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The anti-inflammatory target A_3 adenosine receptor is over-expressed in rheumatoid arthritis, psoriasis and Crohn's disease ☆

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ABSTRACT

The Gi protein associated A_3 adenosine receptor (A_3AR) was recently defined as a novel anti-inflammatory target. The aim of this study was to look at A_3AR expression levels in peripheral blood mononuclear cells (PBMCs) of patients with autoimmune inflammatory diseases and to explore transcription factors involved receptor expression.

Over-expression of A_3AR was found in PBMCs derived from patients with rheumatoid arthritis (RA), psoriasis and Crohn's disease compared with PBMCs from healthy subjects. Bioinformatics analysis demonstrated the presence of DNA binding sites for nuclear factor-κB (NF-κB) and cyclic AMP-responsive element binding protein (CREB) in the A_3AR gene promoter. Up-regulation of NF-κB and CREB was found in the PBMCs from patients with RA, psoriasis and Crohn's disease. The PI3K-PKB/Akt signaling pathway, known to regulate both the NF-κB and CREB, was also up-regulated in the patients' PBMCs.

Taken together, NF-κB and CREB are involved with the over-expression of A_3AR in patients with autoimmune inflammatory diseases. The receptor may be considered as a specific target to combat inflammation.

1. Introduction

Effective anti-inflammatory drugs to treat autoimmune diseases today are mainly monoclonal antibodies against tumor necrosis factor-α (TNF-α) or other inflammatory cytokines. Due to the critical role of these cytokines in regulating normal immune and inflammatory responses, these drugs induce adverse effects and efforts are still directed towards more specific targets which are expressed solely by pathological but not normal cells [1–5].

Recently the A_3 adenosine receptor (A_3AR) was identified as a new target to combat RA due to its unique characteristics. The A_3AR belongs to the Gi protein associated family of adenosine receptors which includes also the A_1, A_{2A} and A_{2B} sub-members. The A_1 and the A_3 adenosine receptors are negatively associated with cAMP whereas activation of the A_{2A} and A_{2B} stimulates adenylyl cyclase and cAMP formation [6].

The A_3AR was found to be over-expressed in cells from synovial and paw tissues of rats with adjuvant induced arthritis (AIA). High receptor expression was also found in PBMCs derived from the AIA rats, reflecting receptor status in the remote inflammatory sites [7–9].

Based on these findings, synthetic highly selective agonists to the A_3AR were introduced for the treatment of AIA. CF101, chemically known as 1-deoxy-1-[6-[[(3-iodophenyl)methyl]amino]-9H-9-yl]-N-methyl-β-D-ribofura-nuronamide (IB-MECA) induced marked amelioration of the clinical and pathological manifestations of AIA. Mechanistically, CF101 decreased the expression levels of PI3K, PKB/Akt, IKK and IκB resulting in down-regulation of NF-κB, inhibition of TNF-α and apoptosis of inflammatory cells. In addition, a direct anti-proliferative effect of CF101 towards auto-reactive T cells was observed [7–11].

Examination of A_3AR expression levels in the PBMCs of RA patients revealed receptor up-regulation in both early diagnosed patients which were not yet under treatment and in patients chronically treated with methotrexate (MTX) [9,12].

These data prompted the initiation of a pre-clinical and clinical programs utilizing CF101 as an anti-inflammatory drug candidate. In a Phase I study in healthy subjects, CF101 was found to be safe

☆ A_3 adenosine receptor is over-expressed in autoimmune inflammatory diseases.

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Table 1
Characteristic of patients with autoimmune inflammatory diseases.

	Age (years)	Disease duration (years)	Treatments
Rheumatoid arthritis	54.5 ± 2.33	6.67 ± 2.06	MTX, prednisone
Psoriasis	47.72 ± 2.65	18.6 ± 2.28	Topicals, Dead sea, phototherapy, MTX, immunobiologicals
Crohn's disease	43.7 ± 3.74	13.07 ± 2.08	Immunosuppressants, anti-inflammatory, immunobiologicals, prednisone, antibiotic

and well tolerated with a linear pharmacokinetic activity [13]. In a Phase IIa study conducted in patients with RA, CF101 administered twice daily for 12 weeks resulted in an improvement of disease signs and symptoms and appeared to be safe and well tolerated. Analysis of A_3AR expression levels at base line showed statistically significant direct correlation with patient responses to CF101, suggesting A_3AR utilization as a biomarker to predict patients' response to the drug prior to treatment initiation [14].

The aim of the present study was to explore whether the A_3AR target is over-expressed in additional autoimmune inflammatory

