Lipopolysaccharide rapidly modifies adenosine receptor transcripts in murine and human macrophages: role of NF-κB in A_{2A} adenosine receptor induction

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The A_{2A} adenosine receptor (A_{2A}AR) mediates anti-inflammatory actions of adenosine in a variety of cell types. LPS (lipopolysaccharide) was reported to induce a small (< 2-fold) increase in the expression of A_{2A}AR mRNA in human monocytes and monocytic cell lines. We investigated the effects of LPS on the expression of adenosine receptor mRNAs in primary mouse IPMΦ (intraperitoneal macrophages), human macrophages and Wehi-3 cells. Treatment with 10 ng/ml LPS for 4 h produced a > 100-fold increase in A_{2A}AR mRNA. LPS-induced increases in mRNA for A_{2A}AR and TNFα (tumour necrosis factor α) are reduced by 90 % in IPMΦ pretreated with the NF-κB (nuclear factor κB) inhibitor, BAY 11-7082 {(E)3-[(4-methylphenyl)sulphonyl]-2-propenenitrile; 10 μM}. In Wehi-3 cells exposed to LPS, A_{2A}AR and A_{2B}AR transcripts are elevated by 290- and 10-fold respectively, the A_1AR transcript is unchanged and the A_3AR transcript is decreased by 67 %. The induction of A_{2A}AR mRNA by LPS is detectable after 1 h, reaches a peak at 6 h at 600 times control and remains elevated beyond 24 h. The ED_{50} (effective dose) of LPS is 2.3 ng/ml. A_{2A}AR receptor number, measured by ^{125}I-ZM241385 binding to whole cells, is undetectable in naïve cells and increases linearly at a rate of 23 receptors · cell^{-1} · min^{-1} to a B_{max} of 348 fmol/mg (28 000 receptors/cell) in 20 h. The increase in receptor number is correlated with an increase in the potency of an A_{2A} agonist (4-{3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl}-cyclohexanecarboxylic acid methyl ester; referred to as ATL146e) to stimulate cAMP in these cells. After LPS pretreatment, the potency of the A_{2A} agonist, ATL146e, to reduce TNFα release from IPMΦ was increased by 200-fold. The results support the hypothesis that regulation of adenosine receptor expression, especially up-regulation of the A_{2A}AR, is part of a delayed feedback mechanism initiated through NF-κB to terminate the activation of human and mouse macrophages.

Key words: adenosine receptor, ATL146e, inflammation, lipopolysaccharide, macrophage, tumour necrosis factor α (TNFα).

INTRODUCTION

Adenosine released from stressed cells can act as a physiological inhibitor of inflammation [1]. Utilization of ATP during periods of high metabolic activity leads to an increased concentration of intracellular adenosine that can be transported across the cell membrane by nucleoside transporters. Adenine nucleotides also are released from stressed cells or necrotic cells or by degranulation of nerves, mast cells or platelets and are dephosphorylated to form adenosine by extracellular nucleotidases [2]. Adenosine acts at the cell surface through four G-protein-coupled adenosine receptors (ARs): A_1, A_{2A}, A_{2B} and A_3. These receptors each have a unique pharmacological and physiological profile that enables adenosine to stimulate a variety of effects depending on its concentration and the distribution of receptors in a given tissue [3]. The G_s-coupled high-affinity A_{2A}AR mediates many anti-inflammatory actions of adenosine in a variety of cell types, including inhibition of neutrophil [4], monocyte [5], platelet [6], and T-cell activation [7,8]. In animal models, A_{2A}AR agonists can prevent death from bacterial LPS (lipopolysaccharide) or sepsis [9]. The G_s/G_q-coupled low-affinity A_{2B}AR is thought to contribute an anti-inflammatory action of adenosine in macrophages [10–12]. The role of the $G_{i/o}$-coupled A_1AR and the A_3AR in macrophages is not clear.

