ on the proliferation in RAW264 and J774 cells. At 72 h after incubation, in RAW264 cells, LPS at only 100 ng/m*l* markedly increased the proliferation, whereas, IFN-γ didn't affect the proliferation at all concentrations tested (Fig. 1, left panel). On the other hand, neither LPS nor IFN-γ affects the proliferation in J774 (Fig. 1, right panel). Moreover, in the presence of IFN-γ, LPS didn't potentiate the proliferation in RAW264 and J774 (Fig. 1).

Alteration of the expression of adenosine receptors by LPS and IFN-γ

Next, we wanted to examine whether LPS and IFN-γ affect the expression of adenosine receptors in RAW264 and J774 cells. In subsequent experiments, we detected the expression of these receptors by using flow cytometry. Representative histograms of RAW264 treated with LPS or IFN-γ at the highest concentration examined, as compared with untreated cells, are shown in Fig. 2 (a). Fluorescent intensities are provided in Fig. 2 (b) where values are the Geo Mean ± SE from three to five different specimens. LPS failed to affect the expression of adenosine receptors at concentrations of up to 100 ng/m*l* at 72 h after incubation. On the other hand, IFN-γ significantly increased the expression of all adenosine receptors at 10^2 and 10^3 unit/m*l* in RAW264.

Similarly, representative histograms of J774 treated with LPS or IFN-γ at a highest concentration examined, as compared with untreated J774, are shown in Fig. 3 (a). Fluorescent intensities are provided in Fig. 3 (b) where values are the Geo Mean ± SE from three to five different specimens. Interestingly, LPS markedly potentiated the expression of A1, A2a, and A2b adenosine receptors at the concentration of 100 ng/m*l* at 72 h after incubation, and potentiated the expression of A3 adenosine receptor at the concentrations of 10 and 100 ng/m*l* at 72 h after incubation. However, IFN-γ failed to affect the expression of adenosine receptors in J774, although IFN-γ increased the expression in RAW264.

To determine the effect of combination of LPS and IFN-γ on the expression of these receptors, RAW264 and J774 cells were treated with IFN-γ at 10^3 unit/m*l* in the presence of LPS at 100 ng/m*l* for 72 h. As shown in Fig. 2 (a, b), in RAW264 cells, LPS didn't potentiate the expression of adenosine receptors by IFN-γ. As shown in Fig. 3 (a, b), in J774 cells, IFN-γ failed to potentiate the expressions by LPS.

Alteration of cell formation by LPS and IFN-γ

Moreover, we investigated the possibility of morphological changes in RAW264 and J774 cells which were incubated with LPS and/or IFN-γ. At 72 h after incubation, a morphological transformation of RAW and J774 cells from macrophage-like cells into dendritic-like cells was clearly observed at all doses of LPS and IFN-γ. As shown in Fig. 4, dendritic morphology was characterized by multiple prominent cytoplasmic processes.

Discussion

The importance of the findings presented in this study is that LPS and IFN-γ that generate the inflammation may increase adenosine receptors that play an important role in termination of inflammation on macrophage cell lines. In general, the production of cytokines by LPS-stimulated macrophages was activated highly from 24 to 48 h after incubation. However, in this study, we investigated the cell proliferation and the adenosine receptor expressions after incubation for 72 h, and we found that LPS and IFN-γ increased adenosine receptors expressions on macrophage cell lines. It thus appears that another cytokines produced by stimulation of LPS and IFN-γ for a long time may result in the alteration of adenosine receptors expressions. It has reported that RAW and J774 cell have the adenosine receptors on its surface[30,31]. Interestingly, the results described in this paper indicated that LPS evidently potentiated the expression of adenosine receptors, A1, A2a, A2b and A3, in J774 cells, whereas IFN-γ potentiated the expression of all adenosine receptors in RAW264. This paper also indicates that adenosine receptors are expressed on macrophages. Namely, LPS did not affect the expression of adenosine receptors in RAW264 cells, and IFN-γ failed to affect the expression of adenosine receptors in J774, indicating that the ability of LPS differs from the ability of IFN-γ. These results suggested that RAW264 and J774 have different specificity for LPS and IFN-γ.

Based on the published data, adenosine receptors are the prominent regulator of cytokine secretion. IFN-γ, which the most prominent cytokines secreted by Th1 cells, modulated the capacity of A2a receptor occupancy to regulate secretion of IL-10 and IL-12 in LPS-activated human monocytic THP-1 cells[32].