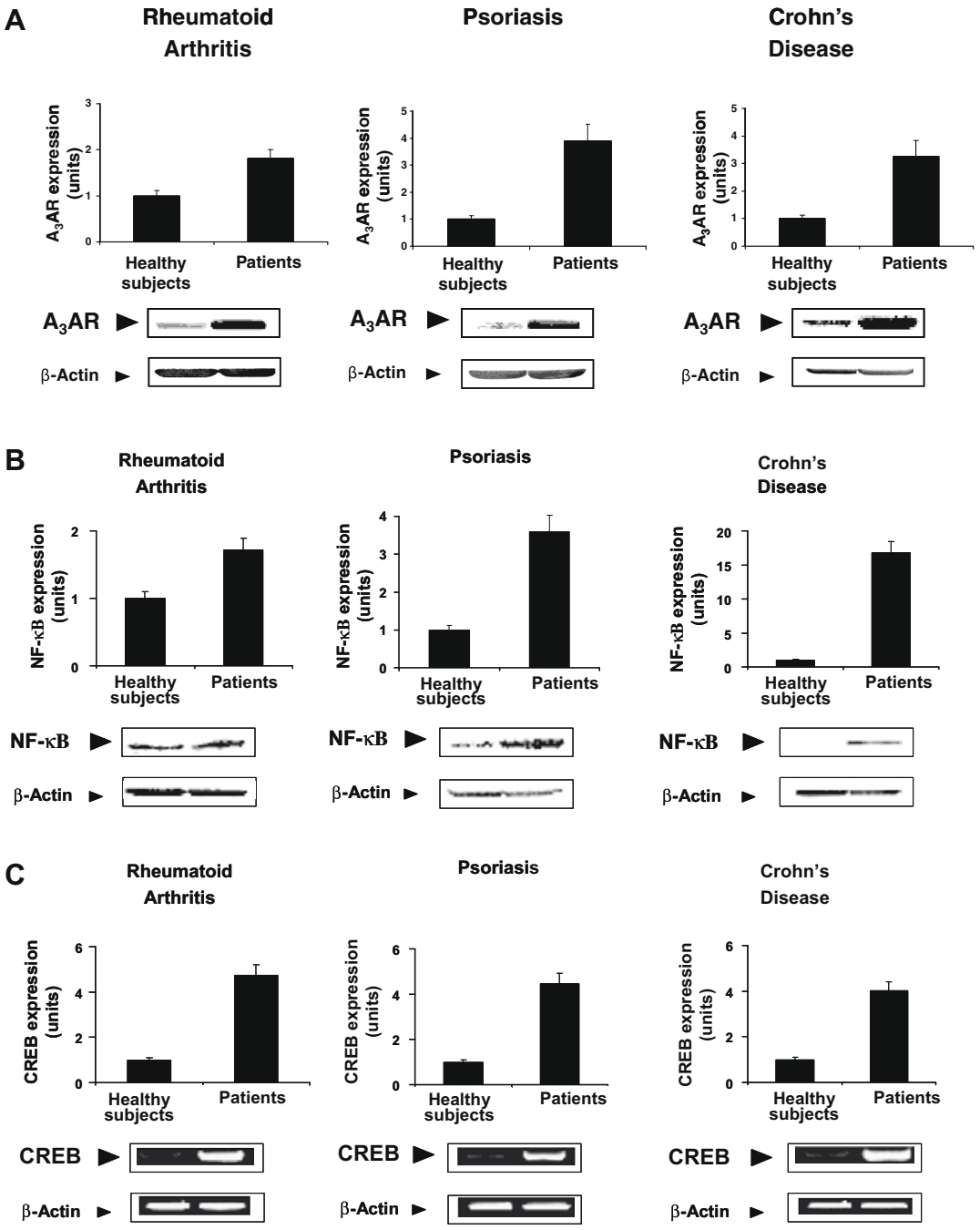


Fig. 1. WB analysis of A_3AR and NF-κB was performed in PBMCs derived from patients ($n = 25$ for each disease) with rheumatoid arthritis, psoriasis and Crohn's. CREB was tested in PBMCs samples utilizing RT-PCR analysis. (A) A_3AR; (B) NF-κB; (C) CREB. (Values of units are the mean and SE, $p < 0.05$).

Table 2
List of transcription factors with relation to the *ADORA3* gene promoter region.

Factor	Extended set of interacting factors
c-Rel	C/EBPα, C/EBPβ, CRE-BP1, ER, NF-κB, Oct-1, PU.1, Sp1, TBP, c-Fos, c-Jun, c-Rel
MyoD	CRE-BP1, ER, GATA-1, GR, MRF4, MyoD, PR, PU.1, c-Jun
c-Fos	AP-1, ATF, C/EBP, C/EBPα, C/EBPβ, C/EBPδ, C/EBPε, CRE-BP1, CREB, ER, GR, MyoD, NF-κB1, Oct-1, Oct-1A, PU.1, USF, c-Rel
GR	AP-1, ATF, C/EBPβ, CRE-BP1, ER, MyoD, Oct-1A, PR, PU.1, c-Fos
CREB	ATF, CRE-BP1, CREB, CREMδC-G, c-Fos, c-Jun
AP-1	GR, Oct-1, Oct-1A, c-Fos, c-Jun
GATA-1	C/EBP, C/EBPβ, MyoD
C/EBP	ATF, C/EBP, C/EBPβ, C/EBPε, CRE-BP1, CREB, CTF-1, NF-κB1, TBP, c-Fos, c-Jun
c-Jun	AP-1, ATF, C/EBP, C/EBPα, C/EBPβ, C/EBPδ, C/EBPε, CRE-BP1, CREB, E47, ER, GR, MyoD, NF-κB1, Oct-1, Oct-1A, PU.1, TBP, c-Fos, c-Jun, c-Rel
PU.1	ATF, C/EBPα, C/EBPβ, CRE-BP1, GR, MyoD, Oct-1, Sp1, TBP, c-Fos, c-Jun, c-Rel
NF-κB	ATF, C/EBP, C/EBPα, C/EBPβ, C/EBPδ, C/EBPε, ER, TBP, c-Fos, c-Jun, c-Rel

diseases. We thus looked at receptor expression levels in PBMCs derived from patients with RA, psoriasis and Crohn's disease. We further studied the molecular mechanism involved with receptor over-expression and performed bioinformatics analysis to look at the transcription factors in the A_3AR promoter gene regulating receptor expression and functionality.

2. Materials and methods

2.1. Reagents

Rabbit polyclonal antibodies against rat A_3AR (developed against amino acids 151–230 with the internal region of the A_3AR, Santa Cruz Biotechnology, CA, USA) and the signaling proteins TNF-α (rat anti TNF-α, R&D systems, MN, USA), PI3K (rabbit polyclonal antibody raised against amino acids 189–390 mapping near the N-terminus of PI3K p110α unit, Santa Cruz Biotechnology, CA, USA), phosphorylated PKB/Akt (PKB/Akt phosphorylated at pSer473, Sigma MI, USA), IκB (rabbit polyclonal antibody against a peptide at the C-terminus of IκB-α, Santa Cruz Biotechnology, CA, USA), NF-κB (NF-κB p65-RelA, Chemicon International, CA, USA) were used.