Bacterial LPS and inflammatory cytokines have been reported to induce a small (< 2-fold) increase in the expression of A_{2A}AR mRNA in human monocytic cell lines [13,14]. Maturation of monocytes to macrophages is associated with increased expression and secretion of TNFα (tumour necrosis factor α) in response to inflammatory stimuli [15]. Macrophages pretreated with LPS have been noted to have an exaggerated response to A_{2A}AR agonists [16,17]. In mouse IPMΦ (peritoneal macrophages), LPS was found to elicit a 15-fold increase in mRNA for the A_{2B}AR [18]. Although macrophages provide an important defence against bacterial pathogens, their overactivation can cause damage to inflamed host tissues, and such overactivation may be prevented by adenosine. In order to further examine the regulation of adenosine receptors in macrophages, we have investigated the effects of LPS on the expression of mouse and human adenosine receptor mRNAs and receptor number. LPS causes a very strong induction of A_{2A}AR mRNA in macrophages and corresponding

Abbreviations used: AR, adenosine receptor; ADA, adenosine deaminase; ATL146e, 4-{3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl}-cyclohexanecarboxylic acid methyl ester; BAY 11-7082, (E)3-[(4-methylphenyl)sulphonyl]-2-propenenitrile; EIA, enzyme immunoassay; FBS, foetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ^{125}I-ZM241385, 4-(2-[7-amino-2-(2-furyl) [1,2,4]-triazolo[2,3-a][1,3,5]-triazin-5-yl amino]ethyl)-3-[^{125}I]iodophenol; IPMΦ, intraperitoneal macrophages; LPS, lipopolysaccharide; NECA, 5′-*N*-ethylcarboxamidoadenosine; NF-κB, nuclear factor κB; TLR, Toll-like receptor; TNFα, tumour necrosis factor α.

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increases in A_{2A}AR density and potency to inhibit macrophage activation.

EXPERIMENTAL

Materials

ZM241385 {4-(2-[7-amino-2-(2-furyl) [1,2,4]-triazolo[2,3-a]-[1,3,5]triazin-5-yl amino]ethyl)phenol} [19] was purchased from Tocris Cookson (Ellisville, MO, U.S.A.). Carrier-free ^{125}I-ZM241385 was synthesized and HPLC-purified as described in [20,21]. ATL146e (4-{3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl}-cyclohexanecarboxylic acid methyl ester) [22] was a gift from Dr J. Rieger of Adenosine Therapeutics (Charlottesville, VA, U.S.A.). ADA (adenosine deaminase) was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN, U.S.A.). BAY 11-7082 {(E)3-[(4-methylphenyl)sulphonyl]-2-propenenitrile} was purchased from Calbiochem (San Diego, CA, U.S.A.). Cell-culture media and reagents were purchased from Gibco BRL (Grand Island, NY, U.S.A.). The following reagents were purchased from Sigma (St. Louis, MO, U.S.A.): NECA (5′-*N*-ethylcarboxamidoadenosine), HCl, PMSF, LPS, leupeptin, pepstatin and aprotinin.

Cell culture

Wehi-3 cells (A.T.C.C.) were grown in suspension at 0.2–1.0 $\times$ 10^6 cells/ml in Iscove's medium supplemented with 10 % (v/v) FBS (foetal bovine serum) and 5 mM 2-mercaptoethanol. Cultures were kept at 37 °C in a humidified incubator with 5 % CO_2. All experiments used cells below passage 20.

IPMΦ harvest

Balb/c mice injected with 2 ml of 39.8 g/l sterile thioglycolate solution were killed after 7 days. The peritoneal cavity was washed twice with 10 ml of PBS + 2 mM EDTA to make a cell suspension containing 3–6 $\times$ 10^5 cells/ml. After centrifugation at 300 ***g*** for 8 min, the cells were resuspended in medium and added to tissue-culture plates. After 3 h, non-adherent cells were removed and the adherent cells were washed with PBS. The resulting macrophage preparations were cultured in Dulbecco's modified Eagle's medium with high glucose, 10 % heat-inactivated FBS and penicillin/streptomycin for up to 48 h.

Human monocyte-derived macrophages

Peripheral venous blood was obtained from healthy adult volunteers with informed written consent. The blood was anticoagulated with heparin (10 units/ml), and the monocytes were enriched with Rosettesep™ according to the manufacturer's instructions. This isolation yielded approx. 5 $\times$ 10^6 monocytes (> 80 %)/10 ml of blood. By FACS, the cells had a phenotype of $CD14^+$, $CD24^-$ and $CD3^-$. The monocytes (1–2 $\times$ 10^6 cells/well) were cultured for 3 days in 24-well tissue-culture-treated plates (37 °C and 5 % CO_2) in RPMI 1640 growth medium containing penicillin/streptomycin, 5 % (v/v) autologous serum and 10 ng/ml recombinant human macrophage colony-stimulating factor, resulting in macrophages expressing high non-specific esterase staining, high CD14 and low MHC Class II, consistent with a macrophage phenotype.

