Overexpression of A_3 Adenosine Receptor in Peripheral Blood Mononuclear Cells in Rheumatoid Arthritis: Involvement of Nuclear Factor-κB in Mediating Receptor Level

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ABSTRACT. ***Objective.*** A_3 adenosine receptor (A_3AR) upregulation has been found in cells of synovial tissue and in peripheral blood mononuclear cells (PBMC) of rats with adjuvant-induced arthritis. We investigated A_3AR levels in PBMC of patients with rheumatoid arthritis (RA) and in mitogen-activated PBMC from healthy subjects. We examined the role of nuclear factor-κB (NF-κB), a transcription factor present in the A_3AR promoter, in mediating receptor upregulation.

Methods. A_3AR and NF-κB protein levels were evaluated in PBMC of RA patients (n = 23) and healthy subjects by Western blot. A_3AR and NF-κB levels were also analyzed in phytohemagglutinin (PHA) and lipopolysaccharide (LPS)-stimulated PBMC in the presence and absence of antibodies against interleukin 2 (IL-2) and tumor necrosis factor-α (TNF-α). Reverse transcription-polymerase chain reaction was performed in PHA-stimulated PBMC of healthy subjects to determine A_3AR expression.

Results. A_3AR was overexpressed in PBMC of RA patients compared to healthy subjects and was directly correlated to an increase in NF-κB. Similar findings were observed in PHA and LPS-stimulated PBMC from healthy subjects. Antibodies against IL-2 or TNF-α prevented the increase in A_3AR and NF-κB expression.

Conclusion. Overexpression of A_3AR was found in PBMC of RA patients. Receptor upregulation was induced by inflammatory cytokines controlling the expression of the A_3AR transcription factor NF-κB. (J Rheumatol 2007;34:20–6)

Key Indexing Terms:
A_3 ADENOSINE RECEPTOR — RHEUMATOID ARTHRITIS — NUCLEAR FACTOR-κB
IB-MECA — PHYTOHEMAGGLUTININ — LIPOPOLYSACCHARIDE

A_3 adenosine receptor (A_3AR) belongs to the family of the Gi-protein-associated cell membrane receptors. Receptor activation leads to inhibition of adenylyl cyclase activity, cAMP formation, and protein kinase A expression, resulting in the initiation of various signaling pathways[1].

Recent studies suggested that A_3AR activation by a specific agonist results in antiinflammatory and anti-tumor effects[2-8]. The selective A_3AR agonist 1-deoxy-1-[6-[[(3-iodophenyl)methyl]amino]-9H-purine-9-yl]-N-methyl-ß-D-ribofura-nuronamide (IB-MECA) inhibited the production of tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein-1α *in vitro* and prevented the development of collagen-induced arthritis in DBA1 mice and adjuvant-induced arthritis (AIA) in rats[8,9]. The mechanistic pathway of the antiinflammatory effect mediated by the A_3AR includes downregulation of A_3AR expression upon receptor activation. Subsequently, decreased protein expression of PKB/Akt was found in protein extracts derived from the synovial tissue. PKB/Akt is known to control the nuclear factor-κB (NF-κB) signal transduction pathway. Decreased PKB/Akt levels failed to phosphorylate IκB kinase, thus preventing IκB degradation and the release of NF-κB from its complex. These events hamper the translocation of NF-κB to the nucleus and the transcription of TNF-α and additional inflammatory cytokines[8,10].

Interestingly, the A_3AR was found to be overexpressed in the synovial and paw inflammatory tissues compared to the relevant normal tissues. Moreover, receptor upregulation was also observed in peripheral blood mononuclear cells (PBMC) of AIA rats compared to controls[8]. These results supported a recent study by Gessi, *et al* showing upregulation of A_3AR expression in phytohemagglutinin (PHA)-stimulated PBMC derived from healthy subjects[11].

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It thus seems that A_3AR expression correlates to cell activation or pathogenicity.

In this study we show that A_3AR is overexpressed in PBMC of patients with rheumatoid arthritis (RA). To investigate the regulation of A_3AR overexpression, we utilized an *in vitro* system of mitogen-stimulated PBMC in which A_3AR upregulation was found. We observed that A_3AR overexpression is associated with an increase in the production of inflammatory cytokines [TNF-α, interleukin 2 (IL-2)] that increase the expression of NF-κB. Bio-informatic studies revealed the presence of NF-κB in the A_3AR promoter, demonstrating the role of this transcription factor in determining A_3AR expression levels.