Phytohemagglutinin (PHA) and Wortmannin were purchased from Sigma (Chemical Co., St. Louis, MO, USA).

2.2. Blood sample collection and separation

Blood samples were collected from patients with RA, psoriasis, Crohn's disease and healthy subjects upon ethical committee approval. The patients signed an informed consent prior to blood withdrawal.

To separate PBMCs, blood (16 ml) was collected in CPT vacutainer (BD, Franklin Lakes, NJ, USA), centrifuged according to manufacturer instructions and washed with PBS.

2.3. In vitro activation of PBMCs with PHA

PBMCs (2×10^6/ml) from healthy subjects were incubated with 5 μg/ml PHA in RPMI 1640 supplemented with 10% FBS for 24 h. Wortmannin (200 nM) was added 30 min. prior to PHA (5 μg/ml) stimulation. At the end of the incubation the PBMCs were collected and protein extracts were prepared.

2.4. Western blot analysis of A_3AR and additional signaling proteins in PBMCs

Western blot analysis (WB) was carried out according to the following protocol. Samples were rinsed with ice-cold PBS and resuspended in ice-cold lysis buffer (TNN buffer, 50 mM Tris buffer, pH 7.5, 150 mM NaCl, NP 40) and proteinase inhibition cocktail. The samples were then incubated on a shaker for 1 h at 4 °C. Cell debris was removed by centrifugation for 10 min, at maximum speed. Protein concentrations were determined using the Bio-Rad protein assay dye reagent. Equal amounts of the sample (50 μg) were separated by SDS–PAGE, using 10% polyacrylamide gels (invitrogels). The resolved proteins were then electro-blotted onto nitrocellulose membranes (Schleicher & Schuell, Keene, NH, USA). Membranes were blocked with 5% BSA and incubated with the desired primary antibody (dilution 1:1000) in 1% BSA for 24 h at 4 °C. Blots were then washed and incubated with a secondary antibody (1:10,000) for 1 h at room temperature. Bands were recorded using BCIP/NBT color development kit (Promega, Madison, W1, USA).

Blots of protein extracts derived from human subjects were quantified by densitometry analysis and the ratio of patients/standard was calculated. Blots of mitogen stimulated cells were normalized against the housekeeping protein, β-actin. The optical density of the bands was quantified using an image analysis system and corrected by the optical density of the corresponding standard/β-actin bands. The control values were designated as 1 U.

2.5. RT-PCR analysis of CREB expression in PBMCs

Total RNA was extracted from PBMCs derived from patients with RA, psoriasis and Crohn's disease utilizing RNeasy mini kit (QIAGEN). The reverse-transcription reaction was performed at 50 °C for 30 min from 1 μg total RNA. For human CREB amplification one Step RT-PCR with Platinum Taq (Invitrogen) was utilized and the primers for CREB 5′-ATGACCATGGAATCTGGA and 3′-TTAATCTGATTTGTGGCAG were added. The PCR reaction was performed by heating to 99 °C for 5 min, 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 70 °C for 1 min.

For amplification of human β-actin the primer 5′-TGGGA ATGGGTCAGAAGGACT and 3′-TTTCACGGTTGGCCTTAGGGT were used. The PCR condition included heating to 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 56 °C for 1 min and 30 s, and 73 °C for 45 s. The PCR Products were electrophoresed on 2% Agarose gels, stained with Ethidium Bromide and visualized with UV illumination.

2.6. Bioinformatics analysis of A_3AR (*ADORA3*)

General data were obtained from release 3.3 of the Transfac database [15] and from the iHOP data collection [16]. Post processing of the information included eliminating redundancies due to nomenclature differences and synonyms. The processed data were used for establishing a hash structure which served the analysis and mapping program. Two types of data were produced, direct (A–B interacting) and first level extended set. The first level

Table 3
Analysis of transcription factors binding sites 3050 bp region, upstream the ADORA3 gene promoter. Probable direct and first level interactions between the various transcription factors are listed with their upstream positions along the promoter.

Factor:	c-Fos	c-Jun	NF-κB	NF-κB1
Upstream positions:	−1666, −1118	−215	−1754, −1598	−1598
Interactions:	ATF: −95 C/EBPbeta: −1617, −11 c-Jun: −215 CRE-BP1: −94	ATF: −95 c-Fos: −1666, −1118 C/EBPbeta: −1617, −11 PU.1: −2833, −426, −408 CRE-BP1: −94 GR: −2840, −2746, −2186, −2163, −2138, −2068, −1725, −1246 MyoD: −2014, −39	c-Rel: −2058, −1560	ER: −2208, −1920, −782, −109 c-Rel: −2058, −1560 C/EBPbeta: −1617, −11 CRE-BP1: −94
First level interactions:	ATF: −95	ATF: −95	TBP: −2983, −2806, −2528, −663	ATF: −95
	C/EBPalpha: −2963, −2914, −2646, −2619, −2490, −1851, −1756, −1714, −1601, −1457, −1323, −1047, −1000, −874, −614, −452, −143	C/EBPalpha: −2963, −2914, −2646, −2619, −2490, −1851, −1756, −1714, −1601, −1457, −1323, −1047, −1000, −874, −614, −452, −143	c-Rel: −2058, −1560	C/EBPalpha: −2963, −2914, −2646, −2619, −2490, −1851, −1756, −1714, −1601, −1457, −1323, −1047, −1000, −874, −614, −452, −143
		E47: −39	NF-kappaB1: −1598	c-Fos: −1666, −1118
	Oct-1A: −1598	Oct-1A: −1598		TBP: −2983, −2806, −2528, −663
	GR: −2840, −2746, −2186, −2163, −2138, −2068, −1725, −1246	c-Fos: −1666, −1118		TBP: −2983, −2806, −2528, −663
	Oct-1: −3021, −2972, −2463, −2051, −1730, −1331, −1310, −1154, −1015, −793, −591, −490, −411, −232	Oct-1: −3021, −2972, −2463, −2051, −1730, −1331, −1310, −1154, −1015, −793, −591, −490, −411, −232		C/EBP: −2988, −1509
	USF: −2903, −2571, −2432, −2359, −2337, −879, −561	AP-1: −2627, −2626, −2456, −2367, −1043, −841, −482, −204		C/EBPepsilon: −1876
	AP-1: −2627, −2626, −2456, −2367, −1043, −841, −482, −204	ER: −2208, −1920, −782, −109		c-Rel: −2058, −1560
	ER: −2208, −1920, −782, −109	C/EBP: −2988, −1509		C/EBPbeta: −1617, −11
	C/EBP: −2988, −1509	NF-kappaB1: −1598		C/EBPdelta: −1950
	NF-kappaB1: −1598	C/EBPepsilon: −1876 MyoD: −2014, −39		CRE-BP1: −94
	C/EBPepsilon: −1876	c-Rel: −2058, −1560		c-Jun: −215
	c-Rel: −2058, −1560	C/EBPbeta: −1617, −11		ER: −2208, −1920, −782, −109
	c-Jun: −215	PU.1: −2833, −426, −408		
	PU.1: −2833, −426, −408	C/EBPdelta: −1950		
	C/EBPdelta: −1950	CREB: −2435, −94		
	CREB: −2435, −94	CRE-BP1: −94		
	MyoD: −2014, −39	TBP: −2983, −2806, −2528, −663		
	C/EBPbeta: −1617, −11	c-Jun: −215		
	CRE-BP1: −94	GR: −2840, −2746, −2186, −2163, −2138, −2068, −1725, −1246		

