Nicotinic α7 receptor inhibits the acylation stimulating protein-induced production of monocyte chemoattractant protein-1 and keratinocyte-derived chemokine in adipocytes by modulating the p38 kinase and nuclear factor-κB signaling pathways

ZHOU-YANG JIAO[1*], JING WU[2*], CHAO LIU[1], BING WEN[1], WEN-ZENG ZHAO[1] and XIN-LING DU[3]

Departments of [1]Cardiovascular Surgery and [2]Pediatrics, First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052; [3]Department of Cardiovascular Surgery, Xiehe Hospital, Huazhong University of Science and Technology, Wuhan, Hubei 430022, P.R. China

Received July 16, 2015; Accepted July 12, 2016

DOI: 10.3892/mmr.2016.5630

Abstract. Obesity is associated with chronic low-grade inflammation, which is characterized by increased infiltration of macrophages into adipose tissue. Acylation stimulating protein (ASP) is an adipokine derived from the immune complement system, which constitutes a link between adipocytes and macrophages, and is involved in energy homeostasis and inflammation. The purpose of the present study was to preliminarily investigate *in vitro*, whether functional α7nAChR in adipocytes may suppress ASP-induced inflammation and determine the possible signaling mechanism. Studies have reported associations between the expression of α7 nicotinic acetylcholine receptor (α7nAChR) and obesity, insulin resistance and diabetes. Additionally, α7nAChRs are important peripheral mediators of chronic inflammation, which is a key contributor to health problems in obesity. The primary aim of the present study was to evaluate the impact of exogenous ASP and α7nAChR on macrophage infiltration in adipose tissue and to examine the potential underlying molecular mechanism. Western blot analysis revealed that recombinant ASP increased the expression levels of monocyte chemoattractant protein-1 (MCP-1) and keratinocyte-derived chemokine (KC) by 3T3-L1 adipocytes. However, nicotine significantly inhibited the production of ASP-induced cytokines via the stimulation of α7nAChR. It was also found that α7nAChR inhibited the ASP-induced activation of p38 kinase and nuclear factor-κB (NF-κB), and the production of MCP-1 and KC. These data indicated that α7nAChR caused the inhibition of ASP-induced activation of p38 kinase and NF-κB to inhibit the production of MCP-1 and KC.

Correspondence to: Dr Jing Wu, Department of Pediatrics, First Affiliated Hospital of Zhengzhou University, 1 Jianshe East Road, Zhengzhou, Henan 450052, P.R. China
E-mail: wu2006jing@163.com

*Contributed equally

Key words: α7 nicotinic acetylcholine receptor, acylation stimulating protein, p38 kinase, nuclear factor κB

Introduction

Obesity is considered to be a systemic, chronic low-grade inflammation, characterized by increased serum levels of pro-inflammatory proteins and accumulation of macrophages within white adipose tissue (1). Adipose tissues secrete a variety of proinflammatory mediators, including tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1) and keratinocyte-derived chemokine (KC). MCP-1 and KC are involved in driving macrophage accumulation and activation, which are important steps towards establishing inflammation in adipose tissues (2). Acylation stimulating protein (ASP) is an adipokine, which is produced by adipocytes and interacts with C5aR-like receptor 2, a seven transmembrane G protein-coupled receptor. In humans, circulating levels of ASP are increased in obesity, and in insulin resistance, diabetes, cardiovascular diseases and metabolic syndrome, even in the absence of obesity. By contrast, the levels of ASP decrease with weight loss or exercise (3). These previous findings suggest the possibility that ASP is involved in the regulation of adipose tissue inflammation. Nicotine, a selective cholinergic agonist, is involved in cholinergic anti-inflammatory activities *in vitro* and *in vivo*, by acting through the α7 nicotinic acetylcholine receptor (α7nAChR). The anti-inflammatory activity of α7nAChR has been demonstrated in various disease models, including arthritis, septic shock and endotoxemia (4-6). Previous studies have reported that an α7nAChR-selective agonist, TC-7020, reduces food intake and weight gain, levels of circulating glucose and triglycerides, and expression levels of proinflammatory cytokines. These effects are reversed by the α7nAChR antagonist, methyllycaconitine, supporting the involvement

of α7nAChR (7). In addition, the expression of α7nAChR is downregulated in obese adults, compared with adults of a healthy weight, and weight loss has been found to partially restore the expression of α7nAChR (8). These studies indicate the possibility that α7nAChR affects the inflammation of adipose tissues and may be a promising target for therapies aimed at obesity-associated inflammatory diseases.

Nuclear factor-κB (NF-κB) is a ubiquitous, rapid response transcription factor, which is involved in inflammatory reactions, and exerts its actions by translating several cytokines, chemokines and cell adhesion molecules. Several reviews have provided evidence that NF-κB inflammatory pathways promote metabolic diseases, including insulin resistance and atherosclerosis (9-11), and several studies have shown that p38 kinase is a key member of the mitogen-activated protein kinase (MAPK) family, which is in adipocyte differentiation and adipogenesis, and in regulating cell proliferation, inflammation and immune responses (12-17). The anti-inflammatory actions of α7nAChR are mediated by the inhibition of NF-κB and p38 kinase in several types of cells, including monocytes, macrophages and endothelial cells (17-20). However, the specific role of p38 kinase and NF-κB in ASP signaling nd the possible molecular mechanism underlying the intracellular signal transduction from α7nAChR leading to the anti-inflammatory action in adipocytes remain to be fully elucidated.

In the present study, the involvement of α7nAChR on ASP-induced cytokine production, and its mechanisms of action, were investigated. It was found that the activation of α7nAChR in 3T3-L1 adipocyte cells inhibited the ASP-induced production of MCP-1 and KC by inhibiting the ASP-induced activation of p38 kinase and NF-κB.

