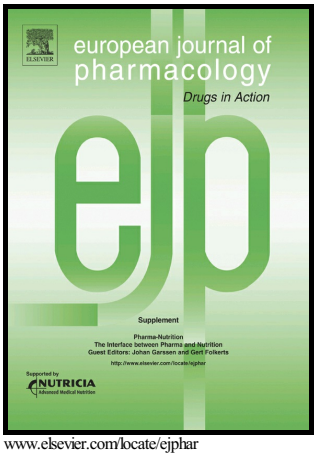

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PII: S0014-2999(18)30245-0
DOI: https://doi.org/10.1016/j.ejphar.2018.04.028
Reference: EJP71777

To appear in: *European Journal of Pharmacology*

Received date: 25 March 2018
Revised date: 20 April 2018
Accepted date: 25 April 2018

Cite this article as: Shiyao Wu, Yaou Zhou, Sijia Liu, Huali Zhang, Hui Luo, Xiaoxia Zuo and Tong Li, Regulatory effect of nicotine on the differentiation of Th1, Th2 and Th17 lymphocyte subsets in patients with rheumatoid arthritis, *European Journal of Pharmacology,* https://doi.org/10.1016/j.ejphar.2018.04.028

Regulatory effect of nicotine on the differentiation of Th1, Th2 and Th17 lymphocyte subsets in patients with rheumatoid arthritis

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Abstract

Previous research has demonstrated that nicotine have protective role in rheumatoid arthritis (RA). However, the immunologic mechanisms of nicotine’s effect have not been fully elucidated. Herein, the effects of nicotine on the differentiation of Th1, Th2, and Th17 cells were assessed. Peripheral blood mononuclear cells (PBMCs) and CD4+T cells were separated from patients with RA. PBMCs were stimulated with anti-CD3/anti-CD28 in the absence or presence of nicotine. CD4+T cells were cultured in the Th cell differentiation condition in the absence of nicotine or nicotine and alpha- bungarotoxin (αBgt) (the antagonist of nicotine) combined. Levels of T cell cytokines were detected with ELISA and flow cytometry. The expression of specific transcription factors (retinoic orphan re- ceptor c (RORc), T-box transcription factor (T-bet), and GATA Binding

Protein 3 (GATA-3)) and signaling molecules (P-ERK1/2 and T-ERK1/2) were determined by Western blot. The results showed nicotine reduced IL-17A and increased IL-4 produced by stimulated PBMCs. During Th17 differentiation conditions, nicotine reduced the levels of IL-17A and RORc, induced the phosphorylation of ERK1/2. Meanwhile, nicotine increased the levels of IL-4 and GATA3 during Th2 differentiation. α-Bgt blocked the effects of nicotine on Th2 and Th17 differentiation. However, nicotine had no effect on the expression of IFN-γ and T-bet in CD4+T cells during Th1differentiation. These results demonstrate that nicotine suppresses Th17 differentiation, promotes Th2 differentiation and improves Th1/Th2 imbalance in RA patients, providing a new justification for its application in the treatment of rheumatoid arthritis.

Keywords: Nicotine, Th cell differentiation, α7nAch receptor, Rheumatoid arthritis

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease, characterized by joint synovial inflammation and destruction of cartilage and bone. Although the pathogenesis of RA is still unclear, Th1, Th2 and Th17 cells as three important subtypes of CD4+Th cells are associated with RA. The specific transcription factors promoting their differentiation are T-box transcription factor (T-bet); GATA Binding Protein 3 (GATA-3); and retinoic orphan receptor c (RORc) respectively. Th1 cells and its hallmark cytokine interferon (IFN)-γ, play an important role in RA inflammation. Inhibition of the Th1 responses is a valid treatment for RA patients (Guggino et al., 2014; Nissinen et al., 2003). Low numbers of Th2 cells are present in RA and it may have a protective role in RA(Guggino et al., 2014). Th2 cells and its cytokine interleukin (IL)-4 is thought to exert a protective function in RA and