extended set of data included the assumption that if there are interactions known between A–B, A–C, B–D and C–E than a complex may include A–E where D and E are considered part of the first level extended set.

2.7. Promoter analysis

Sequence information of the promoter region (3050 bp upstream of the ATG start site) of the ADORA3 gene transcript was derived from entry ENST00000241356 of the ENSEMBL database [17]. This entry is one of nine potential splice variants. It is the most established splice variant. Transcription factors binding sites (TRBS) analysis was performed by several programs and results were compared. The program ALIBABA was chosen for the main analysis (http://www.gene-regulation.com/pub/programs/alibaba2/index.html). The output of TRBS analysis programs is a linear list of positions. Such TRBS can be found along sequences without any relevance to actual binding. Binding of factors to appropriate sites in promoter regions is enhanced by the formation of complex-related interactions. To map possible transcription factors complexes, we wrote two special programs (transcription factors cluster and draw Cluster) in the Python programming language [18]. Transcription factors cluster uses an internal hash structure containing direct and first level interactions between various transcription factors s with linear data from the transcription factors analysis program to form transcription factor clusters which are possible candidates for real transcription factor complexes.

2.8. Statistical analysis

The results were evaluated using the 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.

3. Results

3.1. A_3AR is highly expressed in PBMCs derived from patients with autoimmune inflammatory diseases

Based on former studies which showed that A_3AR over-expression in the inflammatory tissues is reflected in the PBMCs, we looked at the A_3AR expression levels in PBMCs from patients with

RA, psoriasis and Crohn's disease ($n = 25$ from each disease) and from healthy subjects ($n = 50$). Table 1 depicts patients' characteristics.

Over-expression of the A_3AR protein was noted in the PBMCs of the three diseases: RA – 1.8 ± 0.18-, psoriasis – 3.9 ± 0.62-, Crohn's disease – 3.26 ± 0.57-fold higher than the level in healthy subjects (Fig. 1A).

3.2. Bioinformatics analysis of the A_3AR gene promoter

Screening of the entire array of transcription factors with relation to the ADORA3 gene promoter region revealed meaningful high scoring transcription factors which are listed in Table 2.

Mapping of first level interactions clearly enhanced the feasibility of certain transcription factors to form transcription factor