MATERIALS AND METHODS

Reagents. The A_3AR agonist 1-deoxy-1-[6-[[(3-iodophenyl)methyl]-amino]-9H-purine-9-yl]-N-methyl-ß-D-ribofura-nuronamide (IB-MECA) and the antagonist 2,3-diethyl-4,5-dipropyl-6-phenylpyridine-3-thiocarboxylate-5-carboxylate (MRS 1523) were purchased from Sigma (St. Louis, MO, USA). A stock 10 mM solution was prepared in DMSO, and further dilutions in RPMI medium were performed. RPMI, phosphate buffered saline (PBS), fetal bovine serum (FBS), and antibiotics for cell cultures were obtained from Beit Haemek (Haifa, Israel). Rabbit polyclonal antibodies against human A_3AR, and NF-κB, were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-IL-2 and anti-TNF-α antibodies were purchased from R&D Systems (Minneapolis, MN, USA). The mitogens PHA and lipopolysaccharide (LPS) were purchased from Sigma.

Blood sample collection and separation. Blood samples were collected from healthy subjects and from patients with RA scheduled to enter a clinical protocol in which a 1 month washout was requested. This study was approved by the hospitals' ethical committees including the blood sample collection. Healthy subjects and RA patients gave signed informed consent prior to blood withdrawal. Patients' particulars are summarized in Table 1.

To separate PBMC, heparinized blood (20 ml) was subjected to Ficoll-Hypaque gradient centrifugation. The PBMC were then washed with PBS, and protein extracts were prepared as detailed below.

Cell cultures of mitogen-activated PBMC. PBMC (2×10^6/ml) from healthy subjects were incubated with 5 μg/ml PHA or 1 μg/ml LPS in RPMI-1640 supplemented with 10% FBS for 24 h. Antibodies against IL-2 (1 μg/ml) or TNF-α (2 μg/ml) were added to the PHA and LPS-treated cultures, respectively, for the whole culture period. The effect of IB-MECA (10 nM) in the presence or absence of the A_3AR antagonist MRS1532 (10 nM) on the expression of the receptor was also evaluated in the PBMC mitogen-activated culture system. At the end of the incubation PBMC were collected from the culture plates, and protein extracts were prepared.

Table 1. Characteristics of symptoms in patients with RA upon 4 weeks of washout from medication.

Characteristic	Mean	SE
Age, yrs	54.13	2.25
Disease duration, yrs	6.57	2.15
CRP, mg/l	15.15	3.3
ESR, mm/h	40.13	6.1
Swollen joint count	12.43	0.87
Tender joint count	18	0.89
Patient global assessment by VAS	70.96	2.78
Rheumatoid factor, IU/ml	161.77	95.01
CRP, DAS28-4	6.01	0.17
ESR, DAS28-4	6.73	0.17

CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, VAS: visual analog scale, DAS: Disease Activity Score 28-joint count.

Western blot analysis of A_3AR and additional signaling proteins in PBMC. Western blot analysis was carried out according to the following protocol. Samples were rinsed with ice-cold PBS and transferred to ice-cold lysis buffer (TNN buffer, 50 mM Tris buffer, pH 7.5, 150 mM NaCl, NP 40). Cell debris was removed by centrifugation for 10 min at 7500 g. Protein concentrations were determined using the Bio-Rad protein assay dye reagent. Equal amounts of the sample (50 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, using 12% polyacrylamide gels. The resolved proteins were then electroblotted onto nitrocellulose membranes (Schleicher & Schuell, Keene, NH, USA). Membranes were blocked with 1% BSA and incubated with the desired primary antibody (dilution 1:1000) for 24 h at 4°C. Blots were then washed and incubated with a secondary antibody for 1 h at room temperature. Bands were recorded using a BCIP/NBT color development kit (Promega, Madison, WI, USA).

Analysis of A_3AR and NF-κB protein expression in patients' PBMC was as follows. Each 4 RA patients' samples were run in the same gel with a pool of 4 healthy subject samples designated as standard. The blots were quantified by densitometric analysis, and the ratio of RA patient/standard was calculated.

Blots of mitogen-stimulated cells were quantified against ß-actin.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis of A_3AR expression in PHA-stimulated PBMC. Total RNA was isolated from PHA-stimulated PBMC from healthy subjects utilizing TRI-reagent (Sigma). The reverse transcription reaction was performed at 45°C for 45 min from 1 μg total RNA extracted with TRI-reagent. One-step RT-PCR with Platinum Taq (Invitrogen) was utilized for A_3AR amplification and the 5'-ACG GTG AGG TAC CAC AGC TTG TG and 3'-ATA CCG CGG GAT GGC AGA CC primers for A_3AR were added. The reverse-transcription reaction was performed by heating to 99°C for 5 min, 50 cycles of 94°C for 30 s, 59°C for 45 s, and 73°C for 45 s.