collagen-induced arthitis (CIA)(Schulze-Koops & Kalden, 2001; Myers et al., 2002). The Th1/Th2 ratio in RA patients is increased and is positively correlated with disease activity. Disease modifying anti-rheumatic drugs are able to correct the imbalance of Th1/Th2 cells in RA (Lina et al., 2011). The role of Th17 and its cytokine IL-17A in RA has been identified recently(Agarwal et al., 2008; Sato et al., 2006): Th17 cells induce the production of tissue destructive enzymes matrix metallo-proteinase-1 (MMP-1) and MMP-3 from RA synovial fibroblasts (van Hamburg et al., 2011). Th17 cells also participate in bone degradation by increasing the expression of receptor activator of nuclear factor κβ in osteoblasts (Sato et al., 2006). IL-17A is able to induce synovial cells to secrete cytokines and MMPs, resulting in bone destruction.(Agarwal et al., 2008). In conclusion, Th1 and Th17 cells accelerate RA, while Th2 cells may have a protective role in RA.

The vagus nerve alleviates inflammation via the α7 nicotinic acetylcholine receptor (α7nAch receptor). Nicotine, an α7nAch receptor agonist, exerts anti-inflammatory effects in multiple diseas es. For example, nicotine is able to alleviate inflammation of ulcerative colitis and acute lung inflammation (Lakhan & A. Kirchgessner, 2011; Mabley et al., 2011). Interestingly, *in vivo* and *in vitro*, nicotine plays a protective role in RA and experimental arthritis (van Maanen et al., 2009). Our previous research showed that nicotine can reduce the degree of joint inflammation in CIA (Li et al., 2010). Previous studies demonstrated that nicotine inhibits tumor necrosis factor-α induced IL-6 and IL-8 secretion in fibroblast-like synoviocytes from patients with RA (Zhou et al., 2012). van Maanen, et al. demonstrated that knockout of the α7nAch receptor aggravated CIA (van Maanen et al., 2010). Our recent research found that nicotine decreases the distribution of Th17 cells and increases the

distribution of Th2 cells in CIA (Wu et al., 2014). However, how nicotine modifies the distribution of these two cell types is unclear.

The α7nAch receptor on the immune cells (such as T cells, B cells, and macrophages), is believed to contribute significantly to the anti-inflammatory effects of nicotine (Skok et al., 2007; Wang et al., 2003). For example, the nicotine anti-inflammatory effect on macrophages can be counteracted by α7nAch receptor antagonist alpha-bungarotoxin (αBgt) (de Jonge et al., 2005). The serum levels of IFN and IL-6 in α7nAch receptor-/-mice were higher than in wild type mice (van Maanen et al., 2010).

In this study, we examined the effects of nicotine on the differentiation of Th1, Th2, and Th17 cells of RA patients and investigated the importance of α7nAch receptor in the observed effects.

2. Materials and methods

2.1. Ethical approval

Procedures were conducted incompliance with the National Institutes of Health Guidelines for the policy of human subjects. The protocol was approved by the Committee on the Ethics of human subjects of Central South University. All subjects signed the informed consent approved by the ethics committee.

2.2. Patients

We studied 32 patients (9 men and 23 women) aged 20–78 years who fulfilled the 2009 American College of Rheumatology revised criteria for RA, DAS28: 5.1–8.5. All of the subjects were non-smokers and untreated. Patients with complications were excluded (The complications

include infection, Hypertension, diabetes, coronary heart disease, tumor and other diseases). Twelve healthy controls aged 19–70 years (4 men and 8 women) were also evaluated.

2.3. Cell preparation

Peripheral blood samples were obtained from RA patients. The PBMCs were separated from heparinized blood by density gradient centrifugation with Ficoll–Hypaque Plus (GE Healthcare, USA). The CD4+T cells were purified (>96%) using a CD4+T cell Isolation Kit MicroBeads (Miltenyi Biotec, Bergisch-Gladbach, Germany), according to the manufacturer's instructions.