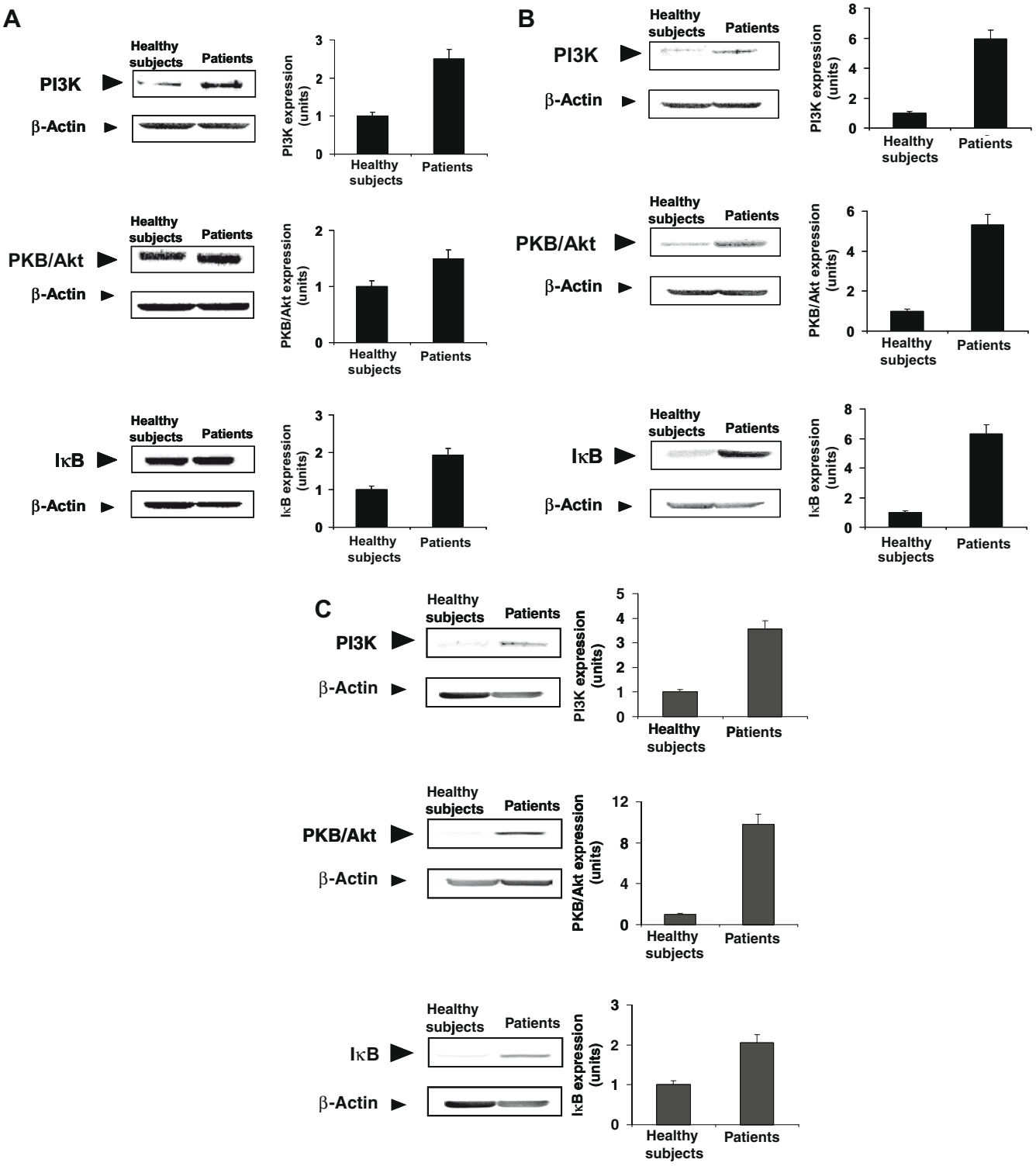


Fig. 2. WB analysis of PI3K, PKB/Akt and IκB in protein extracts of PBMCs derived from patients with (A) rheumatoid arthritis; (B) Psoriasis; (C) Crohn's disease. (Values of units are the mean and SE, $p < 0.05$.)

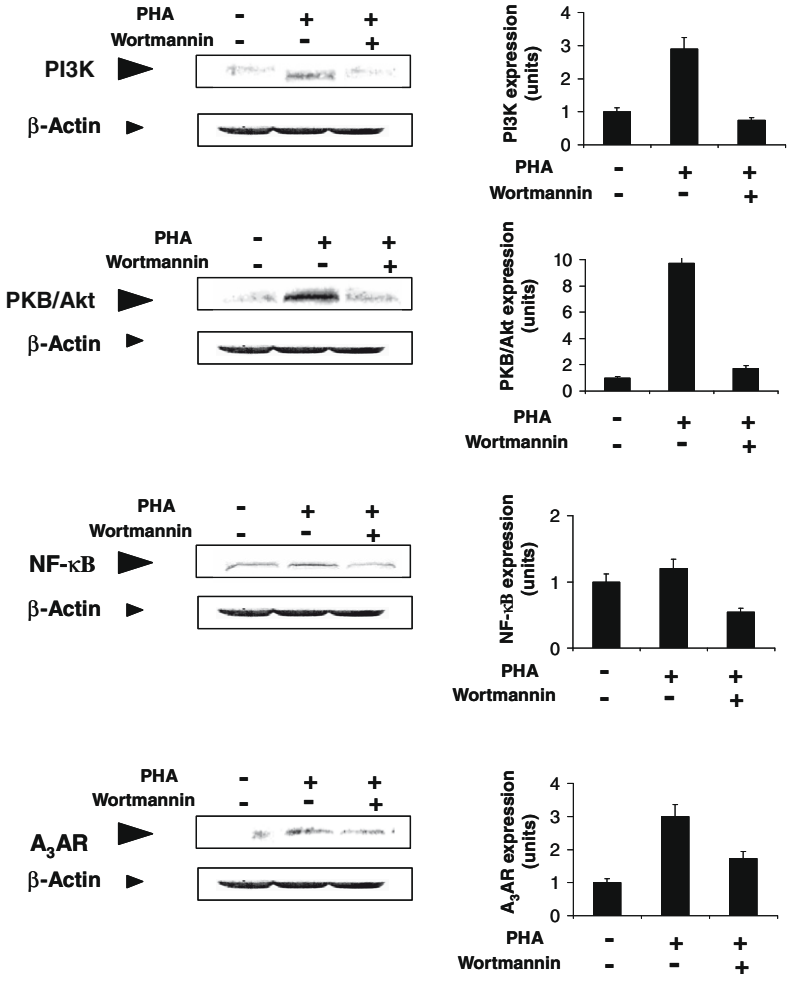


Fig. 3. PBMCs (2×10^6/ml) derived from healthy subjects were stimulated with PHA (5 μg/ml, for 24 h) in the presence and absence of Wortmannin. Protein analysis of PI3K, PKB/Akt, NF-κB and A_3AR was performed utilizing WB analysis. (Values of units are the mean and SE, $p < 0.05$.)

complexes. Several key factors for which binding sites are present on the *ADORA3* gene promoter form a tight network of possible interactions. This high level of inter-connectivity is over simplified, as some of the key factors also interact with themselves and with factors which do not bind to binding sites on the promoter itself. In addition we present here a representative set of factors with their interactions: c-fos, c-jun, NF-κB and NF-κB1 (Table 3).

It may be noted that there is an almost complete match between all first levels extended set transcription factors of c-Fos, c-Jun and NF-κB1, including those factors among themselves. The appearance of probable complex forming factors in proximity supports the assumption on their interplay with each other.