Materials and methods

Materials and reagents. Media and anti-KC polyclonal antibody (cat. no. PA1-32924) were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Monocyte chemoattractant protein-1 (MCP-1; cat. no. sc-28879), inhibitor of NF-κB (IκB)α (cat. no. sc-847), NF-κB (cat. no. sc-109) and poly (ADP-ribose) polymerase (PARP; cat. no. sc-8007) antibodies were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA). Antibodies against phosphorylated-p38 kinase (cat. no. 9211) and p38 kinase (cat. no. 9212; Cell Signaling Technology, Inc., Beverly, MA, USA) were used to detect the phosphorylated form of p38 kinase and total p38 kinase, respectively. Anti-β-actin antibody (cat. no. SAB5500001), horseradish peroxidase-labeled goat anti-mouse IgG and the NF-κB inhibitor, BAY-11-7082, were purchased from Sigma-Aldrich (St. Louis, MO, USA). The 3T3-L1 preadipocytes were purchased from American Type Culture Collection (Manassas, VA, USA), and the ProteoExtract™ subcellular proteome extraction kit was purchased from Calbiochem; EMD Millipore (Billerica, MA, USA). Other reagents and laboratory supplies were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture. The mouse 3T3-L1 preadipocytes were seeded at a density of $1.0x10^5$ cells/well in 5-well plates and routinely cultured at 37˚C in a humidified atmosphere of 5% CO_2 in air. The cells were maintained in Dulbecco's modified Eagle's medium and Ham's F-12 nutrient mixture (DMEM/F-12) with 1% penicillin-streptomycin (100 U/ml; 100 μg/ml) and 10% fetal bovine serum (FBS). At confluence, adipocyte differentiation was induced by adding 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine and 1 mg/l insulin for 2 days, followed by 2 days in differentiating medium, containing 10% FBS in DMEM/F12 with 1 mg/l insulin supplementation, and replaced every 2 days. After 8-9 days, the cells exhibited a differentiated morphology (>80% of the cells) with lipid accumulation. Two days post-differentiation, cells were treated with ASP or PBS control for indicated time at 37˚C. Alternatively, cells were pretreated with nicotine (10 μM), α7nAChR antagonist α-BTX (2 μM), p38 kinase inhibitor SB203580 (20 μM), NF-κB inhibitor BAY-11-7082 (5 μM) for 30 min, followed by stimulation with or without ASP (100 nM) for an additional 24 h.

Recombinant ASP. Recombinant human ASP was produced and purified, as described previously (21). To avoid the inactivation of ASP, no denaturing agents were used at any step in the purification process. The purity was assessed using mass spectrometry, and the ASP was confirmed as endotoxin-free.

Preparation of cytoplasmic and nuclear protein fractions and immunoblotting. The 3T3-L1 adipocytes were processed using a ProteoExtract subcellular proteome extraction kit (Calbiochem; EMD Millipore), according to the manufacturer's protocol, to produce cytoplasmic and nuclear protein fractions, which were then analyzed by immunoblotting using the indicated antibodies. PARP and β-actin were used as loading controls of the nuclear and cytoplasmic fractions, respectively (22).

Immunoblotting. The 3T3-L1 adipocytes were lysed in SDS sample buffer, sonicated and centrifuged at 12,000 x g for 15 min at 4˚C. The resulting supernatants were boiled for 5 min in the presence of 50 mmol/l dithiothreitol. To measure the levels of secreted proteins (MCP-1 and KC), the cultured medium of the cells was also boiled for 5 min in SDS sample buffer (23). The fractions were sonicated and clarified by centrifugation 12,000 x g for 15 min at 4˚C, and their protein concentrations were assessed using a BCA Protein Assay kit (Pierce; Thermo Fisher Scientific, Inc.). Equivalent quantities of protein were separated using 7.5~15% (depending on their molecular weight) SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes, blocked for 1 h in phosphate-buffered saline containing 5% nonfat dry milk and 0.1% Tween 20, and subsequently incubated with primary antibodies at 4˚C overnight. The antibodies were as follows: MCP-1 (1:200), KC (1:500), β-actin (1:1,000), NF-κB p65 (1:500), IκBα (1:500), p38 kinase (1:1,000), phosphorylated p38 kinase (1:500) and PARP (1:500). Following incubation with secondary antibodies (1:5,000) at room temperature with agitation for 1 h, the membranes were washed three times with 100 ml of Tris-buffered saline containing 1% (v/v) Tween 20. The proteins were detected using enhanced chemiluminescence (24,25). The PARP and β-actin signals were used for blotting to verify equivalent gel loading. Band densities were determined using Quantity One