Adenosine regulates the production of IL-6 mediated by the adenosine A2b receptor. IL-6 is important cytokine in inflammation and osteoresorping signal in periodontal tissue. And expression of IL-6 also regulated by LPS and IFN-γ[33]. Moreover adenosine affect the production of inflammatory cytokines such as IL-12 and TNF-α[34]. It is well recognized that cytokines stimulate or inhibit the production of other cytokines in an autocrine and paracrine manner, and our findings suggest that adenosine receptors may play a role in mediating this mutual interaction among cytokines. The functional modulation of adenosine receptors is likely a key factor in the regulation of inflammatory conditions involving numerous cytokines. Furthermore, IFN-γ is the major activator of macrophages, and over 200 genes are now known to be regulated by this cytokine[35,36]. IFN-γ modulates the expression of many receptors. In some cases, IFN-γ induces the expression of receptors such as the high affinity IgG receptor[37] and also down-regulates the expression of other receptors, such as the CSF-1 receptor[38]. In this study, we observed that IFN-γ induced an increase in the number of A1, A2a, A2b and A3 adenosine receptors in RAW264 cell surface. It is likely that this progressive increase was due to *de novo* synthesis of the receptor, and it correlated with the higher capacity of adenosine to induce an increase in cAMP levels, thus suggesting that the newly synthesized receptors were functional. As showed in the Introduction, more recent study using animals deficient in the A2aR indicated that A2aR has an important role in the attenuation of inflammation and tissue damage *in vivo*[24]. Indeed, A2aR is a critical part of the physiological negative feedback mechanism for limitation and termination of both tissue-specific and systemic inflammatory responses. The uniqueness of the adenosine receptors may lie in the physiology of accumulation of abundant and ubiquitous purine nucleotides[39,40] in the local inflammatory environment[12–16]. Inappropriate or prolonged inflammation is the main cause of many diseases. It is important to understand the physiological mechanisms that terminate inflammation.

In this study, moreover, we also found that RAW and J774 cells cultured with LPS and/or IFN-γ underwent a remarkable morphological transformation. Considering that cells with dendritic-like morphology were non-existent in control RAW264 and J774 cell culture results in that a significant morphological transformation occurred at all doses of LPS and/or IFN-γ and that maximal morphological transformation occurred at 100 ng/m*l* of LPS and

10^3 unit/m*l* of IFN-γ (date shown at the only maximal concentration). Recently, it has been reported that a murine macrophage cell line RAW264, appears to differentiate into dendritic-like cells upon treatment with bacterial LPS[41], and similar LPS-induced changes in human macrophages have also been suggested[42]. Macrophages are considered to be end-differentiated cells. If RAW264 and J774 cells represent mature macrophages, as it generally believed, their differentiation into dendritic-like form cells may point to a mechanism through which tissue macrophages may be recruited to become dendritic-like cells during localized bacterial infection. However, whether the morphological changes seen in RAW and J774 cells in response to LPS and IFN-γ potentiated the expression of adenosine receptors still remain to be elucidated in future studies.

On the other hand, IFN-γ did not affect the proliferation of RAW264 cells and J774 cells. In contrast, LPS increased the proliferation in RAW264 at the concentration of only 100 ng/m*l* after incubation for 72 h, whereas, it failed to affect the proliferation in J774. The increase of cells by treatment with LPS at 100 ng/m*l* totally resulted in the increase of the number of all receptors. Under this condition of 72 h incubation, we have demonstrated that the proliferation by LPS 100 ng/m*l* in RAW264 cells didn't observe in the presence of IFN-γ. Moreover, we have demonstrated that LPS didn't potentiate the IFN-γ-induced adenosine receptor expressions in RAW264 and IFN-γ didn't potentiate the LPS-induced adenosine receptor expressions in J774. It is likely that the expressions of adenosine receptors were not potentiated in the combination of LPS and IFN-γ compared with LPS alone and IFN-γ alone.

It thus appears that LPS may potentiate the expression of adenosine receptors in J774 cells and IFN-γ may potentiate the expression of adenosine receptors in RAW264 cells. It is likely that the stimulation by LPS and the production of IFN-γ during inflammatory responses would lead to potentiation of the expression of adenosine receptors. We speculate that activation of adenosine receptors by inflammatory cytokines may partly account for the variability in response to adenosine-mediated anti-inflammatory agents used in the treatment of inflammatory diseases.