3.3. Molecular mechanism involved with A_3AR over-expression

NF-κB and CREB, transcription factors found to interact with the *ADORA3* gene promoter, are known to play a pivotal role in the pathogenesis of RA, psoriasis and Crohn's disease (19–24).

A marked increase in the expression levels of NF-κB (protein) and CREB (mRNA) was noted in the PBMCs of all diseases compared to that of healthy subjects (Fig. 1B and C). This was followed by an increase in A_3AR expression level (Fig. 1), suggesting a role for NF-κB and CREB in mediating A_3AR transcription.

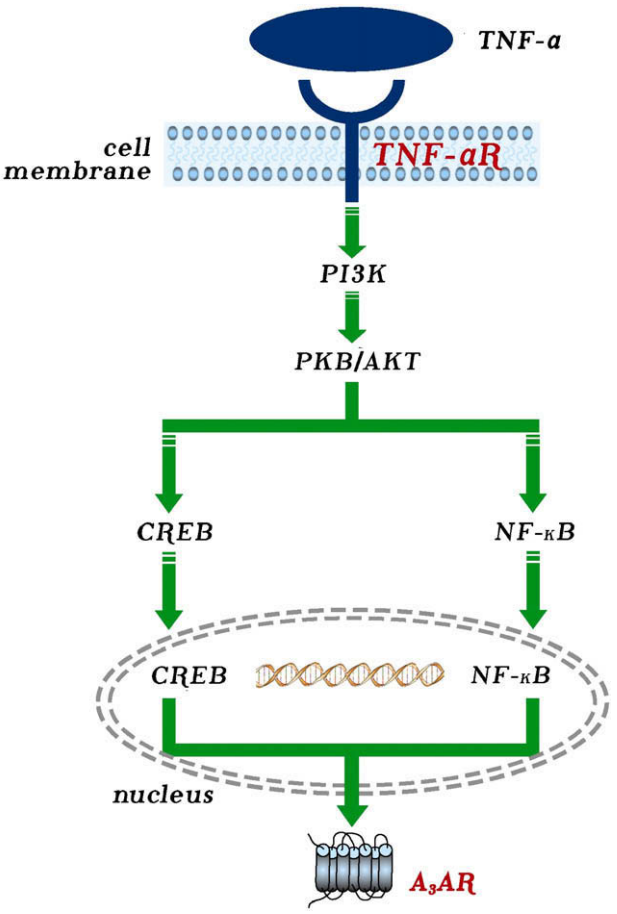
Analysis of the PI3K-PKB/Akt signaling pathway, known to regulate both the NF-κB and CREB expression levels and activity [25–27] was then performed. Up-regulation of PI3K, PKB/Akt and IκB was observed in PBMCs of the three diseases compared to that of healthy subjects (Fig. 2A–C).

A support for the role played by the PI3K signaling pathway in regulating A_3AR expression levels came from in vitro experiments utilizing PHA stimulated PBMCs from healthy subjects. It is well established that upon mitogenic stimulation, A_3AR expression is up-regulated [12,28]. Indeed, introduction of the PI3K inhibitor Wartmannin to this system prevented the PHA induced up-regulation of PI3K, PKB/Akt and NF-κB expression levels, resulting in down-regulation of A_3AR (Fig. 3).

4. Discussion

In this study we first show that the A_3AR is over-expressed in PBMCs derived from RA, psoriasis and Crohn's disease patients in comparison to low receptor expression in PBMCs from healthy subjects.

These data raised the question whether A_3AR up-regulation is a manifestation of the inflammatory condition or does it play a role in mediating disease pathogenesis. Earlier studies showed that A_3AR is up-regulated under hypoxic conditions such as cancer or inflammation. Receptor up-regulation was found to be attributed to adenosine, which accumulates in the extra-cellular environment under stressed conditions, known to regulate the expression of its own receptors via an autocrine pathway [9,29,30].



Scheme 1. The pro-inflammatory cytokine TNF-α induces an increase of the PI3K and PKB/Akt expression levels, resulting in up-regulation of CREB and NF-κB which translocate to the nucleus to act as A_3AR transcription factors.

To further understand the mechanism involved with receptor over-expression we performed bioinformatics analysis which demonstrated the presence of c-Rel, MyoD, c-fos, GR, CREB, AP-1, GATA-1, C/EBP, c-Jun, PU.1 and NF-κB in the *ADORA3* gene promoter. Further analysis also revealed possible interactions between the various transcription factors. Interestingly, most of the transcription factors identified in the A_3AR promoter are known to promote inflammation and among those we selected to further analyze NF-κB and CREB, which were reported earlier to play role in A_3AR transcription [19–24,31–37]. In this study, NF-κB and CREB were found to be up-regulated in the patients' PBMCs in comparison to healthy subjects. It was also noted that the high expression levels of NF-κB and CREB were associated with up-regulation in the expression levels of A_3AR. The PI3K-PKB/Akt pathway found to be up-regulated in the PBMCs of all patient groups, is known to control both NF-κB and CREB expression levels in different cell types [25–27].

These data corroborate former studies showing that adenosine receptor expression levels are controlled by inflammatory cytokines such as TNF-α. In PHA stimulated PBMCs from healthy subjects, A_3AR over-expression was attributed to an increase in TNF-α and Interleukin-2 expression levels. Upon neutralization of these two inflammatory cytokines by monoclonal antibodies, receptor expression levels returned to control values [12]. It thus seems that via an autocrine pathway TNF-α, which is up-regulated in the inflammatory microenvironment and PBMCs, binds to its receptor, thereby activating the PI3K-PKB/Akt pathway. This chain of events result in up-regulation of CREB and NF-κB, which subsequently increase the expression levels of A_3AR (Scheme 1).

A support for this hypothesis came from in vitro data of the present study showing that in PHA stimulated PBMCs, PI3K and PKB/Akt were up-regulated followed by an increase in A_3AR expression levels. A specific PI3K inhibitor reversed PKB/Akt, NF-κB and A_3AR expression levels to control values, demonstrating the role of this pathway in mediating A_3AR expression levels.