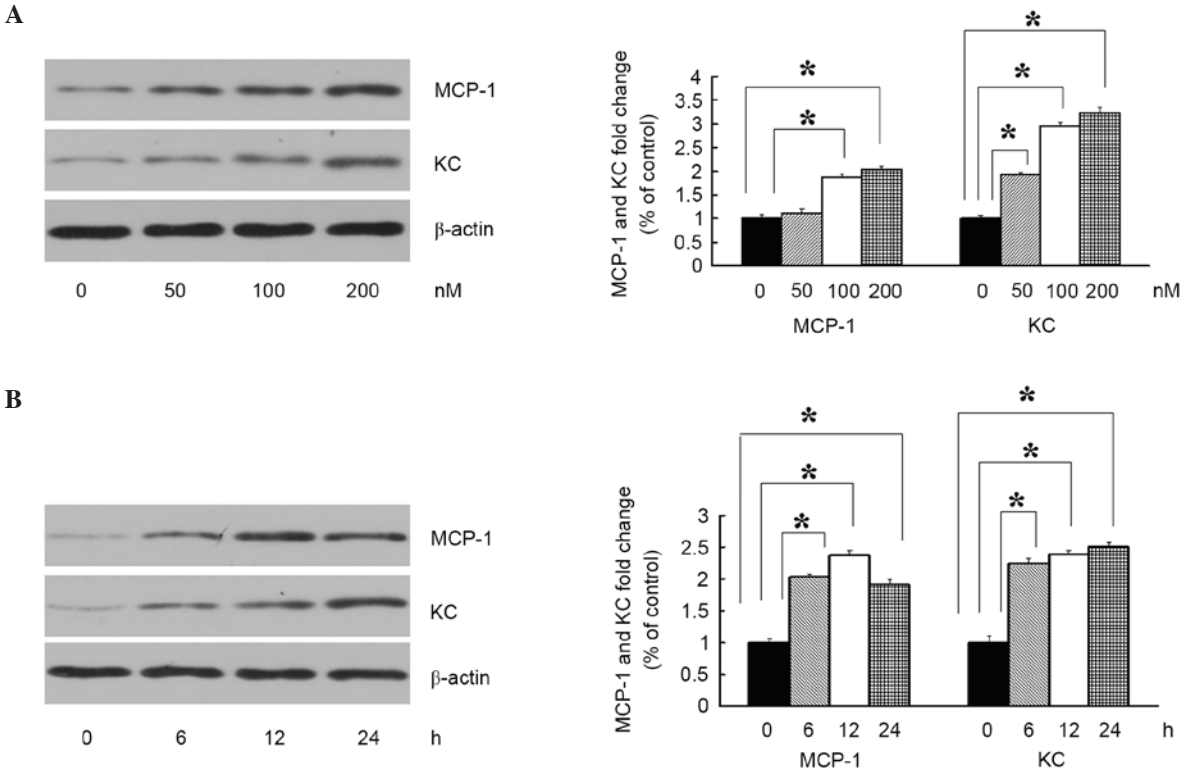

Figure 1. ASP promotes the expression of inflammatory cytokines. ASP increased the expression levels of KC and MCP-1 in a (A) concentration- and (B) time-dependent manner. The levels of KC and MCP-1 were determined using immunoblot and densitometric analyses. Data are presented as the mean ± standard error of the mean (n=3). *P<0.05, vs. untreated control. ASP, acylation stimulating protein; KC, keratinocyte-derived chemokine; MCP-1, monocyte chemoattractant protein-1.

software version 4.62 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. The results are presented as the percentage of control values, as the mean ± standard error of the mean. Differences between mean values of normally distributed data were assessed by Student's t-test for single comparisons between treatment and control. For data with multiple comparisons, one-way analysis of variance followed by Dunnett's test were used. P<0.05 was considered to indicate a statistically significant difference. The data were analyzed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA).

Results

ASP increases the expression levels of MCP-1 and KC. To determine the effect of ASP on the expression of cytokines by differentiated adipocyte cultures, the present study examined the levels of MCP-1 and KC using immunoblotting. As shown in Fig. 1A, the 3T3-L1 adipocytes were treated with increasing concentrations of ASP (0, 50, 100 and 200 nM), and the levels of MCP-1 and KC appeared to increase gradually with increasing concentrations of ASP, compared with the media control at 12 h, which suggested that ASP increased the expression of cytokines in a concentration-dependent manner. The effect of ASP on the expression of cytokines was also time-dependent (Fig. 1B). A fixed concentration of ASP (100 nM) increased the expression levels of MCP-1 and KC between 6 and 4 h, reaching a maximum at 24 h. These results indicated that ASP promoted adipocyte inflammation by enhancing the expression of MCP-1 and KC.

α7nAChR inhibits the ASP-induced expression of KC and MCP-1. To determine the involvement of α7nAChR in the production of proinflammatory mediators, the 3T3-L1 adipocytes were treated with vehicle, nicotine or α-bungarotoxin (α-BTX), prior to stimulation with ASP. Treatment of the 3T3-L1 adipocytes with ASP (100 nM; 24 h) alone significantly induced the expression of KC and MCP-1, compared with the control (Fig. 2). However, the production of KC and MCP-1 induced by ASP was inhibited significantly by preincubation with nicotine (10 μM; 30 min). In addition, the suppression of cytokine production by nicotine was prevented by the addition of α-BTX (2 μM; 30 min), which is an antagonist of α7nAChR. These results indicated that nicotine eliminated the ASP-induced production of inflammatory factors from adipocytes via the stimulation of α7nAChR.

Inhibition of ASP-stimulated p38 kinase phosphorylation by α7nAChR inhibits the expression of KC and MCP-1. The pathways involving p38 kinase are crucial in the regulation of pro-inflammatory molecules in cellular responses. The present study hypothesized that nicotine inhibits the ASP-induced production of inflammatory cytokines by interfering with the p38 kinase signaling pathway, therefore, the effect of ASP on the activation of p38 kinase was investigated. As shown in Fig. 3A, ASP (100 nM; 24 h) resulted in a significant increase in the phosphorylation of p38 kinase, without affecting the overall level of total p38. Subsequently, whether nicotine inhibited the ASP-induced production of cytokines by modulating ASP-induced p38 kinase activation was examined. As expected, the results of the immunoblot analysis