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References

1) Wright, S.D., Ramos, R.A., Tobias, P.S., Tobias, P.S., Ulevitch, R.J. and Mathison, J.C.: CD14 a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* **249**: 1431–1433, 1990.
2) Wright, S.D., Ramos, R.A., Hermanowski-Vosatka, A., Rockwell, P. and Detmers, P.A.: Activation of the adhesive capacity of CR3 on neutrophils by endotoxin: dependence on lipopolysaccharide binding protein and CD14. *J Exp Med* **173**: 1281–1286, 1991.
3) Unanue, E.R.: Antigen-presenting function of the macrophage. *Annu Rev Immunol* **2**: 395–428, 1984.
4) Mosmann, T.R., Cherwinski, H., Bond, M., Giedlin, M.A. and Coffman, R.L.: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted protein. *J Immunol* **136**: 2348–2357, 1986.
5) Bancroft, G.J., Schreiber, R.D., Bosma, G.C., Bosma, M.J. and Unanue, E.R.: A T-cell independent mechanism of macrophage activation by interferon γ. *J Immunol* **139**: 1104–1107, 1987.
6) Wherry, J.C., Schreiber, R.D. and Unanue, E.R.: Regulation of gamma interferon production by natural killer cells in scid mice: role of tumor necrosis factor and bacterial stimuli. *Infect Immun* **59**: 1709–1715, 1991.
7) Vogel, S.N.: Lipopolysaccharide-induced interferons. *In:* Bacterial Endotoxic Lipopolysaccharides, Vol.2, Immunopharmacology and Pathophysiology of LPS. (Ryan, J.L. and Morrison, D.C. eds.). CRC Press, Boca Roton, FL, 1992, p. 165.
8) Pace, J.L., Russell, S.W., LeBlanc, P.A. and Murasko, D.M.: Comparative effects of various classes of mouse interferon on macrophage activation for tumor cell killing. *J Immunol* **134**: 977–978, 1985.
9) Hogan, M.M. and Vogel, S.N.: Rapid A-associated proteins provide an alternate 'second signal' in the activation of recombinant interferon-γ-primed C3H/HeJ macrophages to a fully tumoricidal state. *J Immunol* **139**: 3697–3702, 1987.
10) Adams, D.O.: Molecular interactions in macrophage activation. *Immunol Today* **10**: 33–35, 1989.
11) Ralevic, A. and Burnstock, G.: Receptors for purines and pyrimidines. *Pharmacol Rev* **50**: 413–492, 1998.
12) Winn, H.R., Rubio, R., Berne, R.M.: Brain adenosine