For amplification of human ß-actin we used the primers 5'-TGG GAA TGG GTC AGA AGG ACT and 3'-TTT CAC GGT TGG CCT TAG GGT. The PCR conditions included heating to 94°C for 2 min, 30 cycles of 94°C for 30 s, 56°C for 90 s, and 73°C for 45 s.

Transcription factor binding site analysis. Analysis of the sequence stretch of 3050 bp upstream from the ATG transcription start site of the transcript ENST00000241356 (Chromosome 1, positions 111840578–111843582 of the human genome) at ENSEMBL V.39 was carried out. Basic analysis of transcription factor binding sites was performed with the ALIBABA server using the recommended default parameters. Information on interactions between transcription factors was derived from the literature and assembled into a hash structure. Based on this information we further analyzed the various binding sites for potential interactions. A program written in the Python programming language parsed the output of the ALIBABA analysis and mapped the various transcription factors according to their potential interactions with each other.

Statistical analysis. Results were evaluated using Student's t test, with statistical significance set at $p < 0.05$. Comparison between the mean values of different experiments was carried out. All data are reported as mean ± SD.

RESULTS

A_3AR expression in PBMC of RA patients. We investigated A_3AR expression in PBMC of patients with RA (Table 1). We also looked at the involvement of inflammatory cytokines in mediating A_3AR expression via their ability to control NF-κB expression, found in this study to be associated with the transcription of A_3AR. Figure 1A depicts a

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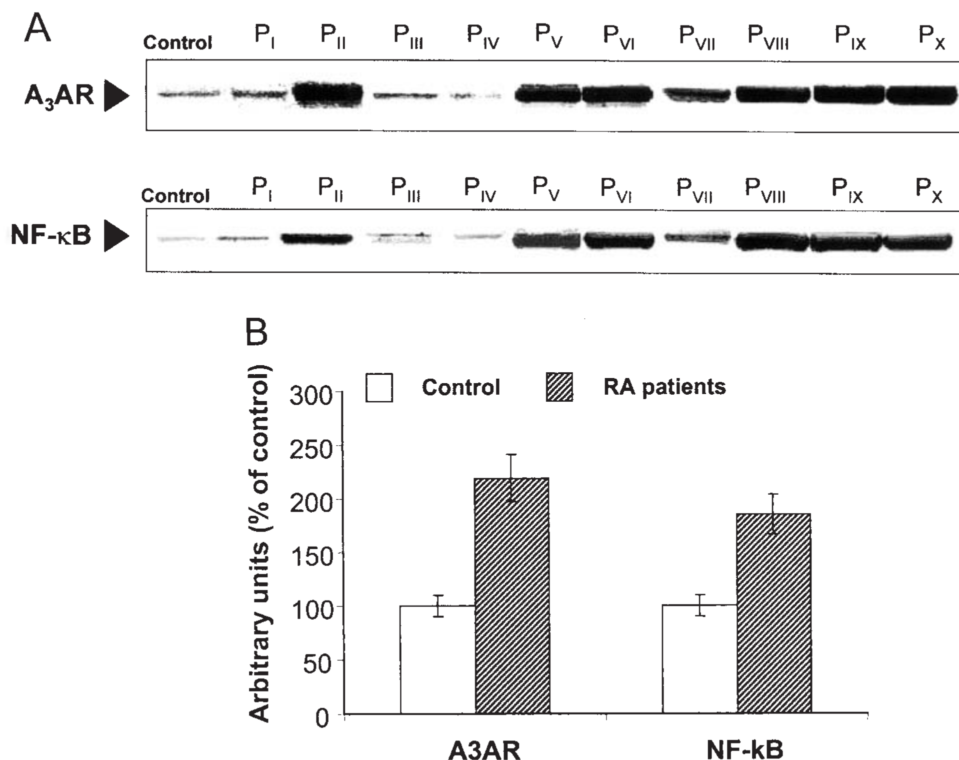

Figure 1. Western blot analysis of A_3AR and NF-κB protein expression in PBMC derived from RA patients. A. Representative Western blots of 10 patients compared to a representative healthy subject (one of 20). B. Summary of A_3AR and NF-κB protein levels in PBMC derived from 23 different RA patients. Results are shown as means ± SE.

representative blot of 10 RA patients (out of 23) in which A_3AR and NF-κB are upregulated compared to the standard. Figure 1B is a summary of expression of the 2 proteins in all patients compared to healthy subjects. A_3AR and NF-κB protein expression in RA patients was significantly higher than that measured in healthy subjects ($p < 0.001$).