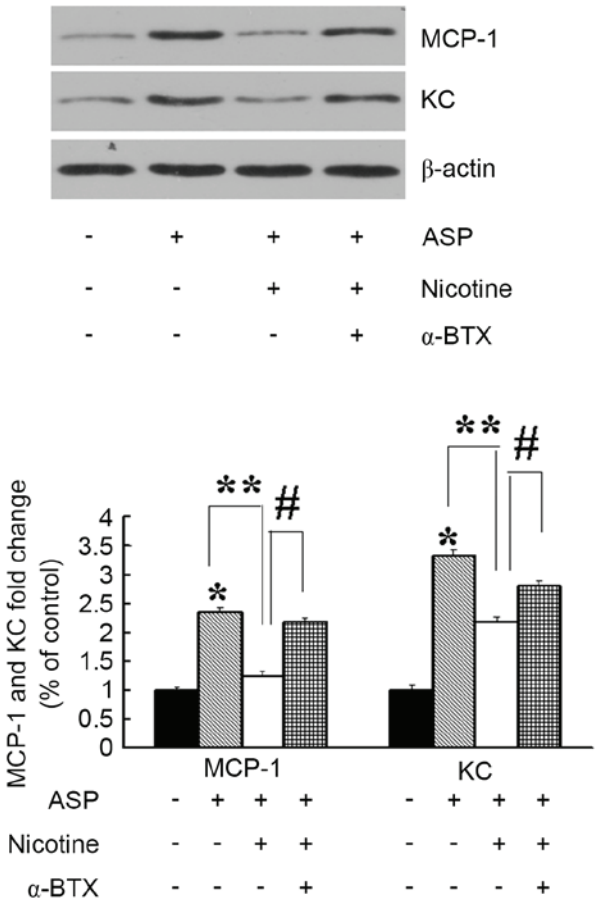

Figure 2. α7nAChR inhibits the ASP-induced expression of KC and MCP-1. Adipocytes were pretreated with nicotine 10 μM in the absence or presence of α-BTX (2 μM; 30 min prior to nicotine treatment) for 30 min and then challenged with ASP (100 nM) for 24 h. The expression levels of KC and MCP-1 were determined using immunoblot and densitometric analyses. Data are presented as the mean ± standard error of the mean (n=3). *P<0.05, vs. untreated control; **P<0.05, vs. ASP; #P<0.05, vs. nicotine+ASP. α7nAChR, α7 nicotinic acetylcholine receptor; ASP, acylation stimulating protein; α-BTX, α-bungarotoxin; KC, keratinocyte-derived chemokine; MCP-1, monocyte chemoattractant protein-1.

showed that pretreatment with 10 μM nicotine suppressed the ASP-induced phosphorylation of p38 kinase. In addition, this was completely reversed by α-BTX (2 μM; 30 min). These data indicated that nicotine, through its actions on α7nAChR, inhibited the expression of ASP-induced cytokines via the suppression of p38 kinase signal transduction. The significance of inhibiting the ASP-induced p38 kinase activation by nicotine in the production of cytokines was further confirmed with by p38 kinase inhibitor, SB203580 (20 μM; 30 min). As shown in Fig. 3B and C, pretreatment with SB203580 markedly inhibited the ASP-induced production of MCP-1 and KC, and phosphorylation of p38 kinase. These data demonstrated that nicotine suppressed the ASP-induced production of cytokines through downregulation of p38 kinase activity in the 3T3-L1 cells.

Inhibition of ASP-stimulated NF-κB activation by α7nAChR inhibits the expression of KC and MCP-1. NF-κB is a transcription factor, which modulates the expression of a variety of genes involved in inflammatory responses. In an unstimulated cell, NF-κB resides in the cytoplasm as an inactive NF-κB-IκB complex. When the cell is stimulated, IκB becomes phosphorylated and is subsequently degraded, allowing NF-κB to translocate into the nucleus (19,20). As the degradation of IκB is an essential step in NF-κB activation by various stimuli, the present study used immunoblot analysis to examine the levels of total IκBα in the 3T3-L1 adipocytes of the different treatment groups. As shown in Fig. 4A, the level of IκBα was markedly decreased in the ASP (100 nM; 24 h) groups, compared with the untreated control. Pretreatment with nicotine prevented ASP-induced IκBα degradation. In addition, the suppression of IκBα degradation by nicotine was prevented by the addition of α-BTX. These results demonstrated that α7nAChR inhibited the ASP-induced production of cytokines, partially by preventing the degradation of IκBα. As the activation and nuclear translocation of NF-κB is an essential step in the regulation of cytokine production, the present study performed immunoblot analysis of the nuclear and cytosolic extracts to assess whether nicotine altered the nuclear translocation of NF-κB p65 induced by ASP. As shown in Fig. 4B and C, the nuclear translocation of NF-κB p65 was induced upon treatment with ASP. However, pretreatment with nicotine significantly attenuated the ASP-induced translocation of NF-κB p65. In addition, the nicotinic-induced suppression of translocation was prevented by the addition of α-BTX. These results demonstrated that α7nAChR inhibited the production of MCP-1 and KC in the ASP-stimulated adipocytes by inhibiting the translocation of NF-κB p65. The present study further confirmed the inhibition of ASP-induced NF-κB activation by nicotine in the production of cytokines using the NF-κB inhibitor, BAY-11-7082 (5 μM; 30 min). As shown in Fig. 4D and E, pretreatment with BAY-11-7082 markedly inhibited the ASP-induced production of MCP-1 and KC, and inhibited the degradation of IκBα. These data demonstrated that nicotine suppressed the ASP-induced production of cytokines through downregulation of NF-κB activity in 3T3-L1 cells.

Discussion

Obesity is accompanied by low-level inflammation, and this has been regarded to be the mechanistic link between obesity and associated cardiovascular and diabetic complications (26). MCP-1 and KC are important in the accumulation and activation of macrophages in inflamed adipose tissue. In the present study, ASP increased the expression levels of MCP-1 and KC in a concentration- and time-dependent manner, as revealed by immunoblot assays. Consistent with these results, Tom *et al* (3), reported that ASP treatment at 200 nM increased the secretion of KC and MCP-1, as measured using ELISA kits. These findings suggest that ASP contributes to obesity-mediated inflammation and adipose tissue macrophage invasion. A previous histological study showed that ASP increases the numbers of M1 macrophages in the adipose tissue, liver and skeletal muscle of mice (27). In addition, ASP exerts a direct concentration-dependent effect, which increase migration and M1 activation of cultured macrophages (27).

There are at least three families of MAPKs, including extracellular signal-regulated kinase, c-Jun-N-terminal kinase and p38 kinase, existing in mammalian cells. As described previously, p38 kinase is considered to be involved in the regulation of inflammatory responses (13). In the