2.4. Cell culture and stimulation

The cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 g/ml streptomycin at 37°C in a 5% CO_2 atmosphere. The PBMCs (1×10^6 cells/ml) were cultured for 72 h in 24-well plates, and subsequently stimulated with anti-CD3 (clone HIT3a) (3 μg/ml, BD Biosciences, USA) and anti-CD28 (clone CD28.2) (5 μg/ml, BD Biosciences) in the presence or absence of differing concentrations (0.01 μmol/l, 0.1 μmol/l, and 1 μmol/l) of nicotine (Sigma, USA). Cell culture supernatants were stored at −80°C for measurement of IFN-γ, IL-4 and IL-17A.

In vitro Th cell differentiation

Purified CD4+ T cells (1×10^6 cells/ml) were polarized using anti-CD3-coated 96-well plates (Biocoat anti-human CD3 T-cell activation plates, BD Biosciences) and anti-CD28 (5 μg/ml, BD Biosciences). Th1 cell differentiation conditions were: IL-12, 15 ng/ml (Peprotech); and anti-IL-4, 4 μg/ml (Peprotech). The Th2 cell differentiation conditions were: IL-4, 10 ng/ml (Peprotech); and

anti-IFN-γ, 20 ng/ml (Peprotech). Th17 cell differentiation conditions were: IL-6, 10 ng/ml (Peprotech); TGF-β, 2.5 ng/ml (Peprotech); IL-21, 20 ng/ml (Peprotech); anti-IL-4, 10 μg/ml (Peprotech); and anti-IL-12, 10 μg/ml (Peprotech). Cells were cultured in the presence or absence of nicotine. In some experiments, CD4+T cells were pre-incubated with α-bungarotoxin (α-Bgt) for 30 min. In some experiments, after culture in Th17 differentiated condition for 12 days, the Th17-differentiated cells were pre-incubated with MEK1/2 inhibitor U0126 for 2 h prior to adding nicotine and then cultured for 1 h (ERK1/2 detection) or 24 h (IL-17A detection).

2.5. Cell proliferation and cytotoxicity analysis

PBMC and CD4+ T cells proliferation and cytotoxicity were analyzed with AlamarBlue (Abdserotec, UK)(Ahmed et al., 1994; O'Brien et al., 2000). Cells that were in the logarithmic phase of growth were harvested; cells were counted and adjusted to 1×10^6 cells/ml. The cells were plated and exposed to test agents, nicotine(1 μmol/l) (Sigma), and α-Bgt(1 μmol/l) (Sigma). The samples were mixed by shaking, and subsequently AlamarBlue was aseptically added in an amount equal to 10% of the volume of the well. AlamarBlue was incubated with the cultures for 8 h. Absorbance was measured at wavelengths of 570 nm and 600 nm after the required incubation.

2.6. Flow cytometric analysis

After culture for 72 h, the Th1, Th2, and Th17 differentiated CD4+T cells were stimulated for 5 h with Leukocyte Activation Cocktail and BD GolgiPlug (BD Biosciences). The Th1 and Th2-differentiated CD4+T cells were harvested and stained extracellularly with PE Cy5- CD3 mono-clonal antibody (mAb) (eBiosciences, USA), fluorescein isothiocyanate-CD8 mAb

(eBiosciences), and subsequently fixed and permeabilized with a Cytofix/Cytoperm Fixation/ Permeabilization Kit, followed by intracellular staining with phycoerythin- IFN-γ, PE- IL-4, and PE-IL-17A mAb (eBiosciences). Cells were assessed using a fluorescence-activated cell sorting (FACS) Calibur flow cytometer and analyzed using CellQuest software (BD Biosciences).