NF-κB in the A_3AR promoter. Analysis of 3050 bp upstream of the ATG start site of the A_3AR gene revealed 2 binding sites for NF-κB/NF-κB1 at positions –1754 and –1598. Binding sites for c-REL[29] and for other transcription factors that are known to interact with NF-κB (e.g., C/EBP[28], CREBP1[30]) were mapped to this region.

A_3AR and NF-κB protein expression in mitogen-stimulated PBMC. To investigate the cellular and molecular mechanisms involved with receptor upregulation we used an *in vitro* system of mitogen-activated PBMC from healthy subjects. In PHA-stimulated PBMC, A_3AR mRNA and protein expression were upregulated (Figure 2A, 2B). NF-κB expression was also upregulated upon cell activation (Figure 3).

We then looked at the effect of IL-2, a marker of lymphocyte activation, in mediating the upregulation of A_3AR in the PHA-stimulated PBMC. Antibodies against IL-2 abrogated the increase in A_3AR and NF-κB expression (Figure 3). Similar data were observed in PBMC activated with LPS. A_3AR and NF-κB were upregulated upon cell activation with LPS. Antibodies against TNF-α, defined as the mediator of the inflammatory response in RA, induced downregulation of A_3AR and NF-αB (Figure 4).

Together, our findings suggest that A_3AR level is determined by inflammatory cytokines that control the level of the transcription factor NF-κB participating in the regulation of A_3AR expression.

Effect of IB-MECA on expression of A_3AR in mitogen-stimulated PBMC. In an additional set of experiments we analyzed the effect of the A_3AR agonist IB-MECA on A_3AR protein expression. IB-MECA downregulated the level of A_3AR in PHA-activated cells (Figure 5). A_3AR expression was also downregulated upon treatment of LPS-stimulated cells with IB-MECA (Figure 6A).

The antagonist MRS1532 reversed receptor downregulation induced by IB-MECA in both the PHA and LPS-treated cells (Figure 5B And 6B, respectively). Moreover, addition of IB-MECA to LPS-stimulated PBMC inhibited the production of TNF-α (Figure 6C).

DISCUSSION

We showed that A_3AR is upregulated in PBMC of patients with RA and that its high level is directly correlated to an increase in the expression of NF-κB. Receptor was also

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Figure 2. A_3AR expression in PHA-stimulated PBMC from healthy subjects. A_3AR mRNA (A) and protein level (B) were upregulated.

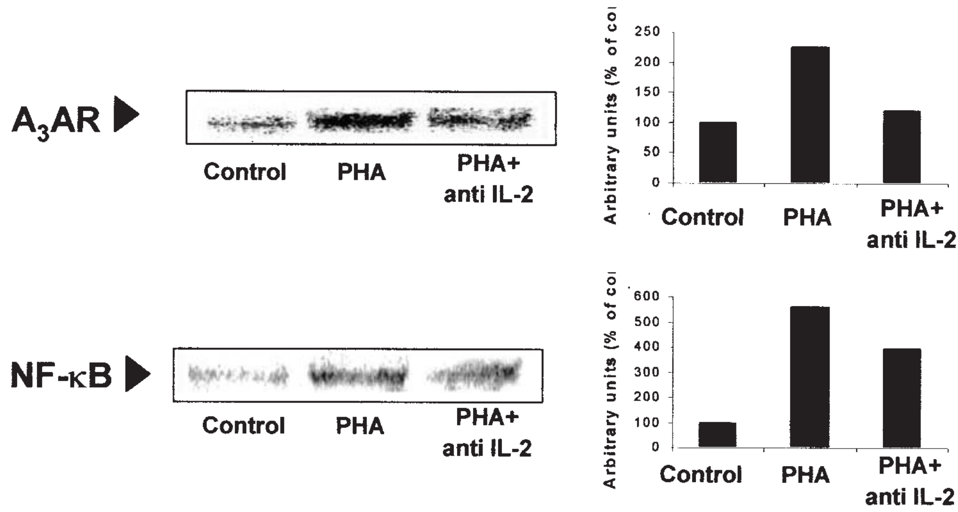

Figure 3. Effect of antibodies against IL-2 on A_3AR and NF-κB protein expression in PHA-stimulated PBMC from healthy subjects. PBMC were stimulated in the presence or absence of antibodies against IL-2. A_3AR and NF-κB levels were upregulated in PHA-stimulated PBMC. Antibodies against IL-2 abrogated the increase in A_3AR and NF-κB expression.