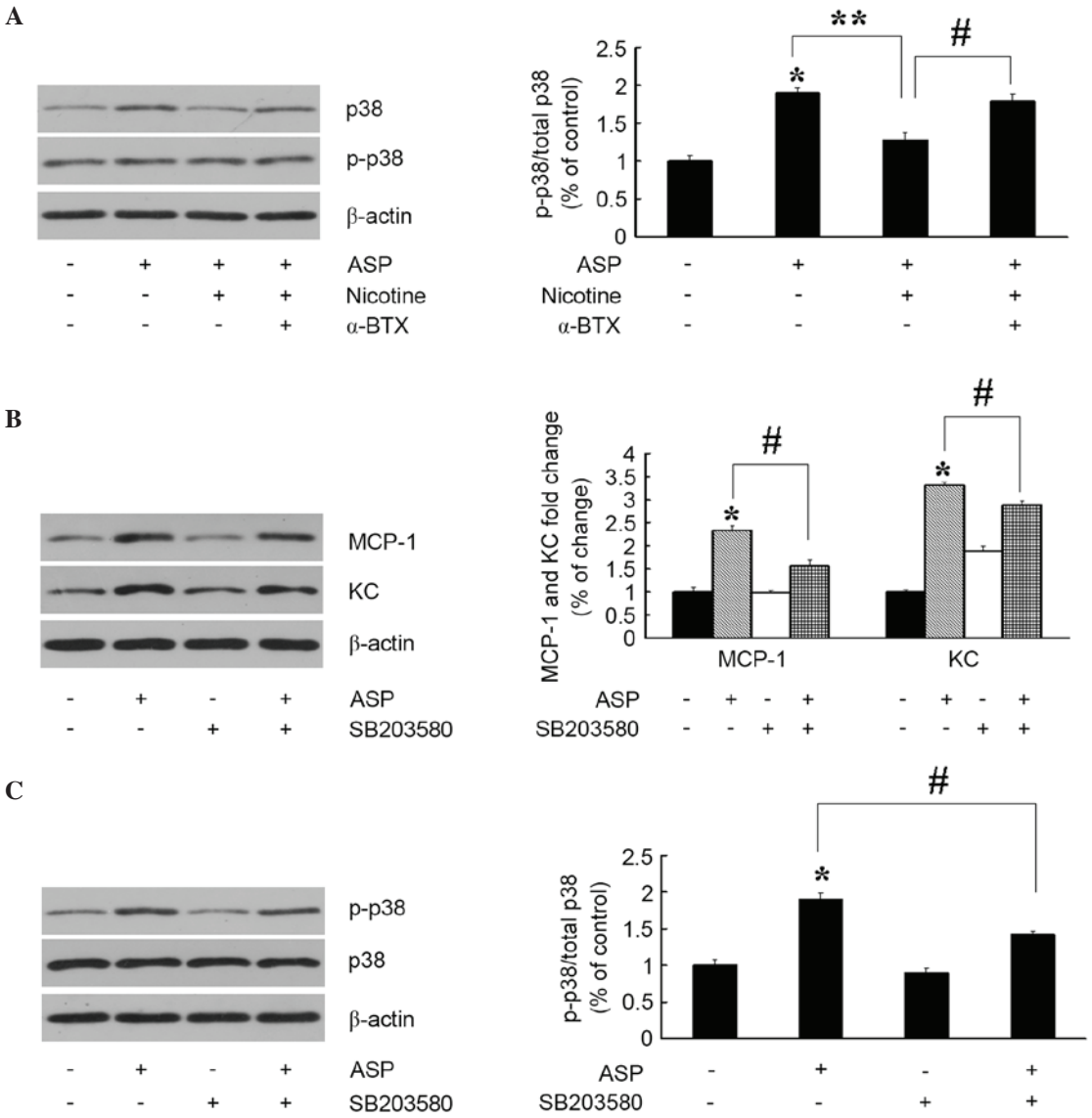

Figure 3. Inhibition of ASP-stimulated p38 kinase phosphorylation by α7nAChR inhibits the expression of KC and MCP-1. (A) Adipocytes were pretreated with nicotine (10 μM) in the absence or presence of α-BTX (2 μM) for 30 min and then challenged with ASP (100 nM) for 24 h. Following treatment, levels of p38 kinase and p-p38 kinase were determined using immunoblot and densitometric analysis. Data are presented as the mean ± standard error of the mean (n=3) *P<0.05, vs. untreated control; **P<0.05, vs. ASP; $^{\#}$P<0.05, vs. nicotine+ASP. Adipocytes were incubated with ASP (100 nM) for 24 h and/or the p38 kinase inhibitor, SB203580 (20 μM; 30 min). The levels of (B) KC and MCP-1, and (C) p38 kinase and p-p38 kinase were determined using western blot and densitometric analysis. Data are presented as the mean ± standard error of the mean (n=3). *P<0.05, vs. untreated control; $^{\#}$P<0.05, vs. ASP. α7nAChR, α7 nicotinic acetylcholine receptor; ASP, acylation stimulating protein; KC, keratinocyte-derived chemokine; MCP-1, monocyte chemoattractant protein-1; p-, phosphorylated.

present study, the increased production of the ASP-stimulated cytokines, MCP-1 and KC, in the 3T3-L1 cells was partly mediated by the p38 kinase phosphorylation pathways. The inhibition of p38 kinase following the administration of SB203580, a selective p38 kinase inhibitor, suppressed the ASP-induced phosphorylation of p38 kinase and production of cytokines. The results of the present study are concordant with those from a study on adipocytes stimulated with TNF-α, which selectively increased the expression of MCP-1 via the activation of p38 kinase (28).

α7nAChR is involved in mediating cholinergic anti-inflammatory activities *in vitro* and *in vivo*. In the present study, nicotine pretreatment significantly inhibited the ASP-induced expression of KC and MCP-1, and this was prevented by the α7nAChR antagonist, α-BTX. The fact that nicotine significantly reduced the phosphorylation of p38 kinase demonstrated that the effects of α7nAChR on the ASP-induced production of KC and MCP-1 were mediated by inhibition of the p38 kinase pathway. The results of the present study are consistent with previous reports that α7nAChR inhibits the lipopolysaccharide-induced release of TNF-α in microglial cells, and is associated with the suppression of p38 kinase activity (29,30). By contrast, Aicher *et al* (31) showed that, in dendritic cells, nicotine induced α7nAChR-mediated T-cell proliferation and cytokine secretion by partly activating p38 kinase. The most likely explanation for the discrepant results is that, in these systems, regulation is cell type- and stimulus-dependent.