Quantitative PCR

Using the iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, U.S.A.), cDNA was made from 1 μg of RNA, using mixed random and oligo-dT primers, following the manufacturer's instructions. The reaction mixture was then diluted to the equivalent of 5 μg/ml RNA, and 5 μl of the diluted mixture was incorporated into the quantitative-PCR reaction mixture. Quantitative PCR was performed using the Quantitect SYBR® Green PCR kit (Qiagen, Valencia, CA, U.S.A.). A typical reaction contained 25 μl of the kit reaction mixture, 17 μl of molecular biology grade water, 1.5 μl each of 10 μM primer stocks and 5 μl of cDNA or plasmid standard. Standard curves were produced using diluted plasmids with known copy numbers of the gene of interest. Real-time PCR was performed using the iCycler iQ Real-Time PCR Detection System from Bio-Rad using the supplied software. The thermal cycler tracks fluorescence levels over 40 amplification cycles. A melt curve was performed at the end of each run to verify that there was a single amplification product and a lack of primer dimers. All samples were normalized to the amount of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) or β-actin (human cells) mRNAs present in the sample. The relative amount of a given mRNA of interest was determined using the $\Delta\Delta C_T$ method [23].

Primers for the real-time PCR were designed from sequences in GenBank® database. The PCR primers were validated by sequencing the reaction products after TA-cloning (Invitrogen, Carlsbad, CA, U.S.A.). The following primers were used (forward, reverse): (i) mouse: A_1AR (5′-CCTGAGTGAGGTGGAACAAG-3′, 5′-ACCAGAGGAGGCTGACAC-3′); A_{2A}AR (5′-TGGCTTGGTGACGGGTATG-3′, 5′-CGCAGGTCTTTGTGGAGTTC-3′); A_{2B}AR (5′-CTGGGACACGAGCGAGAG-3′, 5′-CTGGGACACGAGCGAGAG-3′; A_3AR (5′-GAGACGGACTGGCTGAACATC-3′, 5′-GAGACGGACTGGCTGAACATC-3′); TNFα (5′-CCTCCCTCTCATCAGTTCTATGG-3, 5′-CGTGGGCTACAGGC TTGTC-3′); and GAPDH (5′-TTCACCACCATGGAGAAGGC-3′, 5′-GGCATGGACTGTGGTCATGA-3′). (ii) Human: A_{2A}R (5′-AGTTCCGCCAGACCTTCC-3′, 5′-ACCTGCTCTCCGTCACTG-3′) and human β-actin (5′-CCCTGGCACCCAGCAC-3′, 5′-GCCGATCCACACGGAGTA-3′).

Radioligand binding

Wehi-3 or IPMΦ cells were resuspended in PBS with 1.5 units/ml ADA at a concentration of 2 $\times$ 10^6 cells/ml. Aliquots (100 μl) of the cell suspensions were placed into wells of a 96-well Millipore Multiscreen® GF/C filter plate. Various concentrations of the radiolabelled antagonist, ^{125}I-ZM241385 were added in 50 μl of PBS with 2 units/ml ADA. After incubating the assays at 4 °C for 2–3 h, binding reactions were terminated by rapid filtration on a cell harvester (Brandel, Gaithersburg, MD, U.S.A.) followed by 4 $\times$ 150 μl washes for 30 s with ice-cold 10 mM Tris/HCl (pH 7.4) and 10 mM $MgCl_2$. Non-specific binding was measured in the presence of 50 μM NECA.

cAMP assays

Cells were removed from their media, washed twice by centrifugation with PBS and resuspended in PBS supplemented with 2 units/ml ADA at a concentration of 1 $\times$ 10^6 cells/ml. Aliquots (200 μl) of the cell suspension were added to 75 mm polypropylene tubes with 50 μl of 5 $\times$ ATL146e in various concentrations. All tubes received 50 μM rolipram. After incubation for 15 min at 37 °C, the reaction was stopped by the addition of 0.5 ml of 0.15 M HCl. The cells were pelleted by centrifugation and the supernatants were frozen for cAMP analysis by EIA (enzyme immunoassay; Assay Designs, Ann Arbor, MI, U.S.A.).