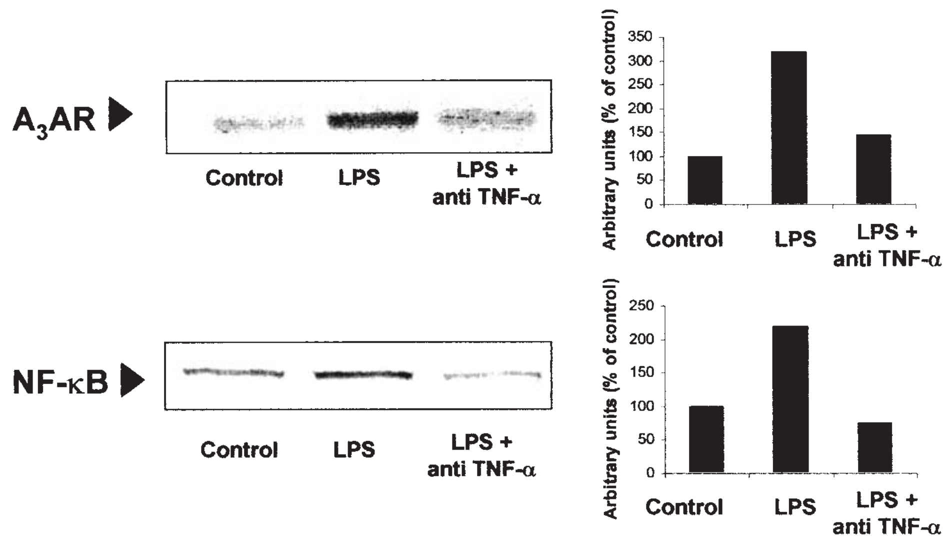

Figure 4. Effect of antibodies against TNF-α on A_3AR and NF-κB protein expression in LPS-stimulated PBMC from healthy subjects. PBMC were stimulated with LPS in the presence or absence of antibodies against TNF-α. A_3AR and NF-κB levels were upregulated upon cell activation with LPS. Antibodies against TNF-α induced downregulation of A_3AR and NF-κB levels.

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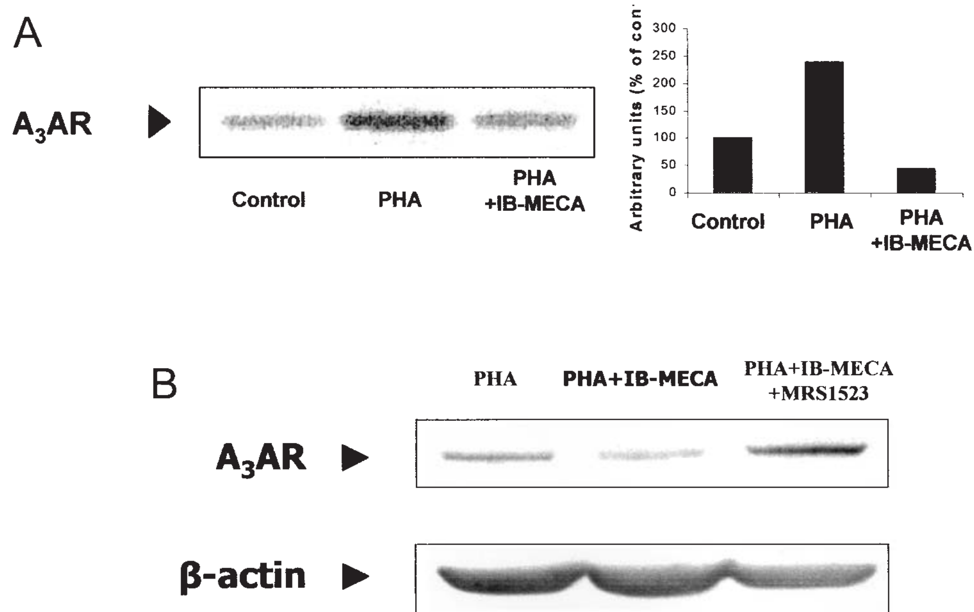

Figure 5. Effect of A_3AR agonist and antagonist on receptor protein expression in PHA-stimulated PBMC from healthy subjects. A. IB-MECA (10 nM) downregulated the increase in A_3AR levels. B. MRS1523 (10 nM) reversed the effect of IB-MECA.