NF-κB is composed of two subunits, p50 and p65, and is retained in the cytoplasm of unstimulated cells by a non-covalent

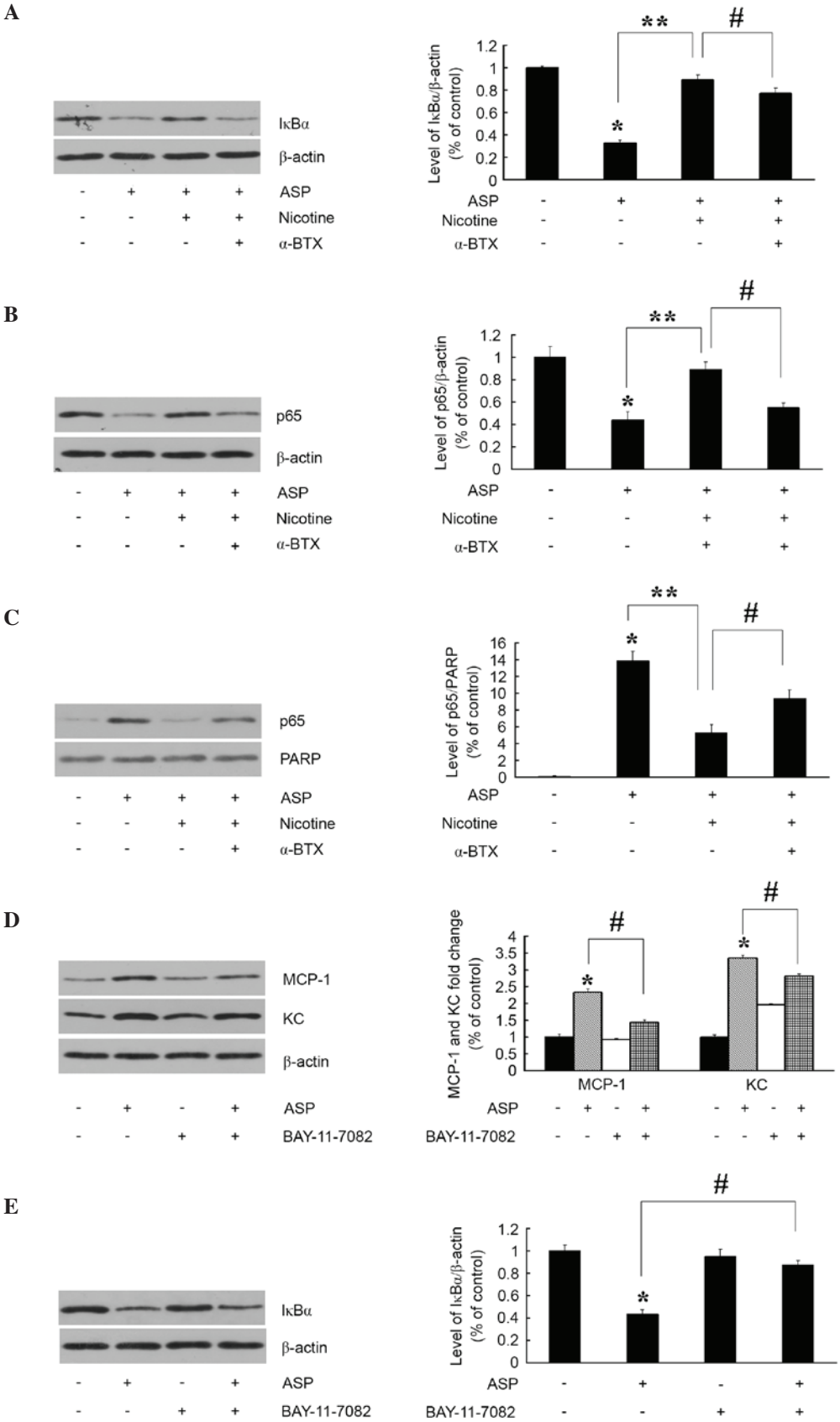

Figure 4. Inhibition of ASP-stimulated NF-κB activation by α7nAChR inhibits the expression of KC and MCP-1. Adipocytes were pretreated with nicotine 10 μM in the absence or presence of α-BTX (2 μM) for 30 min and then challenged with ASP (100 nM) for 24 h. The expression levels of (A) IκBα and the (B) p65 subunit of NF-κB in the cytosol extracts and (C) nuclear extracts were determined using immunoblot and densitometric analysis. Data are presented as the mean ± standard error of the mean (n=3). *P<0.05, vs. untreated control; **P<0.05, vs. ASP; $^{\#}$P<0.05, vs. nicotine+ASP. Adipocytes were incubated with ASP (100 nM) for 24 h or the NF-κB inhibitor, BAY-11-7082 (10 nM), and the expression levels of (D) KC and MCP-1, and (E) IκBα were determined using immunoblot and densitometric analysis. Data are presented as the mean ± standard error of the mean (n=3), *P<0.05, vs. untreated control; $^{\#}$P<0.05, vs. ASP. α7nAChR, α7 nicotinic acetylcholine receptor; ASP, acylation stimulating protein; α-BTX, α-bungarotoxin; NF-κB, nuclear factor-κB; IκBα, inhibitor of NF-κB; KC, keratinocyte-derived chemokine; MCP-1, monocyte chemoattractant protein-1; PARP, poly (ADP-ribose) polymerase.

interaction with the inhibitory molecule, IκB. Following activation by a number of physiological and non-physiological stimuli, IκB dissociates from NF-κB within minutes, and undergoes ubiquitination and degradation (19,20). Upon release, NF-κB is translocated to the nucleus, where it regulates the transcription of inflammatory genes (32). The data in the present study showed that treatment of the adipocytes with ASP promoted the degradation of IκBα and translocation of NF-κB. Pretreatment with the NF-κB inhibitor, BAY-11-7082, inhibited the ASP-induced production of MCP-1 and KC, and degradation of IκBα. These findings are consistent with those of a previous study, which reported that ASP increased the phosphorylation of Ser(468) and Ser(536) of p65 NF-κB, which is required for the transactivation of gene expression, in a time- and concentration-dependent manner (3). Pretreatment of the cells with nicotine in the present study inhibited the ASP-induced activation of NF-κB, and production of MCP-1 and KC. Consistent with these results, previous studies have reported that, in different cells, including monocytes, mast cells and endothelial cells, α7nAChR may prevent inflammation by inhibiting NF-κB transcriptional activity (17,19,33). By contrast, other studies have shown that α7nAChR signaling proceeds through intracellular pathways, leading to the upregulated expression and transactivation of NF-κB (34,35). These findings suggest that a different stimulus, or stimulus intensity, targeted to the same receptor may either inhibit or activate the same signaling system. The mechanism used by nicotine to modulate the response of NF-κB to ASP remains to be elucidated. The present study hypothesized that nicotine may also activate intracellular anti-inflammatory signal transduction pathways, including the Janus kinase 2-signal transducer and activator of transcription 3-suppressor of cytokine signaling 3 pathway (14,36), the cyclic adenosine 3',5'-monophosphate (cAMP) response element binding protein or the cAMP-dependent protein kinase (19), which can inhibit the ASP-induced activation and nuclear translocation of NF-κB.

