Disruption of the A3 Adenosine Receptor Gene in Mice and Its Effect on Stimulated Inflammatory Cells*

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The A3 adenosine receptor (A3AR) is one of four receptor subtypes for adenosine and is expressed in a broad spectrum of tissues. In order to study the function of A3AR, a mouse line carrying a mutant A3 allele was generated. Mice homozygous for targeted disruption of the A3AR gene, A3AR−/−, are fertile and visually and histologically indistinguishable from wild type mice. The lack of a functional receptor in the A3AR−/− mice was confirmed by molecular and pharmacological analyses. The absence of A3AR protein expression in the A3AR−/− mice was demonstrated by lack of N6-(4-amino-3-iodobenzyl)adenosine binding to bone marrow-derived mast cell membranes that were found to express high levels of A3AR in wild type mice. In A3AR−/− mice, the density of A1 and A2A adenosine receptor subtypes was the same as in A3AR+/+ mice as determined by radioligand binding to brain membranes. Additionally, A3AR receptor transcript expression was not affected by ablation of the A3AR gene. A3AR−/− mice have basal heart rates and arterial blood pressures indistinguishable from A3AR+/+ mice. Functionally, in contrast to wild type mice, adenosine and the A3AR-specific agonist, 2-chloro-N6-(3-iodobenzyl)-adenosine-5’-N-methylcarboxamide (2-Cl-IB-MECA), elicited no potentiation of antigen-dependent degranulation of bone marrow-derived mast cells from A3AR−/− mice as measured by hexosaminidase release. Also, the ability of 2Cl-IB-MECA to inhibit lipopolysaccharide-induced tumor necrosis factor-α production in vivo was decreased in A3AR−/− mice in comparison to A3AR+/+ mice. The A2A adenosine receptor agonist, 2-p(2-carboxyethyl)phenylamino)-5’-N-ethylcarboxamidoadenosine, produced inhibition of lipopolysaccharide-stimulated tumor necrosis factor-α production in both A3AR−/− and A3AR+/+ mice. These results show that the inhibition in vivo can be mediated by multiple subtypes, specifically the A3 and A2A adenosine receptors, and A3AR activation plays an important role in both pro- and anti-inflammatory responses.

Adenosine is a naturally occurring nucleoside that exhibits diverse and potent physiological responses in the central nervous system and the cardiovascular, renal, pulmonary, and immune systems. The actions of adenosine are mediated through G-protein-coupled receptors classified into four subtypes, A1, A2A, A2B, and A3, on the basis of their affinity order profiles for agonists and antagonists (1–3). The A3 adenosine receptor (A3AR)1 is the most recent subtype identified, since it had remained pharmacologically obscure until its gene was cloned in the early 1990s. The A3AR has been cloned from multiple species including rat (4, 5), human (6, 7), sheep (8), and rabbit (10).

The physiological importance of the A3 adenosine receptor has not been established. A3AR is expressed in a broad spectrum of tissues. The most abundant expression in human is found in lung and aorta (7). The evaluation of the functional role of the A3AR has been hampered by the presence of multiple adenosine receptor subtypes in target tissues and lack of selective A3AR agonists and antagonists. In addition, the unique species difference between rat and human receptors in their ability to bind xanthine antagonists and differences in tissue distribution of expression has contributed to the difficulty in selecting an appropriate model to study the function of the A3AR (3). Despite these obstacles, a number of studies have been reported in which the A3AR has been linked to a variety of physiological processes including degranulation of antigen-stimulated rat basophilic leukemic cell line, RBL-2H3 (11), mast cell-dependent constriction of hamster cheek pouch arterioles (12), mast cell mediation of hypotension in rats (13, 14), induction of apoptotic cell death (15), inhibition of TNFα production from LPS-stimulated murine (16) and human (17) macrophage-like cell lines, inhibition of platelet-activating factor-induced chemotaxis of human eosinophils (18), neural (19) and cardiac (20, 21) protection, and depression of locomotor activity in mice (22).