Taken together, A_3AR up-regulation is most probably a result of the inflammatory condition and is not involved with disease pathogenesis. A support for this hypothesis came from recent data of a Phase IIa clinical study in rheumatoid arthritis patients. A_3AR expression levels were analyzed at base line, prior to treatment with the CF101 drug, an A_3AR agonist. A statistically significant direct correlation between receptor expression prior to treatment and patient response to the drug was observed, demonstrating that patients with high receptor expression at base line responded positively to the drug [14]. Therefore, A_3AR may be suggested as a biological predictive marker in autoimmune inflammatory diseases.

References

[1] A. Alonso-Ruiz, J.I. Pijoan, E. Ansuategui, A. Urkaregi, M. Calabozo, A. Quintana, Tumor necrosis factor alpha drugs in rheumatoid arthritis: systematic review and metaanalysis of efficacy and safety, BMC Musculoskelet. Disord. 19 (2008) 52.
[2] R.O. Williams, E. Paleolog, M. Feldmann, Cytokine inhibitors in rheumatoid arthritis and other autoimmune diseases, Curr. Opin. Pharmacol. 7 (2007) 412–417.
[3] C. Scheinecker, K. Redlich, J.S. Smolen, Cytokines as therapeutic targets: advances and limitations, Immunity 28 (2008) 440–444.
[4] S.E. Gabriel, Tumor necrosis factor inhibition: a part of the solution or a part of the problem of heart failure in rheumatoid arthritis?, Arthritis Rheum 58 (2008) 637–640.
[5] A.J. Branten, Risk of infectious complications during anti-TNFalpha therapy, Neth. J. Med. 66 (2008) 50–52.
[6] S.A. Poulsen, R.J. Quinn, Adenosine receptors: new opportunities for future drugs, Bioorg. Med. Chem. 6 (1998) 619–641.
[7] P. Fishman, S. Bar-Yehuda, L. Madi, L. Rath-Wolfson, A. Ochaion, S. Cohen, E. Baharav, The PI3K–NF-κ B signal transduction pathway is involved in mediating the anti-inflammatory effect of IB-MECA in adjuvant-induced arthritis, Arthritis Res Ther 8 (2006) 1–9.
[8] L. Rath-Wolfson, S. Bar-Yehuda, L. Madi, A. Ochaion, S. Cohen, A. Zabutti, P. Fishman, IB-MECA, an A3 adenosine receptor agonist prevents bone resorption in rats with adjuvant induced arthritis, Clin. Exp. Rheumatol. 24 (2006) 400–406.
[9] A. Ochaion, S. Bar-Yehuda, S. Cohn, L. Del Valle, G. Perez-Liz, L. Madi, F. Barer, M. Farbstein, S. Fishman-Furman, T. Reitblat, A. Reitblat, H. Amital, Y. Levi, Y. Molad, R. Mader, M. Tishler, P. Langevitz, A. Zabutti, P. Fishman, Methotrexate enhances the anti-inflammatory effect of CF101 via up-regulation of the A3 adenosine receptor expression, Arthritis Res. Ther. 8 (2006) R169.
[10] E. Baharav, S. Bar-Yehuda, L. Madi, D. Silberman, L. Rath-Wolfson, M. Halpren, A. Ochaion, A. Weinberger, P. Fishman, The anti-inflammatory effect of A3 adenosine receptor agonists in murine autoimmune arthritis models, J. Rheumatol. 32 (2005) 469–476.
[11] .S. Bar-Yehuda, M.H. Silverman, W.D. Kerns, A. Ochaion, S. Cohen, P. Fishman, The anti-inflammatory effect of A3 adenosine receptor agonists: a novel targeted therapy for rheumatoid arthritis, Expert. Opin. Invesig. Drugs 16 (2007) 1601–1613.
[12] .L. Madi, S. Cohen, A. Ochaion, S. Bar-Yehuda, F. Barer, P. Fishman, Over-expression of A3 adenosine receptor in PBMNC of rheumatoid arthritis patients: involvement of NF-κB in mediating receptor level, J. Rheumatol. 34 (2007) 20–26.
[13] A.R. van Troostenburg, E.V. Clark, W.D. Carey, S.J. Warrington, W.D. Kerns, I. Cohn, M.H. Silverman, S. Bar-Yehuda, K.L. Fong, P. Fishman, Tolerability, pharmacokinetics, and concentration-dependent hemodynamic effects of oral CF101, an A3 adenosine receptor agonist, in healthy young men, Int. J. Clin. Pharmacol. Ther. 42 (2004) 534–542.
[14] M.H. Silverman, V. Strand, D. Markovits, M. Nahir, T. Reitblat, Y. Molad, I. Rosner, M. Rozenbaum, R. Mader, M. Adawi, D. Caspi, M . Tishler, P. Langevitz, A. Rubinow, J. Friedman, L. Green, A. Tanay, A. Ochaion, S. Cohen, W.D. Kerns, I. Cohn, S. Fishman-Furman, M. Farbstein, S. Bar Yehuda, P. Fishman, Clinical evidence for utilization of the A3 adenosine receptor as a target to treat rheumatoid arthritis: data from a Phase II clinical trial, J. Rheumatol. 35 (2008) 1–7.
[15] E. Wingender, A.E. Kel, O.V. Kel, H. Karas, T. Heinemeyer, P. Dietze, R. Knüppel, A.G. Romaschenko, N.A. Kolchanov, TRANSFAC, TRRD and COMPEL: towards a federated database system on transcriptional regulation, Nucleic Acids Res. (1997) 265–268.
[16] R. Hoffmann, A. Valencia, A gene network for navigating the literature, Nat. Genet. 34 (2004) 664.
[17] E. Birney, T.D. Andrews, P. Bevan, M. Caccamo, Y. Chen, L. Clarke, G. Coates, J. Cuff, V. Curwen, T. Cutts, T. Down, E. Eyras, X.M. Fernandez-Suarez, P. Gane, B.