The majority of reports state that p38 kinase can positively regulate NF-κB activity, albeit through various mechanisms (14,16,37). By contrast, in certain studies, the inhibition of p38 kinase significantly increased NF-κB activity (15,28). Although examining the association between NF-κB and p38 kinase is beyond the scope of the present study, it is important to determine whether there is reciprocal cross-talk between NF-κB and p38 kinase in the system, and further investigation of their interaction is required. The present study may provide novel insights into obesity treatment and various approaches toward the development of novel anti-obesity therapeutic agents.

In conclusion, the results of the present study suggested a novel anti-inflammatory function of α7nAChR in the regulation of chemokine production by adipocytes in response to ASP. Of note, α7nAChR appeared to exert its effects through modulation of the p38 kinase pathway and the canonical NF-κB pathway.

Acknowledgements

The authors would like to thank Mr Marc Lapointe (Laval University, Québec, Canada) for the preparation and purification of recombinant ASP. This study was supported by the National Natural Science Foundation of China (grant no. 81300685) to Dr Jing Wu (First Affiliated Hospital of Zhengzhou University, Zhengzhou, China).

References

1. Coenen KR, Gruen ML, Chait A and Hasty AH: Diet-induced increases in adiposity, but not plasma lipids, promote macrophage infiltration into white adipose tissue. Diabetes 56: 564-573, 2007.
2. Neels JG, Badeanlou L, Hester KD and Samad F: Keratinocyte-derived Chemokine in obesity: Expression, regulation, and role in adipose macrophage infiltration and glucose homeostasis. J Biol Chem 284: 20692-20698, 2009.
3. Tom FQ, Gauvreau D, Lapointe M, Lu H, Poursharifi P, Luo XP and Cianflone K: Differential chemoattractant response in adipocytes and macrophages to the action of acylation stimulating protein. Eur J Cell Biol 92: 61-69, 2013.
4. van Maanen MA, Papke RL, Koopman FA, Koepke J, Bevaart L, Clark R, Lamppu D, Elbaum D, LaRosa GJ, Tak PP and Vervoordeldonk MJ: Two novel α7 nicotinic acetylcholine receptor ligands: In vitro properties and their efficacy in collagen-induced arthritis in mice. PLoS One 10: e0116227, 2015.
5. Peña G, Cai B, Liu J, van der Zanden EP, Deitch EA, de Jonge WJ and Ulloa L: Unphosphorylated STAT3 modulates alpha7 nicotinic receptor signaling and cytokine production in sepsis. Eur J Immunol 40: 2580-2589, 2010.
6. Kim TH, Kim SJ and Lee SM: Stimulation of the α7 nicotinic acetylcholine receptor protects against sepsis by inhibiting Toll-like receptor via phosphoinositide 3-kinase activation. J Infect Dis 209: 1668-1677, 2014.
7. Marrero MB, Lucas R, Salet C, Hauser TA, Mazurov A, Lippiello PM and Bencherif M: An alpha7 nicotinic acetylcholine receptor-selective agonist reduces weight gain and metabolic changes in a mouse model of diabetes. J Pharmacol Exp Ther 332: 173-180, 2010.
8. Cancello R, Zulian A, Maestrini S, Mencarelli M, Della Barba A, Invitti C, Liuzzi A and Di Blasio AM: The nicotinic acetylcholine receptor α7 in subcutaneous mature adipocytes: Downregulation in human obesity and modulation by diet-induced weight loss. Int J Obes (Lond) 36: 1552-1557, 2012.
9. Berg AH, Lin Y, Lisanti MP and Scherer PE: Adipocyte differentiation induces dynamic changes in NF-kappaB expression and activity. Am J Physiol Endocrinol Metab 287: E1178-E1188, 2004.
10. Aggarwal BB: Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. Annu Rev Nutr 30: 173-199, 2010.
11. Dandona P: Insulin resistance and endothelial dysfunction in atherosclerosis: Implications and interventions. Diabetes Technol Ther 4: 809-815, 2002.
12. He Z, Zhu HH, Bauler TJ, Wang J, Ciaraldi T, Alderson N, Li S, Raquil MA, Ji K, Wang S, *et al*: Nonreceptor tyrosine phosphatase Shp2 promotes adipogenesis through inhibition of p38 MAP kinase. Proc Natl Acad Sci USA 110: E79-E88, 2013.
13. Roux PP and Blenis J: ERK and p38 MAPK-activated protein kinases: A family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev 68: 320-344, 2004.
14. Yoon SW, Goh SH, Chun JS, Cho EW, Lee MK, Kim KL, Kim JJ, Kim CJ and Poo H: alpha-Melanocyte-stimulating hormone inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in leukocytes by modulating protein kinase A, p38 kinase and nuclear factor kappa B signaling pathways. J Biol Chem 278: 32914-32920, 2003.
15. Bowie AG and O'Neill LA: Vitamin C inhibits NF-kappa B activation by TNF via the activation of p38 mitogen-activated protein kinase. J Immunol 165: 7180-7188, 2000.
16. Feng M, Wang Y, Chen K, Bian Z, Jinfang Wu and Gao Q: IL-17A promotes the migration and invasiveness of cervical cancer cells by coordinately activating MMPs expression via the p38/NF-κB signal pathway. PLoS One 9: e108502, 2014.
17. Saeed RW, Varma S, Peng-Nemeroff T, Sherry B, Balakhaneh D, Huston J, Tracey KJ, Al-Abed Y and Metz CN: Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. J Exp Med 201: 1113-1123, 2005.