The generation of a mouse line that does not express the A3AR subtype provides a useful tool to dissect out the role of this receptor from other adenosine receptor subtypes. By complementing pharmacological analyses with targeted gene disruption, evaluation of the functional role of the A3AR in various physiological processes can be more readily addressed. In this report, we describe the initial characterization of the A3AR−/− mouse, and we evaluate the consequence of A3AR

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1 The abbreviations used are: A3AR, A3 adenosine receptor; A3AR−/−, A3 adenosine receptor-deficient; A3AR+/−, wild type; BMMC, bone marrow-derived mast cells; CGS21680, 2-p(2-carboxyethyl)phenylamino)-5’-N-ethylcarboxamidoadenosine; 2-Cl-IB-MECA, 2-chloro-N6-(3-iodobenzyl)-adenosine-5’-N-methylcarboxamide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; ES, embryonic stem; I-ABA, N9-(4-amino-3-iodobenzyl)adenosine; IB-MECA, N9-(3-iodobenzyl)9-[5-(methylcarba- moyl)-β-ribofuranosyl]adenosine; LPS, lipopolysaccharide; TNFα, tumor necrosis factor-α; kb, kilobase pair; Pipex, 1,4-piperazinediethanesulfonic acid APNEA, N9-2-(4-aminophenyl)ethyladenosine.
gene ablation on adenosine receptor-mediated bone marrow-derived mast cell degranulation and in vivo inhibition of LPS-stimulated TNFα production. Our findings illustrate an important role for the A3AR on stimulated inflammatory cells.

**EXPERIMENTAL PROCEDURES**

**Generation of A3R−/− Mice**—The mouse genomic DNA utilized to construct the targeting vector was obtained by polymerase chain reaction screening (5′-GCTGGCATGCGGCTCGTGTG and 5′-GAGCGGATAAAGTGCCATCT) of a P1 129 Mouse ES Library (Genome Systems, Inc.). The targeting vector was introduced into the embryonic stem (ES) line E14Tg2a (23) by electroporation, and G418-resistant colonies were identified by established protocols (24). Two A3AR-targeted ES cell clones were injected into 3.5-day-old blastocysts from C57BL/6 mice and reimplanted into pseudopregnant foster mothers. Resultant progeny were screened initially by coat color chimerism as the indicator of ES cell contribution. Chimeras were bred to B6D2 and C57BL/6 mice and heterozygote mice determined by genomic Southern blot of tail biopsy DNA. Heterozygote mice were intercrossed to produce homozygote A3AR−/− animals and wild type (A3AR+/+) littermate controls.

**Southern Blot Analysis**—Genomic DNA was isolated from ES cell clones and tail biopsies by modifications of standard procedures (25) and analyzed by genomic Southern blot for targeted recombination. Ten microliters of genomic DNA was digested with one of the restriction enzymes XhoI, electron illuminated on a 0.7% agarose gel, transferred to Hybond-N, and UV cross-linked according to the manufacturer’s suggestion (Amersham Pharmacia Biotech). Filters containing the immobilized DNA were incubated with 2 × 10⁶ cpm/ml of radiolabeled probe in RapidHyb (Amersham Pharmacia Biotech) solution for 3 h at 65 °C. The A3 targeting probe corresponds to a 0.75-kb Xhol-HindIII fragment located immediately upstream of the short arm of the targeting construct. Filters were washed at 65 °C in a solution containing 0.1× SSC and 0.1% SDS and analyzed by autoradiography.

**Purification of Bone Marrow-derived Mast Cell (BMMCs)—**BMMCs were isolated from the femurs of 8–12-week-old A3AR−/− and A3AR+/+ mice according to established procedures (26). BMMCs were cultured in the presence of interleukin-3 and passed weekly to eliminate adherent cells as described (26). Experiments were carried out within 4–8 weeks of culture initiation.