2.7. Western blot

Whole cell lysates were obtained from Th1, Th2, and Th17-differentiated CD4+ T cells cultured in RPMI-1640 with 10% FCS. Time periods of culture were 24 h or 48 h for transcription factor detection. Whole cell lysates were heated for 5 minutes at 90°C in 5× sodium dodecyl sulfate (SDS) loading buffer, separated by SDS-polyacrylamide gel electrophoresis, and transferred to polyvinylidene fluoride membranes. The membranes were blocked for 1 h at room temperature with 5% nonfat milk in 0.05% Tween 20/Tris buffered saline, followed by overnight incubation at 4°C with primary antibody (anti-T-bet antibody, anti-GATA3 antibody, anti-RORc antibody, all from Abcam, USA); anti-p-ERK1/2 antibody (Cell Signaling Technology, USA); anti-t-ERK1/2 antibody; and anti-GAPDH antibody (CST). The blots were incubated subsequently with secondary goat anti-rabbit horseradish peroxidaseH-conjugated immunoglobulin G (IgG) for 2 h at room temperature. The reaction was visualized by chemiluminescence detection.

2.8. Enzyme-Linked Immunosorbent Assay (ELISA)

The cytokine levels in culture supernatants were measured using human IFN-γ, IL-4, and IL-17 Quantikine ELISA kits (R&D Systems, USA). Cytokine concentrations were calculated in pg/ml using recombinant human IFN-γ, IL-4, and IL-17 (R&D Systems) as standards. Absorbance was

measured at 450 nm with an ELISA plate reader, and the reading at 540 nm subtracted to correct for optical imperfections in the plate.

2.9. Statistics analysis

With SPSS 17.0 statistical software, the data were expressed as mean ± standard deviation (S.D.). The significant differences among groups were determined by single factor variance (one-way ANOVA) analysis followed by a multiple comparison test (Student-Newman-Keuls). If the data did not satisfy the homogeneity of variance, the Kruskal Wallis test was used. P values less than 0.05 were considered significant.

3. Results

3.1. Cell proliferation/viability analysis

PBMCs from RA patients were stimulated with anti-CD3/CD28 antibodies, and CD4+T cells were cultured separately with Th1, Th2 or Th17-differentiation conditions, with nicotine or/and α-Bgt. The effects of nicotine or/and α-Bgt on cell proliferation and viability were analyzed using the AlamarBlue assay. Neither nicotine nor the combination of nicotine and α-Bgt had any effect on cell proliferation or viability. (The negative results were not listed).

3.2. Nicotine inhibits IL-17A and promotes IL-4 production by PBMCs from RA patients

PBMCs were cultured for 72 h with anti-CD3/CD28 antibodies alone or with various concentrations of nicotine. Anti-CD3/CD28 stimulated PBMCs from RA patients produced more IL-17A and less IL-4 than those of healthy volunteers. Nicotine inhibited the production of IL-17A (Fig. 1)and increased IL-4 production (Fig. 2) in a dose-dependent manner. Anti-CD3/CD28

stimulated RA PBMCs produced more IFN-γ than healthy controls, while nicotine had no effect on the production of IFN-γ. (The negative results were not listed).

Fig. 1. Nicotine inhibits IL-17A production by PBMCs from patients with RA. PBMCs were pre-incubated with nicotine (0.01, 0.1, and 1 μmol/l) for 30 min, and then cultured with anti-CD3/anti-CD28 for 72 h. IL-17A production in supernatants of PBMC cultures was assessed by ELISA. ## = P < 0.01 versus healthy controls (n=12). * = P < 0.05, ** = P < 0.01, *** = P < 0.001, versus anti-CD3/anti-CD28-stimulated PBMCs from RA patients (n=12).

Fig. 2. Nicotine increases IL-4 production by PBMCs from patients with RA. PBMCs from patients with RA were pre-incubated with nicotine (0.01, 0.1, and 1 μmol/l) for 30 min, and then cultured with anti-CD3/anti-CD28 for 72 h. IL-4 production in supernatants of PBMCs was analyzed with ELISA. ### = P < 0.001 versus healthy controls (n=12). * = P < 0.05, ** = P < 0.01, *** = P < 0.001, versus anti-CD3/anti-CD28-stimulated PBMCs from RA patients (n=10).