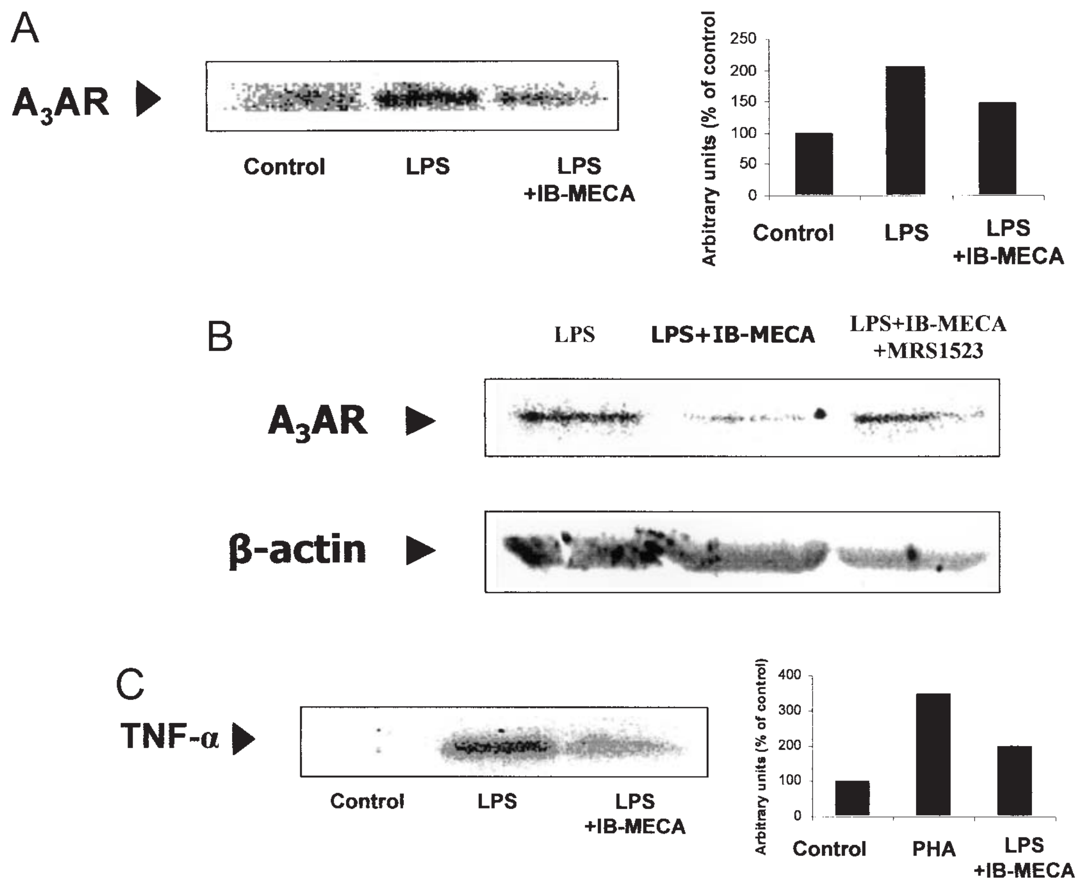

Figure 6. Effect of A_3AR agonist and antagonist on receptor protein expression in LPS-stimulated PBMC from healthy subjects. A. IB-MECA (10 nM) downregulated the increase in A_3AR levels. B. MRS1523 (10 nM) reversed the effect of IB-MECA. C. IB-MECA downregulated the levels of A_3AR and TNF-α.

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upregulated in PHA and LPS-activated PBMC from healthy subjects. In addition, it was shown that antibodies against IL-2 and TNF-α reversed A_3AR upregulation via a decrease in the level of NF-κB.

Upregulation of A_3AR expression was described previously in additional pathologies. A selective, approximately 10-fold upregulation of the A_3AR mRNA and protein was consistently found in the nonpigmented ciliary epithelium of all eyes in pseudoexfoliation syndrome, with and without glaucoma, compared to normal and glaucomatous control eyes[15]. Several recent reports describe A_3AR upregulation in a variety of cancerous diseases. In human melanoma, colon, breast, small-cell lung, and pancreatic carcinoma tissues, higher A_3AR expression in the tumor compared to adjacent non-neoplastic tissue was described. Receptor expression was correlated to disease severity. Interestingly, the lymph node metastasis expressed even more A_3AR mRNA than the primary tumor tissue[16,17]. It was further reported that the high A_3AR expression in the tumor tissue was reflected in PBMC. A high level of A_3AR expression was noted in PBMC from a patient with colon cancer, and returned to normal levels upon tumor removal[18]. Moreover, Gessi, *et al* reported that A_3AR was upregulated in activated PBMC from healthy subjects, and showed that CD4 T cells were the subpopulation that overexpressed the receptor[11]. None of these studies identified a molecular mechanism to explain receptor upregulation.