**Northern Blot Analysis**—Total brain RNA was isolated from A3AR−/− and A3AR+/+ mice using the Trizol Reagent (Life Technologies, Inc.). Poly(A)+ mRNAs was isolated by subjecting total brain RNA to two cycles of oligo(dT)-cellulose chromatography. Total RNA was isolated from A3AR−/−, A3AR+/−, and A3AR+/+ cultured BMMCs using RNAzol (Tel-Test, Inc.) according to the manufacturer’s recommendation. Fifteen micrograms of total BMMCs RNA or 5 μg of poly(A)+ RNA was fractionated on a 1% agarose/formaldehyde gel (27), transferred to Hybond-N, and hybridized in RapidHyb solution containing 2 × 10⁶ cpm/ml radiolabeled probe. Mouse A1, A2A, and A3A cDNA probes were a kind gift from Dr. Diana Marquardt (University of California, San Diego). The mouse A3a probe corresponds to a 431-base pair SacI fragment containing part of the 2nd extracellular loop and stopping 18 amino acids upstream of the stop codon. Filters were washed at 65 °C in a solution containing 0.1× SSC and 0.1% SDS and analyzed by autoradiography.

**Radioligand Binding**—Membranes were prepared from cultured bone marrow-derived mast cells from A3AR−/−, A3AR+/−, and A3AR+/+ mice, and [3H]IgE binding was carried out according to Salvatore et al. (7). Membranes were prepared from mouse whole brain as described (28) and utilized to measure the binding of [3H]DPCPX (8-cyclopentyl-1,3-dipropylxanthine) to A1 adenosine receptors and [3H]CGS21680 (2,6-Di(2-carboxyethyl)phenylamino)-5′-N-ethylcarboxamidoadenosine) to A2A adenosine receptors. Binding to A2A and A3 receptors was performed in 50 mM Tris, pH 7.4, 10 mM MgCl₂, at 25 °C for 2 h. Assays were terminated by rapid filtration on to Skatron glass fiber filters (Skatron Inc., Sterling, VA) with three washes of ice-cold binding buffer. Filter bound radioactivity was determined using a Packard Tri Carb liquid scintillation counter. Equilibrium dissociation constants (Kᵦ) and maximal binding densities were obtained using the program EBDA (Biodesign, Fuggera).
Fig. 1. A, schematic representation of targeted recombination of the mouse A3 adenosine receptor. A3 replacement vector pA3KO (top panel), endogenous A3 adenosine receptor (middle panel), disrupted A3 adenosine receptor (bottom panel) are shown. Restriction sites BamH1 (B), EcoRI (E), HindIII (H), and XhoI (X) are as indicated. The position of 0.75-kb XhoI-HindIII 5' probe used as diagnostic for targeted recombination and expected fragment sizes is indicated. The putative start site of the A3 adenosine receptor is indicated by ATG and the stop site by TAA. B, genomic Southern blot representing the three A3AR genotypes (A3AR+/+ (−−), A3AR+/− (+−), A3AR−/− (++/)).

combination. Four ES cell clones were positive for targeted recombination as determined by a decrease in the size of the BamH1 fragment from 5.5 kb in wild type to 3.1 kb in the mutant allele (Fig. 1B). Two of the targeted clones were injected into 3.5-day-old blastocysts and produced highly chimeric mice. Male chimeras were bred to B6D2 and C57BL/6 mice, and transmission of the mutant allele was determined by coat color and genomic Southern blot of DNA obtained from tail biopsies.