3.3. Nicotine reduces the percentages of IL-17A+T cells and increases the percentages of IL-4+T cells during Th17, Th2 cell-culture differentiation

To examine the direct effects of nicotine on Th1, Th2, and Th17 differentiation, CD4+ T cells were isolated, separated from RA PBMCs using a CD4+ T-cell Isolation Kit MicroBeads and stimulated with nicotine during Th1, Th2, and Th17 cell-culture differentiation. The intracellular expression of IFN-γ, IL-4 and IL-17A was assessed by flow cytometry following cellular activation with phorbol myristate acetate (PMA) and ionomycin. Exposure to PMA internalizes membrane CD4. Hence, the CD3+CD8- T cell population was used as the CD4+ T-cell population. Production

of IFN-γ, IL-4 and IL-17A in culture supernatants were detected with ELISA. Incubation with nicotine (1 μmol/l) reduced the percentage of IL-17A+CD3+CD8-T cells(Fig. 3A–I) and increased the percentage of IL-4+CD3+CD8- T cells(Fig. 4A–I). Nicotine decreased IL-17A(Fig. 3J) and increased IL-4 levels(Fig. 4J) in cell-culture supernatants. Nicotine had no effects on the percentage of IFN-γ+CD3+CD8-T cells or on IFN-γ production (The negative results were not listed). Stimulation with αBgt (1 μmol/l) prior to nicotine was able to increase IL-17A and decease IL-4 expression during Th17 and Th2 cell-culture differentiation (Fig. 3 and Fig. 4).

Fig. 3. Nicotine reduces the percentages of IL-17A+ T cells during Th17 cell-culture differentiation. (A, B, C) Gating strategy used for fluorescence activated cell sorting of CD3+CD8-T cells from RA patients. (D) Isotype control antibody. (E) The percentage of IL-17A+ cells within the CD3+CD8-T cell population after stimulation with anti-CD3/anti-CD28 for 72 h. (F) The percentage of IL-17A+ cells within the CD3+CD8-T cell population after cell-culture in Th17 differentiation conditions for 72 h. (G, H) Effect of nicotine alone (G) or with αBgt (H) on the expression of IL-17A by CD3+CD8-T cells from patients with RA. Representative results are shown. Numbers are the percentages of cells within the quadrants. (I) Quantification of IL-17A+CD3+CD8-T cell percentage. Bars show the mean ± S.D. (n=6), * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. (J)The expression of IL-17A in supernatants of differentiated Th17 CD4+T cell cultures analyzed by ELISA. Bars show mean ± S.D. (n=12), *** = $P < 0.001$.

Fig. 4. Nicotine increases the percentages of IL-4+ T cells in RA CD4+ T cells during Th2 cell-culture differentiation. (A, B, C) Gating strategy used for fluorescence activated cell sorting of

CD3+CD8-T cells from RA patients. (D) Isotype control antibody. (E) The percentages of IL-4+ cells in CD3+CD8-T cells stimulated by anti-CD3/anti-CD28 for 72 h. (F) The percentages of IL-4+ cells in CD3+CD8-T cells with Th2 cell-culture differentiation conditions for 72 h. (G, H) Effect of nicotine alone or αBgt on the expression of Th2 by CD3+CD8-T cells from patients with RA. Representative results are shown. Numbers are the percentages of cells within the quadrants. (I) Quantification of the percentages of IL-4+CD3+CD8-T cells. Bars show the mean ± S.D. (n=6), * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. (J) IL-4 supernatant levels from differentiated Th2 CD4+T cell cultures as assessed with ELISA . Bars are mean ± S.D. (n=12), *** =$P < 0.001$.

3.4. During Th17 and Th2 cell-culture differentiation, nicotine reduces RORc and increases GATA3 levels in CD4+ T cells derived from RA patients

The effect of nicotine on the differentiation of Th17 and Th2 cells was examined. The transcription factors RORc and GATA3 were analyzed by western blotting. Nicotine (1 μmol/l) inhibited RORc expression significantly and promoted GATA3 expression. This effect was blocked by αBgt (1 μmol/l) (Fig. 5 and Fig. 6).

Fig. 5. Nicotine reduces RORc levels in CD4+T cells from RA patients (n=3) during Th17 differentiation. (A) Statistical analysis of the relative expression of RORc in each treatment group. (B) Representative images for the levels of RORc protein and GAPDH in each treatment group. * = $P < 0.05$, ** = $P < 0.01$.