concentration during hypoxia in rat. *Am J Physiol* **241**: H235–242, 1981.
13) Van Belle, H., Goossens, F. and Wynants, J.: Formation and release of purine catabolites during hypoperfusion, anoxia, and ischemia. *Am J Pathol* **252**: H886–893, 1987.
14) Rudolphi, K.A., Schubert, P., Parkinson, F.E. and Fredholm, B.B.: Neuroprotective role of adenosine in cerebral ischemia. *Trends Pharmacol Sci* **13**: 439–445, 1992.
15) Marquardt, D.L., Gruber, H.E. and Wasserman, S.I.: Adenosine release from stimulated mast-cells. *Proc Natl Acad Sci USA* **81**: 6192–9196, 1984.
16) Filippini, A., Taffs, R.E. and Sitkovsky, M.V.: Extracellular ATP in T-lymphocyte activation: possible role in effector functions. *Proc Natl Acad Sci USA* **87**: 8267–8271, 1990.
17) Cronstein, B.N.: Adenosine, an endogenous anti-inflammatory agent. *J Appl Physiol* **76**: 5–13, 1994.
18) Cronstein, B.N.: A novel approach to the development of anti-inflammatory agents: adenosine release at inflamed sites. *J Invest Med* **43**: 50–57, 1995.
19) Firestein, G.S., Boyle, D., Bullough, D.A., Gruber, H.E., Sajjadi, F.G., Montag, A., Sambol, B. and Mullane, K.M.: Protective effect of an adenosine kinase inhibitor in septic shock. *J Immunol* **152**: 5853–5859, 1994.
20) Huang, S., Koshiba, M., Apasov, S. and Sitkovsky, M.: Role of A2a extracellular adenosine receptor-mediated signaling in inhibition of T cell activation and expansion. *Blood* **90**: 1600–1610, 1997.
21) Eigler, A., Greten, T.F., Sinha, B., Haslberger, C., Sullivan, G.W. and Endres, S.: Endogenous adenosine curtails lipopolysaccharide-stimulated tumor necrosis factor synthesis. Scand. *J Immunol* **45**: 132–139, 1997.
22) Sullivan, G.W., Sarembock, I.J. and Linden, J.: The role of inflammation in vascular diseases. *J Leukoc Biol* **67**: 591–602, 2000.
23) Hoskin, D.W., Reynolds, T. and Blay, J.: Adenosine as a possible inhibitor of killer T-cell activation in the microenvironment of solid tumors. *Int J Cancer* **59**: 854–855, 1994.
24) Ohta, A. and Sitkovsky, M.: Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* **414**: 916–920, 2001.
25) Ukai, T., Mori, Y., Onoyama, M. and Hara, Y.: Immunohistological study of interferon-gamma- and interleukin-4-bearing cells in human periodontitis gingiva. *Arch Oral Biol* **46**: 901–908, 2001.
26) Azuma, Y. and Ohura, K.: Alteration of constitutive apoptosis in neutrophils by quinolones. *Inflammation* **27**: 123–130, 2003.
27) Azuma, Y., Watanabe, K., Date, M., Daito, M. and Ohura, K.: Possible involvement of p38 in mechanisms underlying acceleration of proliferation by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and the precursors in leukemia cell line THP-1. *J Pharmacol Sci* **94**: 261–270, 2004.
28) Azuma, Y. and Ohura, K.: Endomorphin-2 modulates productions of TNF-α, IL-1β, IL-10 and IL-12, and alters functions related to innate immune of macrophages. *Inflammation* **26**: 223–232, 2002.
29) Azuma, Y., Watanabe, K., Date, M., Daito, M. and Ohura, K.: Induction of proliferation by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and the precursors in monocytic leukemia U937. *Pharmacology* **71**: 181–191, 2004.
30) Hon, W.M., Moochhala, S. and Khoo, H.E.: Adenosine and its receptor agonists potentiate nitric oxide synthase expression induced by lipopolysaccharide in RAW264.7 murine macrophages. *Life Sci* **60**: 1327–1335, 1997.
31) Bowlin, T.L., Borcherding, D.R., Edwards, C.K. 3rd. and McWhinney, C.D.: Adenosine A3 receptor agonists inhibit murine macrophage tumor necrosis factor-alpha production *in vitro* and *in vivo*. *Cell Mol Biol* **43**: 345–349, 1997.
32) Khoa, N.D., Montesinos, M.C., Reiss, A.B., Delano, D., Awadallah, N. and Cronstein, B.N.: Inflammatory cytokines regulate function and expression of adenosine A2a receptors in human monocytic THP-1 cell. *J Immunol* **167**: 4026–4032, 2001.
33) Murakami, S., Terakura, M., Kamatani, T., Hashikawa, T., Saho, T., Shimabukuro, Y. and Okada, H.: Adenosine regulates the production of interleukin-6 by human gingival fibroblasts via cyclic AMP/protein kinase A pathway. *J Periodontal Res* **35**: 93–101, 2000.
34) Hasko, G., Kuhel, D.G., Chen, J.F., Schwarzschild, M.A., Deitch, E.A., Mabley, J.G., Marton, A. and Szabo, C.: Adenosine inhibits IL-12 and TNF-[alpha] production via adenosine A2a receptor-dependent and independent mechanisms. *FASEB J* **14**: 2065–2074, 2000.
35) Bach, E.A., Aguet, M. and Schreiber, R.D.: The IFN-γ receptor: a paradigm for cytokine receptor signaling. *Annu Rev immunol* **15**: 563–591, 1997.
36) Boehm, U., Klamp, T., Groot, M. and Howard, J.C.: Cellular responses to interferon-γ. *Annu Rev immunol* **15**: 749–795, 1997.
37) Pearse, R.N., Freinman, R., Shuai, K., Darnell, J.E. Jr., Ravetch, J.V.: Interferon γ-induced transcription of the high-affinity Fc receptor for IgG requires assembly of a complex that induces the 91-kDa subunit of transcription factors ISGF3. *Proc Nalt Acad Sci USA* **90**: 4314–4318, 1993.
38) Baccarini, M., Dello, S.P., Buscher, D., Bartocci, A. and Stanley, E.R.: IFN-γ/lipopolysaccharide activation of macrophages is associated with protein kinase C-dependent down-modulation of the colony-stimulating factor-1 receptor. *J Immunol* **149**: 2656–2661, 1992.
39) Fredholm, B.B., Arslan, G., Halldner, L., Kull, B., Schulte, G. and Wasserman, W.: Structure and function of adenosine receptors and their genes. Naunyn-Schmiedeberg's Arch. *Pharmacology* **362**: 364–374, 2000.
40) Burnstock, G.: Purine-mediated signaling in pain

and visceral perception. *Trends Pharmacol Sci* **22**: 182–188, 2001.
41) Saxena, R.K., Vallyathan, V. and Lewis, D.M.: Evidence for lipopolysaccharide-induced differentiation of RAW264.7 murine macrophage cell line into dendritic like cells. *J Biosci* **28**: 129–134, 2003.
42) Lyakh, L.A., Koski, G.K., Telford, W., Gress, R.E., Cohen, P.A. and Rice N.R.: Bacterial lipopolysaccharide, TNF-alpha, and calcium ionophore under serum-free conditions promote rapid dendritic cell-like differentiation in CD14+ monocytes through distinct pathways that activate NK-kappa B. *J Immunol* **165**: 3647–3655, 2000.