Animals heterozygous for the mutant allele were intercrossed, and the progeny of these matings yielded wild type mice, heterozygotes, and mice homozygous for the targeted allele with the expected Mendelian frequency (Fig. 1B). No difference was observed between the A3AR-deficient and wild type littersmates by visual inspection or routine histological analysis of all organs. In addition, despite high levels of the A3AR in maintaining basal blood pressure or heart rate has been previously shown it has been previously shown that A2A and A3 adenosine receptor subtype mRNAs, but not A1 adenosine receptor subtype mRNAs, are expressed in murine BMMCs (33). A3 transcript expression was not detected in this initial analysis of BMMCs. In cultured BMMCs isolated from A3AR−/− mice, high levels of A3AR transcripts were detected (Fig. 2A). In comparison, no A3AR mRNA was detected in cultured BMMCs isolated from A3AR−/− mice. To determine whether the expression of A1, A2A, and A2B adenosine receptor subtypes were affected by the elimination of A3AR expression, mRNA levels were determined by Northern blot analysis. No significant change in levels of either A2A or A2B adenosine receptor transcript was seen in cultured BMMCs from wild type and A3AR−/− mice (Fig. 3). Consistent with previous reports (33), no expression of A1 receptor transcript was detectable in cultured BMMC mRNA from wild type and A3AR−/− mice (data not shown). In order to evaluate A1 receptor expression in A3AR−/− and A3AR−/− mice, RNA was isolated from whole brain and analyzed by Northern blots. Similar to A2A and A2B expression in BMMCs, no differences were observed between A3AR−/− and A3AR−/− mice (Fig. 3).

Binding Analysis of Tissues from A3AR+/+ and A3AR−/− Mice—The lack of functional A3AR protein expression was confirmed by binding assays on BMMC membranes utilizing the A3AR agonist [125I]ABA (Fig. 2B). BMMC membranes were utilized for the binding analysis as they express high levels of A3AR. Membranes prepared from A3AR+/+ mice exhibited specific [125I]ABA binding. Heterozygote mice exhibited a 50% decrease in specific [125I]ABA binding. In comparison, no specific [125I]ABA binding was measured on membranes from A3AR−/− mice (Fig. 2B). The density of A1 and A2A adenosine receptors expressed in whole brain from A3AR+/+ and A3AR−/− mice was measured by radioligand binding of [3H]DPCPX, 8-cyclopentyl-1,3-dipropylxanthine for A1 adenosine receptors, and [3H]CGS21680 for A2A receptors. No differences were observed in the density or affinities of A1 and A2A receptors between A3AR+/+ and A3AR−/− mice. Kd and Bmax values for [3H]DPCPX binding to A1 receptors on A3AR+/+ brain membranes was measured as 0.70 ± 0.31 nM and 1040 ± 106 fmol/mg of protein and 0.90 ± 0.45 nM and 910 ± 82 fmol/mg of protein on A3AR−/− brain membranes. Kd and Bmax values for [3H]CGS21680 binding to A2A receptors was measured as 16.02 ± 1.8 nM and 200.3 ± 23.7 fmol/mg of protein for binding to A3AR−/− brain membranes, and 15.33 ± 2.5 nM and 214.5 fmol/mg of protein on A3AR+/+ brain membranes were measured.

Degranulation of BMMCs from A3AR+/+ and A3AR−/−
BMMCs from activation of the A2A or A2B adenosine receptor expressed on BMMCs, degranulation experiments were carried out in receptor subtype modulated the secretory process. To determine the effect of the A2B receptor and/or another receptor subtype modulated the secretory process. To determine which adenosine receptor subtype mediates the response in BMMCs, degranulation experiments were carried out in BMMCs from A3AR−/− and A3AR+/+ mice. The ability of a selective A3AR agonist, 2-chloro-N6-(3-iodobenzyl)-adenosine-5′-N-methyl-carboxamide (2-Cl-IB-MECA), to potentiate mast cell degranulation was evaluated on antigen-stimulated BMMCs isolated from A3AR−/− and A3AR+/+ mice. BMMCs were preincubated with varying concentrations of 2-Cl-IB-MECA (0.1 nM to 1 μM) prior to antigen challenge. BMMCs from wild type mice demonstrated a dose-dependent increase in hexosaminidase release (81.5 ± 11.5% at 1 μM, p = 0.05), whereas BMMCs from A3AR−/− mice showed no change over antigen alone (Fig. 4). In comparison BMMCs from A3AR−/− mice exhibited a difference to wild type mice in the potentiation of antigen-induced degranulation by prostanoid prostaglandin E2 (115 ± 35 and 113 ± 28%, respectively). To determine whether activation of the A2A or A2B adenosine receptor expressed on BMMCs could also potentiate mast cell degranulation, BMMCs from A2AR+/+ and A3AR−/− mice were preincubated with varying concentrations of adenosine prior to antigen challenge. BMMCs from wild type mice displayed a dose-dependent potentiation of hexosaminidase release with adenosine preincubation, whereas A3AR−/− mice showed no potentiation over antigen alone (Fig. 5). Mean percent hexosaminidase release with antigen alone in A3AR−/− and A3AR+/+ was 27 ± 3 and 27 ± 5%, respectively. Preincubation with 1 × 10−4 M adenosine prior to antigen challenge resulted in an increase in hexosaminidase release by 67.3 ± 11% (p = 0.028) in wild type mice, whereas A3AR−/− mice showed no change (28 ± 5%) over antigen alone. These results support the key role of A3AR activation in enhancing mast cell mediator release.