Our earlier studies in tumor and inflammatory cells showed that, upon receptor activation, signal transduction pathways are initiated leading to inhibition of PKB/Akt; this subsequently deregulates the Wnt and the NF-κB signaling pathways, resulting in downregulation of NF-κB and Lef/Tcf. This chain of events was accompanied by A_3AR downregulation and inhibition of tumor or inflammatory cell growth and development[2,5,6,8]. In this study we show that in PBMC of RA patients as well as in PHA or LPS-stimulated PBMC, the upregulation in A_3AR expression directly correlates to upregulation of the transcription factor NF-κB. A selective A_3AR agonist, IB-MECA, induced receptor downregulation in both PHA and LPS-activated cells, demonstrating the ability of the agonist to "normalize" receptor levels. The specificity of this response was proved by introduction of the A_3AR antagonist MRS1523, which blocked receptor downregulation.

An additional interesting finding was the difference in A_3AR expression in PBMC of RA patients. In 3 out of 10 blots presented in Figure 1, the receptor level was similar to that of the control. This may lead to an assumption that A_3AR expression needs to be analyzed prior to treatment with a specific agonist in order to predict the response.

Inflammatory cytokines are known to control adenosine receptor expression via an autocrine pathway. An increase in cytokine expression results in activation of cytokine receptor, which consequently generates downstream signaling pathways leading to upregulation of transcription factors inducing adenosine receptor upregulation. This loop was described for TNF-α in PBMC from healthy subjects. TNF-α induced by LPS stimulation was found to upregulate the A_{2A} adenosine receptor level[19-22]. Similar findings were observed in our study supporting the role of inflammatory cytokines in mediating A_3AR expression. Upon the introduction of anti-TNF-α and anti-IL-2 antibodies to LPS and PHA-stimulated PBMC, respectively, A_3AR expression was downregulated, demonstrating the role of these cytokines in mediating receptor expression. It thus seems that expression of both $A_{2A}AR$ and A_3AR is regulated by inflammatory cytokines.

Based on our findings we present a model suggesting upregulation of A_3AR expression as a result of TNF-α and IL-2 upregulation upon PBMC activation. The increase in cytokine levels induces upregulation of the transcription factor NF-κB, which subsequently induces receptor upregulation. In an analogy, in the PBMC microenvironment, the level of inflammatory cytokines is upregulated, resulting in overexpression of NF-κB, thereby inducing A_3AR upregulation. The existence of NF-κB binding sites in the A_3AR promoter further supports our theory that upregulation of NF-κB may be attributed to the increase in A_3AR expression[7-9].

Proinflammatory cytokines such as IL-2 and TNF-α play an important role in maintaining cartilage damage and severe destruction of joints due to uncontrolled activation of cellular immunity[23,24]. The presence of these cytokines in the inflammatory system leads to high expression of A_3AR in PBMC and in inflamed tissues. The finding that in activated PBMC, IB-MECA decreased A_3AR expression, resulting in downregulation of TNF-α, supports our concept that A_3AR activation may disconnect the autocrine loop, thus inhibiting synthesis of the inflammatory cytokines. Indeed, in an animal model in which A_3AR expression was upregulated in synovia and PBMC derived from AIA rats, treatment with IB-MECA ameliorated the clinical and pathological manifestations of the disease. This was followed by downregulation of receptor expression in the synovia and PBMC[5].

Our findings indicate inflammatory cytokines are responsible for overexpression of A_3AR in activated cells, which may suggest a new target to combat inflammation.

REFERENCES

1. Schulte G, Fredholm BB. Signaling pathway from the human adenosine A(3) receptor expressed in Chinese hamster ovary cells to the extracellular signal-regulated kinase 1/2. Mol Pharmacol 2002;62:1137-46.
2. Fishman P, Madi L, Bar-Yehuda S, Barer F, Del Valle L, Khalili K. Evidence for involvement of Wnt signaling pathway in IB-MECA mediated suppression of melanoma cells. Oncogene 2002;21:4060-4.
3. Fishman P, Bar-Yehuda S, Madi L, Cohn I. A3 adenosine receptor as a target for cancer therapy. Anticancer Drugs 2002;13:437-43.