Measurement of TNFα concentration

IPMΦ were resuspended in PBS supplemented with 2 units/ml ADA at a concentration of 0.5 $\times$ 10^6 cells/ml. Cells were

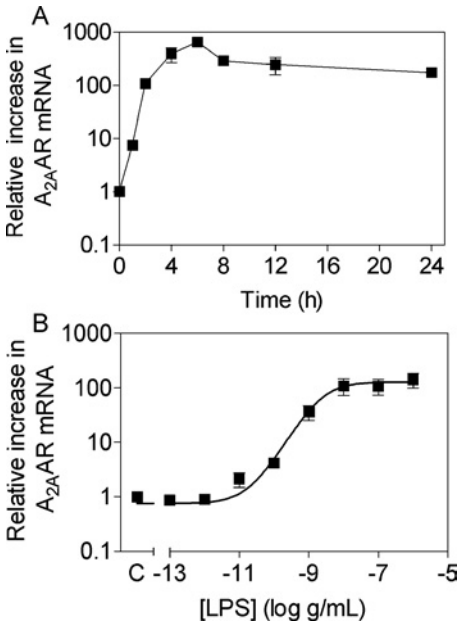

Figure 1 Effect of LPS treatment on expression of A_{2A}AR mRNA in Wehi-3 cells as determined by real-time, quantitative PCR

(**A**) Time course of A_{2A}AR expression upon stimulation with 100 ng/ml LPS. (**B**) Dose–response of A_{2A}AR expression as measured after 4 h of LPS treatment. Transcript levels were normalized to GAPDH levels and all data points are the means ± S.E.M. for at least three independent experiments performed in duplicate and quantitative PCR performed in triplicate.

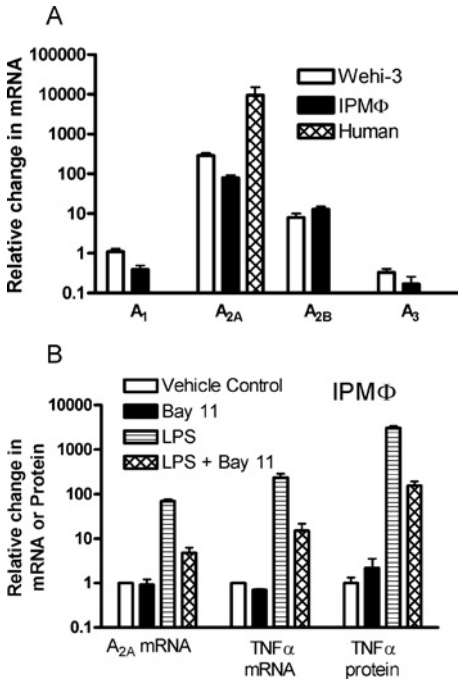

Figure 2 Effects of LPS and BAY 11-7082 on mRNA and protein expression in macrophages

(**A**) Fold changes in transcripts following treatment of various cells with 10 ng/ml LPS for 4 h. (**B**) Effect of pretreating IPMΦ with 10 μM BAY 11-7082 (BAY 11) on the transcription of A_{2A}R, TNFα mRNAs and TNFα protein. Control TNFα levels are 3.6 ± 1.2 pmol/ml. Data are pooled for three to seven experiments.

stimulated to produce TNFα by the addition of 10 ng/ml LPS with various concentrations of ATL146e. After 4 h, the supernatant was removed and assayed for TNFα by ELISA (eBioscience, San Diego, CA, U.S.A.).