Inhibition of TNFα Production in A3AR+/+ and A3AR−/− Mice—Adenosine has been shown to inhibit production of TNFα from stimulated J774.1 murine macrophage-like cells (16) and from the human cell line U937 (17). These reports have suggested that the A3AR mediates the response to adenosine as the inhibition of TNFα in response to adenosine receptor agonists exhibited an affinity order profile similar to the cloned rat A3 receptor. The involvement of the A3AR in the inhibition of TNFα production from LPS-stimulated inflammatory cells was evaluated in vivo in A3AR−/− mice (Fig. 6). Mice injected with vehicle before LPS stimulation had TNFα concentrations of 10.58 ± 1.7 ng/ml (n = 15) and 10.50 ± 2.02 ng/ml (n = 14) for A3AR+/+ and A3AR−/− mice, respectively. Intravenous administration of 50 μg/kg 2-Cl-IB-MECA immediately before LPS injection prevented elevations in plasma TNFα concentrations in A3AR+/+ mice (2.21 ± 0.51 ng/ml, n = 15) but
not in A3AR-/- mice (9.8 ± 1.23 ng/ml, n = 15). These results are in agreement with results reported from in vitro measurements in cultured mouse J774.1 and human U937 cells where A3AR activation was correlated with inhibition of LPS-stimulated TNFα production.

The effect of the A2A adenosine receptor agonist, CGS21680, on inhibition of LPS-stimulated TNFα production was compared in A3AR+/+ and A3AR-/- mice. In contrast to the observation with the A3AR agonist, 2Cl-IB-MECA, CGS21680 produced inhibition of LPS-stimulated TNFα production in both A3AR-deficient and wild type mice. Mice injected with vehicle before LPS stimulation had TNFα concentrations of 7.29 ± 1.02 ng/ml (n = 8) and 9.65 ± 1.15 ng/ml (n = 12) for A3AR+/+ and A3AR-/- mice, respectively. Administration of 100 μg/kg CGS21680 immediately before LPS prevented elevations in plasma TNFα concentrations in both A3AR+/+ (3.17 ± 0.54 ng/ml; n = 8) and A3AR-/- (4.35 ± 0.50 ng/ml; n = 10) mice. These results are comparable to those measured in BALB/c mice where CGS2180 produced inhibition of LPS-stimulated TNFα production in vivo (34). The results obtained in the A3AR+/+ and A3AR-/- mice with 2Cl-IB-MECA and CGS21680 suggest that inhibition of LPS-stimulated TNFα production is mediated via multiple adenosine receptor subtypes, specifically the A3 and A2A adenosine receptor subtypes.