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4. Fishman P, Bar-Yehuda S. Pharmacology and therapeutic applications of A3 receptor subtype. Curr Top Med Chem 2003;3:463-9.
5. Fishman P, Bar-Yehuda S, Rath-Wolfson L, et al. Targeting the A3 adenosine receptor for cancer therapy: inhibition of prostate carcinoma cell growth by A3AR agonist. Anticancer Res 2003;23:2077-83.
6. Fishman P, Bar-Yehuda S, Ohana G, et al. An agonist to the A3 adenosine receptor inhibits colon carcinoma growth in mice via modulation of GSK-3ß and NF-kB. Oncogene 2004;23:2465-71.
7. Baharav E, Bar-Yehuda S, Madi L, et al. The anti-inflammatory effect of A3 adenosine receptor agonists in murine autoimmune arthritis models. J Rheumatol 2005;32:469-76.
8. Fishman P, Bar-Yehuda S, Madi L, et al. The PI3K–NF-kB signal transduction pathway is involved in mediating the anti-inflammatory effect of IB-MECA in adjuvant-induced arthritis. Arthritis Res Ther 2006;8:1-9.
9. Szabo C, Scott GS, Virag L, et al. Suppression of macrophage inflammatory protein (MIP)-1-alpha production and collagen-induced arthritis by adenosine receptor agonists. Br J Pharmacol 1998;125:379-87.
10. Hanada T, Yoshimura A. Regulation of cytokine signaling and inflammation. Cytokine Growth Factor Rev 2002;13:413-21.
11. Gessi S, Varani K, Merighi S, et al. Expression of A3 adenosine receptors in human lymphocytes: up-regulation in T cell activation. Mol Pharmacol 2004;65:711-9.
12. Heinemeyer T, Wingender E, Reuter H, et al. Databases on transcriptional regulation: TRANSFAC, TRRD, and COMPEL. Nucl Acids Res 1998;26:364-70.
13. Bailey TL, Elkan C. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. Proc Int Conf Intell Syst Mol Biol 1994;2:28-36.
14. Heinemeyer T, Chen X, Karas H. Expanding the TRANSFAC database towards an expert system of regulatory molecular mechanisms. Nucl Acids Res 1999;27:318-22.
15. Schlotzer-Schrehardt U, Zenkel M, Decking U, et al. Selective upregulation of the A3 adenosine receptor in eyes with pseudoexfoliation syndrome and glaucoma. Invest Ophthalmol Vis Sci 2005;46:2023-34.
16. Madi L, Ochaion A, Rath-Wolfson L, et al. The A3 adenosine receptor is highly expressed in tumor vs. normal cells: potential target for tumor growth inhibition. Clin Cancer Res 2004;10:4472-9.
17. Merighi S, Mirandola P, Varani K, et al. A glance at adenosine receptors: novel target for antitumor therapy. Pharmacol Ther 2003;100:31-48.
18. Gessi S, Cattabriga E, Avitabile A, et al. Elevated expression of A3 adenosine receptors in human colorectal cancer is reflected in peripheral blood cells. Clin Cancer Res 2004;10:5895-901.
19. Nguyen DK, Montesinos MC, Williams AJ, Kelly M, Cronstein BN. Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells. J Immunol 2003;171:3991-8.
20. Trincavelli ML, Costa B, Tuscano D, Lucacchini A, Martini C. Up-regulation of A(2A) adenosine receptors by proinflammatory cytokines in rat PC12 cells. Biochem Pharmacol 2002;64:625-31.
21. Khoa ND, Montesinos MC, Reiss AB, Delano D, Awadallah N, Cronstein BN. Inflammatory cytokines regulate function and expression of adenosine A(2A) receptors in human monocytic THP-1 cells. J Immunol 2001;167:4026-32.
22. Murphree LJ, Sullivan GW, Marshall MA, Linden J. Lipopolysaccharide rapidly modifies adenosine receptor transcripts in murine and human macrophages: role of NF-kappa B in A(2A) adenosine receptor induction. Biochem J 2005;391:575-8.
23. Ruschpler P, Stiehl P. Shift in Th1 (IL-2 and IFN-gamma) and Th2 (IL-10 and IL-4) cytokine mRNA balance within two new histological main types of rheumatoid arthritis. Cell Mol Biol Noisy-le-grand 2002;48:285-93.
24. Migita K, Eguchi K, Kawabe Y, et al. TNF-alpha-mediated expression of membrane-type matrix metalloproteinase in rheumatoid synovial fibroblasts. Immunology 1996;89:553-7.
25. Redlich K, Hayer S, Maier A, et al. Tumor necrosis factor alpha-mediated joint destruction is inhibited by targeting osteoclasts with osteoprotegerin. Arthritis Rheum 2002;46:785-92.

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