RESULTS

LPS rapidly changes adenosine receptor mRNAs in macrophages

Treatment of Wehi-3 cells, mouse macrophages or human macrophages with LPS evoked rapid and large changes in A_{2A}AR mRNA and smaller changes in mRNAs for the other adenosine receptor subtypes. Figure 1 shows the time-course and dose–response curve for A_{2A}AR mRNA induction in Wehi-3 cells. A_{2A}AR mRNA was increased by over 8-fold within 1 h and reached a peak above 600-fold in 6 h. LPS increased A_{2A}AR mRNA with an ED_{50} of 2.3 ng/ml, with significant induction noted by 10 pg/ml (Figure 1B). This up-regulation was almost completely inhibited by 30 μg/ml polymyxin B (results not shown). LPS elicited a smaller increase in A_{2B}AR transcript and a small decrease in A_3AR transcript in both Wehi-3 cells and mouse IPMΦ (Figure 2A). A_1AR mRNA expression did not change from the initial low-level expression in Wehi-3 cells and it was decreased in IPMΦ. In human macrophages, mRNA in unstimulated cells was very low and strongly induced by LPS, resulting in a very high fold stimulation of A_{2A}AR mRNA.

Role of NF-κB (nuclear factor κB) in A_{2A}AR mRNA induction

NF-κB has been implicated as a mediator of cytokine induction in macrophages. We investigated the effects of the selective NF-κB inhibitor BAY 11-7082 on induction of A_{2A}AR mRNA and, as a positive control, on the induction of TNFα mRNA and protein in IPMΦ. As shown in Figure 2(B), inhibition of NF-κB resulted in a $>90\,\%$ decrease in A_{2A}AR and TNFα mRNAs and TNFα protein.

Increased ^{125}I-ZM241385 binding to LPS-treated Wehi-3 cells

In order to determine if increased A_{2A}AR mRNA levels result in increased A_{2A}AR expression, we measured receptor number in Wehi-3 cells using the specific A_{2A}AR antagonist, ^{125}I-ZM241385. As seen in Figure 3(A), there is no significant specific radioligand binding to vehicle-treated control cells. After treatment with 100 ng/ml LPS, the cells specifically bind ^{125}I-ZM241385 with a B_{max} of 85 fmol/mg after 4 h, 150 fmol/mg after 8 h and 348 fmol/mg after 20 h. Figure 3(B) shows Scatchard transformations of these data, indicating binding to a single saturable site that does not change affinity as a result of receptor up-regulation. We determined that 1 μg of protein corresponds to 7500 Wehi-3 cells. On the basis of this calculation, the number of receptors/cell at 20 h is 28 000 and the receptor density increases at a linear rate of 23 receptors $\cdot$ cell^{-1} $\cdot$ min^{-1} for at least 20 h (Figure 3C). In order to determine whether the newly made A_{2A}ARs on Wehi-3 cells function normally, we measured cAMP levels in cells pretreated with or without 100 ng/ml LPS. Figure 3(D) shows that control cells did not have a significant cAMP response to the A_{2A} agonist ATL146e. In contrast, cells that had been treated overnight with LPS made significant amounts of cAMP in the presence of 10 nM ATL146e. This effect was inhibited by treatment with 50 nM ZM241385.

LPS-pretreated IPMΦs are more sensitive to an A_{2A}AR agonist

We also examined the effect of LPS on A_{2A}AR number on IPMΦ. Since LPS has no effect on ^{125}I-ZM241385 affinity, we used a single concentration of radioligand. Figure 4(A) shows that cells that had been pretreated with LPS overnight displayed over 20 times higher radioligand binding than control cells. In order

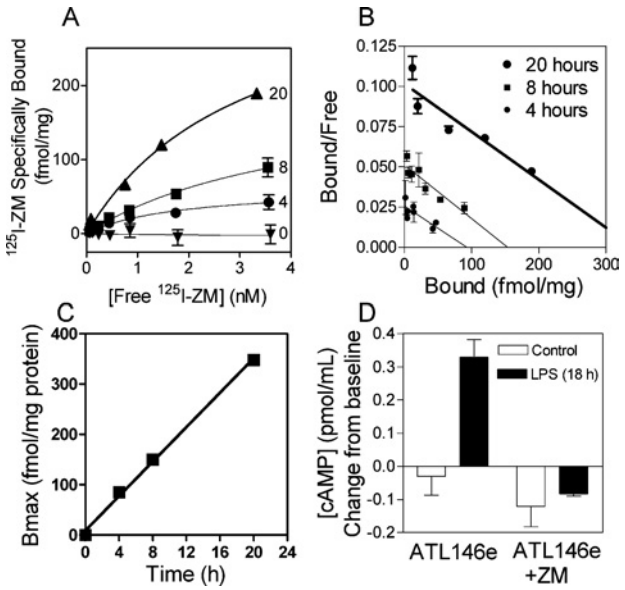

Figure 3 Effect of LPS treatment on A_{2A}AR receptor expression and function in Wehi-3 cells