**DISCUSSION**

In this study, mice deficient in the expression of A3AR were generated. The A3AR-/- mice were determined to be healthy and fertile and exhibited no differences in the development of any organ systems. In addition, the expression of the other adenosine receptor subtypes, A1, A2A, and A2B, was not affected by the ablation of the A3AR gene. The physiological consequence of the loss of A3AR expression was evaluated by measuring the response of antigen-stimulated BMMC degranulation to adenosine and inhibition of LPS-stimulated TNFα production in vitro in A2A+/+ and A2A-/- mice. Our results support the essential role of the A3AR as a mediator in these processes as both functions were found to be eliminated in mice lacking A3AR. The ablation of adenosine or 2Cl-IB-MECA potentiation of antigen-stimulated BMMC degranulation and decrease in 2Cl-IB-MECA inhibition of LPS-stimulated TNFα production are the first phenotypic differences to be reported for the A3AR-/- mice.

Adenosine has been reported to potentiate mediator release from multiple types of mast cells including human lung mast cells (35), rat mast cells (36), murine BMMCs (33), and the rat basophilic leukemic cell line, RBL-2H3 (11, 37), after stimulation with antigen or calcium ionophore. Adenosine has been implicated to contribute to the pathophysiology of asthma as inhaled adenosine provokes bronchoconstriction in asthmatic but not normal patients (38). The response to adenosine is believed to be predominantly mediated through mast cell activation (39). Attempts to elucidate definitively the adenosine receptor subtype that mediates mast cell activation has been pharmacologically difficult due to lack of agonists and antagonists that display sufficient selectivity to discriminate between multiple adenosine receptor subtypes present on these cells. Recent studies employing the rat mast cell-derived basophilic leukemia cell line, RBL-2H3, a model for mast cells, have suggested that the A3AR is the adenosine receptor subtype that mediates mast cell degranulation.

The relationship between A3AR activation and mast cell degranulation was first postulated by the detection of A3AR on RBL-2H3 cells, using the A3AR agonist 125I-labeled aminophenethyladenosine N6-2-(4-aminophenyl) (APNEA) (11). It was further shown that enhancement of antigen-induced secretion from RBL-2H3 cells correlated with an affinity order profile for adenosine receptor agonists similar to that established for the cloned rat A3AR (11). Additional support for the A3AR role in enhancement of antigen-induced degranulation of RBL-2H3 cells came from the observation that dexamethasone increased the amount of 125I-APNEA binding, possibly due to an increase in A3AR expression, and the A3AR-dependent release of hexosaminidase (37).

A correlation between A3AR activation and nonimmunologically dependent mast cell mediator release has recently been demonstrated in vivo. The hypotensive response to A3AR activation by APNEA in the anesthetized rat is qualitatively similar to the mast cell degranulation agent compound 48/80. Additionally, the hypotensive response to APNEA was suppressed by mast cell degranulation inhibitors, and depletion of mast cell mediators with compound 48/80 decreased the effectiveness of APNEA-induced hypotension (14). Van Schaick et al. (40) have shown that intravenous administration of the A3 agonist, 2Cl-IB-MECA, in conscious rats resulted in a hypotensive response coincident with an increase in plasma histamine concentrations indicating the involvement of the A3AR in mediator release from mast cells in vivo.

In this report, we demonstrate high levels of A3AR expression on murine BMMCs. This is the first report of A3AR expression on native mast cells in contrast to established cell lines such as RBL-2H3 cells. In BMMCs from A3AR-/- mice, the potentiation of antigen-dependent degranulation by either adenosine or the A3AR agonist, 2Cl-IB-MECA, was found to be eliminated. The observation of the absence of adenosine or 2Cl-IB-MECA potentiation of BMMC degranulation in A3AR-/- mice supports the conclusions drawn from previous studies with RBL-2H3 cells and in rats in vivo in which the A3AR was implicated in mediating mast cell degranulation.