18. de Jonge WJ and Ulloa L: The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. Br J Pharmacol 151: 915-929, 2007.
19. Yoshikawa H, Kurokawa M, Ozaki N, Nara K, Atou K, Takada E, Kamochi H and Suzuki N: Nicotine inhibits the production of proinflammatory mediators in human monocytes by suppression of I-kappaB phosphorylation and nuclear factor-kappaB transcriptional activity through nicotinic acetylcholine receptor alpha7. Clin Exp Immunol 146: 116-123, 2006.
20. Sun P, Zhou K, Wang S, Li P, Chen S, Lin G, Zhao Y and Wang T: Involvement of MAPK/NF-κB signaling in the activation of the cholinergic anti-inflammatory pathway in experimental colitis by chronic vagus nerve stimulation. PLoS One 8: e69424, 2013.
21. Murray I, Parker RA, Kirchgessner TG, Tran J, Zhang ZJ, Westerlund J and Cianflone K: Functional bioactive recombinant acylation stimulating protein is distinct from C3a anaphylatoxin. J Lipid Res 38: 2492-2501, 1997.
22. Shi D, Pop MS, Kulikov R, Love IM, Kung AL and Grossman SR: CBP and p300 are cytoplasmic E4 polyubiquitin ligases for p53. Proc Natl Acad Sci USA 106: 16275-16280, 2009.
23. Takahashi K, Yamaguchi S, Shimoyama T, Seki H, Miyokawa K, Katsuta H, Tanaka T, Yoshimoto K, Ohno H, Nagamatsu S and Ishida H: JNK- and IkappaB-dependent pathways regulate MCP-1 but not adiponectin release from artificially hypertrophied 3T3-L1 adipocytes preloaded with palmitate in vitro. Am J Physiol Endocrinol Metab 294: E898-E909, 2008.
24. Martins RP, Kaur K, Hwang E, Ramirez RJ, Willis BC, Filgueiras-Rama D, Ennis SR, Takemoto Y, Ponce-Balbuena D, Zarzoso M, *et al*: Dominant frequency increase rate predicts transition from paroxysmal to long-term persistent atrial fibrillation. Circulation 129: 1472-1482, 2014.
25. Wang HF, Lin PP, Chen CH, Yeh YL, Huang CC, Huang CY and Tsai CC: Effects of lactic acid bacteria on cardiac apoptosis are mediated by activation of the phosphatidylinositol-3 kinase/AKT survival-signalling pathway in rats fed a high-fat diet. Int J Mol Med 35: 460-470, 2015.
26. Després JP: Body fat distribution and risk of cardiovascular disease: An update. Circulation 126: 1301-1313, 2012.
27. Fisette A, Poursharifi P, Oikonomopoulou K, Munkonda MN, Lapointe M and Cianflone K: Paradoxical glucose-sensitizing yet proinflammatory effects of acute ASP administration in mice. Mediators Inflamm 2013: 713284, 2013.
28. Weber NC, Blumenthal SB, Hartung T, Vollmar AM and Kiemer AK: ANP inhibits TNF-alpha-induced endothelial MCP-1 expression-involvement of p38 MAPK and MKP-1. J Leukoc Biol 74: 932-941, 2003.
29. Shytle RD, Mori T, Townsend K, Vendrame M, Sun N, Zeng J, Ehrhart J, Silver AA, Sanberg PR and Tan J: Cholinergic modulation of microglial activation by alpha 7 nicotinic receptors. J Neurochem 89: 337-343, 2004.
30. Suzuki T, Hide I, Matsubara A, Hama C, Harada K, Miyano K, Andrä M, Matsubayashi H, Sakai N, Kohsaka S, *et al*: Microglial alpha7 nicotinic acetylcholine receptors drive a phospholipase C/IP3 pathway and modulate the cell activation toward a neuroprotective role. J Neurosci Res 83: 1461-1470, 2006.
31. Aicher A, Heeschen C, Mohaupt M, Cooke JP, Zeiher AM and Dimmeler S: Nicotine strongly activates dendritic cell-mediated adaptive immunity: Potential role for progression of atherosclerotic lesions. Circulation 107: 604-611, 2003.
32. Zhang JZ, Liu Z, Liu J, Ren JX and Sun TS: Mitochondrial DNA induces inflammation and increases TLR9/NF-κB expression in lung tissue. Int J Mol Med 33: 817-824, 2014.
33. Mishra NC, Rir-sima-ah J, Boyd RT, Singh SP, Gundavarapu S, Langley RJ, Razani-Boroujerdi S and Sopori ML: Nicotine inhibits Fc epsilon RI-induced cysteinyl leukotrienes and cytokine production without affecting mast cell degranulation through Alpha7/Alpha9/Alpha 10-nicotinic receptors. J Immunol 2185: 588-596, 2010.
34. Marrero MB and Bencherif M: Convergence of alpha 7 nicotinic acetylcholine receptor-activated pathways for anti-apoptosis and anti-inflammation: Central role for JAK2 activation of STAT3 and NF-kappaB. Brain Res 1256: 1-7, 2009.
35. Chernyavsky AI, Arredondo J, Galitovskiy V, Qian J and Grando SA: Upregulation of nuclear factor-kappaB expression by SLURP-1 is mediated by alpha7-nicotinic acetylcholine receptor and involves both ionic events and activation of protein kinases. Am J Physiol Cell Physiol 299: C903-C911, 2010.
36. Kox M, van Velzen JF, Pompe JC, Hoedemaekers CW, van der Hoeven JG and Pickkers P: GTS-21 inhibits pro-inflammatory cytokine release independent of the Toll-like receptor stimulated via a transcriptional mechanism involving JAK2 activation. Biochem Pharmacol 78: 863-872, 2009.
37. Li X, Zhou X, Ye Y, Li Y, Li J, Privratsky B, Wu E, Gao H, Huang C and Wu M: Lyn regulates inflammatory responses in Klebsiella pneumoniae infection via the p38/NF-κB pathway. Eur J Immunol 44: 763-773, 2014.