(**A**) Saturation binding isotherms for control cells and cells treated for 4–20 h with LPS ($n = 3$; where n is the number of replicates). (**B**) Scatchard transformations of the data in (**A**) fitted with linear regressions demonstrating binding to a single site. (**C**) Change over time in the number of A_{2A}ARs (^{125}I-ZM241385 binding sites) after treatment with LPS. The data are fitted to a straight line (coefficient of determination $r^2 = 0.998$). (**D**) Change in cAMP (TNFα-treated – basal) in response to A_{2A}AR agonist stimulation. After stimulation with or without 10 nM ATL146e and 50 nM ZM241385 (^{125}I-ZM) (in four combinations shown in **D**), cells were incubated for 20 min at 37 °C in the presence of 50 μM rolipram. The cAMP concentrations in the supernatant were measured by EIA. The baseline level of cAMP was 0.2 pmol/ml.

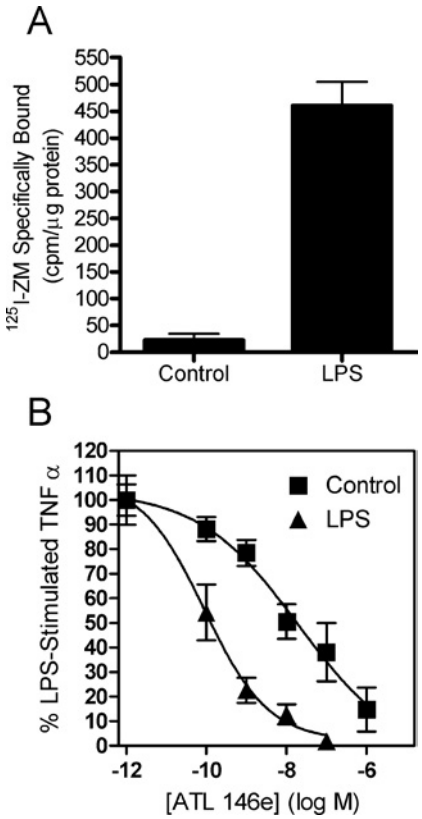

Figure 4 Effect of LPS treatment on whole-cell radioligand binding and functional potency of ATL146e in IPMΦ

Macrophages were harvested from Balb/c mice as described in the Experimental section. (**A**) Cells treated with or without 10 ng/ml LPS for 20 h were removed from the tissue-culture plates with PBS + 10 mM EDTA and gentle scraping. After washing in PBS, cells were resuspended and radioligand binding was performed with approx. 0.3 nM ^{125}I-ZM241385 for 3 h at 4 °C. Non-specific binding was measured in the presence of 100 nM ZM241385. Data represent the means ± S.E.M., $n = 3$. (**B**) Cells were pretreated with or without 10 ng/ml LPS for 18 h, rinsed twice with PBS, and then restimulated with 10 ng/ml LPS ± ATL146e in various concentrations. Cell supernatants were harvested after 4 h and assayed for TNFα. The IC_{50} of ATL146e for control and LPS-pretreated cells was 20 and 0.1 nM respectively. Each point represents the mean ± S.E.M. for three independent experiments.

to confirm the functional significance of A_{2A}AR up-regulation, we examined the sensitivity of these cells to A_{2A}AR-mediated inhibition of TNFα release in response to acute LPS treatment. Cells were treated overnight with or without (±) 10 ng/ml LPS, washed with PBS, then rechallenged for 4 h with 10 ng/ml LPS and ATL146e at various doses. As seen in Figure 4(B), the EC_{50} for ATL146e in LPS-pretreated cells was 0.1 nM, approx. 200-fold more potent than the EC_{50} in control cells (20 nM).