Previous reports have implicated the A2B adenosine receptor to mediate BMMC degranulation through demonstration of an inability of pertussis toxin to affect adenosine-induced calcium mobilization (41). In other species, specifically, canine and human, the A2B receptor has also been reported to mediate mast cell degranulation (42–44). This discrepancy in identifying which adenosine receptor subtype mediates mast cell degranulation may be due to a number of variables including differences in the levels of adenosine receptor subtype expression between cells selected for evaluation, the tissue source for mast cells, species differences or whether mast cells are immunologically primed prior to degranulation measurements. For exam-
ple, RBL-2H3 cells and mouse BMMCs express high levels of A3ARs as measured by both transcript expression and radio-labeled binding. BMMCs are more similar phenotypically to the rat RBL-2H3 tumor cell line in which the A3AR has been reported to predominate the potentiation of mast cell mediator release. In contrast, dog BR mastocytoma cells express low levels of A3ARs. Degranulation of non-immunologically stimulated BR mastocytoma cells in response to adenosine receptor agonists was found to exhibit an affinity order profile most consistent with the A3R1 adenosine receptor (42). In the human mast cell line, HMC-1, A3R agonist activation has been shown to couple to mobilization of intracellular calcium and interleukin-8 secretion (43, 44). The adenosine receptor subtype that mediates these responses has not been defined on HMC-1 cells and warrants further study with selective agonists, such as 2Cl-IB-MECA, to determine if the A3AR is involved as we have shown in the mouse. Recently, it has been reported that calcium mobilization in HMC-1 cells is stimulated with a potency order of 5’-N-ethylcarboxamidoadenosine > N6-(2-iodobenzyl)-5’-N-methylcarboxamidoadenosine (IB-MECA) consistent with activation of A2B receptors (45). It is important to note that native human mast cells have not been evaluated for A3AR-mediated responses.

In this report, we also evaluated the potential role of A3AR activation in the inhibition of LPS-stimulated TNFα production through a comparison of the responses of A3AR+/− and A3AR−/− mice. A number of published reports have linked A3AR activation with inhibition of LPS-stimulated TNFα production in vitro from cultured human monocytes and mouse and human macrophage-like cell lines. The possible involvement of A3AR was first postulated when adenosine receptor agonists were shown to inhibit TNFα production in endotoxin-stimulated human monocytes with a pharmacological profile uncharacteristic of either A1 or A2B adenosine receptor subtypes (46). In J774.1 cells, a murine macrophage-like cell line, LPS-induced TNFα secretion and TNFα gene expression were inhibited by adenosine receptor agonists with an affinity order profile similar to the cloned rat A3 receptor (16). In human U937 cells, micromolar concentrations of the A3 agonists, IB-MECA, decreased LPS-stimulated TNFα production, and it was concluded that A3AR activation mediated this response (17). Recently, inhibition of LPS-stimulated TNFα production by high concentrations (1 μM) IB-MECA was confirmed on human monocytes; however, the inhibition could be completely blocked with an A2A adenosine receptor-selective antagonist indicating that IB-MECA was acting via A2A and not A3 receptor activation (47). The availability of the A3AR−/− mouse provided the opportunity to evaluate specific receptor activation in vivo. Our results suggest that activation of either A1 or A2A adenosine receptors can mediate inhibition of LPS-stimulated TNFα production in vivo in mice. These results are consistent with published reports obtained with murine cell lines where A3AR activation has been implicated to mediate the inhibition of LPS-stimulated TNFα production (16) and with in vitro studies in BALB/c mice where A2A receptor activation was demonstrated to mediate inhibition of TNFα production (34).

In summary, our characterization of the A3AR−/− mouse has shown that A3AR activation can produce both pro-inflammatory and anti-inflammatory effects. Specifically, we have demonstrated that the A3AR is the adenosine receptor subtype mediating potentiation of antigen-induced degranulation in murine BMMCs. We have also shown that A1 and A2A receptor activation inhibits LPS-stimulated increases in TNFα in vivo. Further characterization of the A3AR−/− mouse may provide important information about the functional role of the A3AR in more complex inflammatory responses.

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REFERENCES