Gibbins, J. Gilbert, M. Hammond, H.R. Hotz, V. Iyer, K. Jekosch, A. Kahari, A. Kasprzyk, D. Keefe, S. Keenan, H. Lehvaslaiho, G. McVicker, C. Melsopp, P. Meidl, E. Mongin, R. Pettett, S. Potter, G. Proctor, M. Rae, S. Searle, G. Slater, D. Smedley, J. Smith, W. Spooner, A. Stabenau, J. Stalker, R. Storey, A. Ureta-Vidal, K.C. Woodwark, G. Cameron, R. Durbin, A. Cox, T. Hubbard, M. Clamp, An overview of ensemble, Genome Res. 14 (2004) 925–928.

[18] A. Watters, G. van Rossum, J.C. Ahlstrom, Internet Programming with Python, MIS Press/Henry Holt publishers, New York, 1996.

[19] R.E. Simmonds, B.M. Foxwell, Signalling, inflammation and arthritis: NF-kappaB and its relevance to arthritis and inflammation, Rheumatology 47 (2008) 584–590.

[20] S. Bell, K. Degitz, M. Quirling, N. Jilg, S. Page, K. Brand, Involvement of NF-kappaB signalling in skin physiology and disease, Cell. Signal. 15 (2003) 1–7.

[21] I. Atreya, R. Atreya, M.F. Neurath, NF-kappaB in inflammatory bowel disease, J. Intern. Med. 263 (2008) 591–596.

[22] Y. Takeba, N. Suzuki, S. Wakisaka, M. Takeno, A. Kaneko, T. Asai, T. Sakane, Involvement of cAMP responsive element binding protein (CREB) in the synovial cell hyperfunction in patients with rheumatoid arthritis, Clin. Exp. Rheumatol. 18 (2000) 47–55.

[23] A.T. Funding, C. Johansen, K. Kragballe, L. Iversen, Mitogen- and stress-activated protein kinase 2 and cyclic AMP response element binding protein are activated in lesional psoriatic epidermis, J. Invest. Dermatol. 127 (2007) 2012–2019.

[24] C. Taylor, Regulation of intestinal epithelial gene expression in hypoxia, Kidney Int. 66 (2004) 528–531.

[25] K. Hazeki, K. Nigorikawa, O. Hazeki, Role of phosphoinositide 3-kinase in innate immunity, Biol. Pharm. Bull. 30 (2007) 1617–1623.

[26] L. Caravatta, S. Sancilio, V. di Giacomo, R. Rana, A. Cataldi, R. Di Pietro, PI3-K/Akt-dependent activation of cAMP-response element-binding (CREB) protein in Jurkat T leukemia cells treated with TRAIL, J. Cell. Physiol. 214 (2008) 192–200.

[27] S.W. Tas, P.H. Remans, K.A. Reedquist, P.P. Tak, Signal transduction pathways and transcription factors as therapeutic targets in inflammatory disease: towards innovative antirheumatic therapy, Curr. Pharm. Des. 11 (2005) 581–611.

[28] S. Gessi, K. Varani, S. Merighi, E. Cattabriga, A. Avitabile, R. Gavioli, C. Fortini, E. Leung, S. Mac Lennan, P.A. Borea, Expression of A3 adenosine receptors in human lymphocytes: up-regulation in T cell activation, Mol. Pharmacol. 65 (2004) 711–719.

[29] M. Zenkel, E. Pöschl, K. von der Mark, C. Hofmann-Rummelt, G.O. Naumann, F.E. Kruse, U. Schlötzer-Schrehardt, Differential gene expression in pseudoexfoliation syndrome, Invest. Ophthalmol. Vis. Sci. 46 (2005) 3742–3752.

[30] U. Schlötzer-Schrehardt, M. Zenkel, U. Decking, D. Haubs, F.E. Kruse, A. Jünemann, M. Coca-Prados, G.O. Naumann, Selective upregulation of the A3 adenosine receptor in eyes with pseudoexfoliation syndrome and glaucoma, Invest. Ophthalmol. Vis. Sci. 46 (2005) 2023–2034.

[31] M. Grossmann, Y. Nakamura, R. Grumont, S. Gerondakis, New insights into the roles of ReL/NF-kappa B transcription factors in immune function, hemopoiesis and human disease, Int. J. Biochem. Cell Biol. 31 (1999) 1209–1219.

[32] R. Chandran, T.J. Knobloch, M. Anghelina, S. Agarwal, Biomechanical signals upregulate myogenic gene induction in the presence or absence of inflammation, Am. J. Physiol. Cell Physiol 293 (2007) C267–C276.

[33] A. Laudanna, M.I. Nogueira, M. Mariano, Expression of Fos protein in the rat central nervous system in response to noxious stimulation: effects of chronic inflammation of the superior cervical ganglion, Braz. J. Med. Biol. Res. 31 (1998) 847–850.

[34] E.F. Wagner, Eferl R Fos/AP-1 proteins in bone and the immune system, Immunol. Rev. 208 (2005) 126–140.

[35] V. Poli, The role of C/EBP isoforms in the control of inflammatory and native immunity functions, J. Biol. Chem. 273 (1998) 29279–29282.

[36] G.L. Johnson, K. Nakamura, The c-jun kinase/stress-activated pathway: regulation, function and role in human disease, Biochim. Biophys. Acta 1773 (2007) 1341–1348.

[37] L. Ribas, N. Roher, M. Martínez, J.C. Balasch, C. Doñate, F.W. Goetz, D. Iliev, J.V. Planas, L. Tort, S. Mackenzie, Characterization and expression of the transcription factor PU.1 during LPS-induced inflammation in the rainbow trout (*Oncorhynchus mykiss*), Fish Shellfish Immunol. 24 (2008) 35–45.