DISCUSSION

TLRs (Toll-like receptors) on macrophages recognize microbial products (e.g. lipoproteins, peptidoglycan, LPS, flagellin and bacterial DNA) and initiate the transcription of many cytokines that promote phagocytosis in response to bacterial pathogens. The present study demonstrates that activation of macrophages with LPS also initiates a mechanism to terminate inflammation after a delay, by causing the induction of anti-inflammatory adenosine receptors A_{2B} and principally A_{2A}. There is also a reduction of G_i-coupled A_1 and A_3 mRNAs that may also contribute to inactivation of macrophages exposed to adenosine after LPS. Changes in the macrophage response to adenosine may also be modulated by altered expression of the β-subunits of heterotrimeric G-proteins [24–26]. The magnitude of A_{2A}AR mRNA induction, > 100-fold in Wehi-3 cells, IPMΦ and human macrophages, is far greater than the induction noted in the human monocyte-like THP-1 cell line and mouse endothelial cells [14,26]. The large A_{2A}AR mRNA and protein induction may be an important adaptation to limit collateral damage to host tissues during infection.

The activation of the G_s-coupled A_{2A}AR in a variety of cell types leads to inhibition of pro-inflammatory pathways. A_{2A}AR activation inhibits aggregation in platelets [28–30], inhibits adhesion and oxidative burst in neutrophils [4,31] and decreases pro-inflammatory cytokines in monocytes [5,32,33] and macrophages [34,35]. Genetic deletion of the A_{2A}AR gene, *adora2a*, leads to a hyperactive immune response after chemical or ischaemic liver injury, suggesting that the A_{2A}AR is an important endogenous anti-inflammatory pathway [1,36]. Previous work from our group has shown that A_{2A}AR activation improves survival in murine models of endotoxaemia and sepsis [9].

It is noteworthy that our findings demonstrate for the first time that the anti-inflammatory potency of A_{2A}AR activation is enhanced after inflammation due to the large induction of radioligand-binding sites and functional A_{2A} receptors. Hence, A_{2A}AR or A_{2B}AR agonists or adenosine are probably most effective as anti-inflammatory agents when administered during infection or other inflammatory conditions that provokes the induction of these

receptors. The increase in A_{2A}AR mRNA and functional potency was associated with an increase in receptor density measured by radioligand binding. Leibovich and colleagues [16,17] did not observe an increase in A_{2A}AR immunoreactivity by Western blotting following treatment of macrophages with LPS. Our experience is that radioligand binding is more sensitive and less prone to non-specific binding than Western blotting as a means to detect the A_{2A}AR. For example, some antibodies detect immunoreactive proteins near the molecular mass of the A_{2A}AR, even in tissues from mice in which the A_{2A}AR gene has been deleted [38]. No specific radioligand binding is detected in such mice. The results of the present study clearly demonstrate an increase in A_{2A}AR receptor density over time after exposure of Wehi-3 cells, IPMΦ or human macrophages to LPS.

Pretreatment of IPMΦ with the NF-κB inhibitor BAY 11-7082 inhibited the induction of A_{2A}AR and TNFα mRNAs. A role for NF-κB in the induction of cytokines, along with inhibition by BAY 11-7082, has been previously demonstrated in human macrophages [39]. The AP-1 family of transcription factors comprising the Jun, Fos, Maf and ATF subfamilies can be activated by LPS in macrophages to stimulate cytokine production [40,41]. Analysis of the 5′-end of the *adora2a* gene by Fredholm et al. [42] shows a putative AP-1 binding site approx. 1 kb upstream of the transcriptional start site, suggesting that transcription of cytokines and A_{2A}ARs may be regulated by some of the same transcription factors.

In summary, we have shown that LPS rapidly and markedly increases the expression of A_{2A}AR mRNA in human and mouse macrophages. The newly formed receptors are functional as evidenced by A_{2A}AR agonist-stimulated cAMP accumulation, enhanced radioligand binding and an increased potency of an agonist to inhibit TNFα release from these cells. LPS causes a smaller increase in A_{2B}AR mRNAs and decreases A_1 and A_3 mRNA in mouse macrophages. The up-regulation of the A_{2A}AR may provide an endogenous and inducible anti-inflammatory pathway to limit the activity of the immune system in response to bacterial infection and other inflammatory stimuli.

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