Fig. 6. Nicotine increases GATA3 expression in CD4+T cells from RA patients (n=3) during Th2 differentiation. (A) Statistical analysis of the relative expression of GATA3 in each treatment

group. (B) Representative images for the levels of GATA3 protein and GAPDH in each treatment group. * =P < 0.05, ** = P < 0.01, *** = P < 0.001.

3.5. Nicotine induced phosphorylation of ERK1/2 in Th17 cells

Based on the foregoing, the effect of nicotine on ERK1/2 during Th17 cell-culture differentiation was assessed by western blot. The results showed that nicotine promotes phosphorylation of ERK1/2 in Th17 cells, with the most dramatic effect after 1 h of stimulation. ERK1/2 is activated by MEK1/2, which can be blocked with U1026. Th17 cells were incubated with U0126 for 2 h, stimulated with nicotine for 1 h, and cellular protein levels assessed with western blot. In addition, IL-17A levels were assessed in cell-culture supernatants with ELISA. Results showed that U0126 inhibited the nicotine-induced phosphorylation of ERK1/2. As judged with ELISA, the inhibitory effect on IL17A was reversed by U0126.(Fig. 7 and Fig. 8)

Fig. 7. Nicotine induces phosphorylation of ERK1/2 in Th17 cells. Th17 cells were incubated with nicotine for differing time periods. P-ERK1/2 and t-ERK1/2 levels were detected with western-blot (n=3). (A) Statistical analysis of the relative expression of P-ERK1/2 in each treatment group. (B) Representative images for the levels of P-ERK1/2 protein and t-ERK1/2 in each treatment group. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Fig. 8. U0126 inhibits the phosphorylation of ERK1/2 and increases production of IL-17A in nicotine-treated Th17 cells. Th17 cells were incubated with nicotine alone or combined with U0126. P-ERK1/2 and t-ERK1/2 were detected by western blot (n=3). IL-17A was detected with ELISA (n=4). (A) Results of statistical analysis for the relative expression of P-ERK1/2 in each treatment

group. (B) Representative images for the levels of P-ERK1/2 protein and t-ERK1/2 in each treatment group. (C) Results of statistical analysis for the levels of IL-17A in each treatment group. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

4. Discussion

We have demonstrated previously that the cholinergic agonist, nicotine, affects the distribution of Th1, Th2 and Th17 in CIA mice(Wu et al., 2014). However, the mechanism of nicotine's action was not determined. In this study, we have explored the effects of nicotine on the differentiation of Th1, Th2 and Th17 cells derived from RA patients.

The present study found that the IL-17A levels in supernatants of RA PBMCs were significantly higher than that of normal control individuals. These results are consistent with previous research (Colin et al., 2010). This study further found a nicotine concentration effect on IL-17A production by RA PBMCs. Very low concentrations of nicotine (0.01 μmol/l) had an inhibitory effect on IL-17A production, while 1 μmol/l nicotine had a significant inhibitory effect on IL-17A production. A previous study showed that the plasma nicotine levels 2-120 mins after smoking is 12.44 ± 1.91 – 147.22 ± 16.51 μmol/l (peak plasma nicotine levels) (Mello et al., 2013). Therefore, the concentration of nicotine in the present study is very low and is in a safe level. Lee-J and co-workers descrybed the proinflammatory role of nicotine in RA: nicotine drives neutrophil extracellular trap formation and accelerates collagen-induced arthitis(J. Lee et al., 2017). Our findings herein do not contradict the Lee–J study. Our previous experiments indicated that a low doses of nicotine played a protective role in CIA, while a high doses aggravated CIA joint inflammation. The concentration of nico-

tine was 250 μg/kg per day in our experiments, while Lee–J used 15 mg/kg per day, in vivo. In vitro, the concentration of nicotine was 1 μmol/l in the present study while that of Lee–J was 5 mmol/l.

Further, we demonstrated that nicotine inhibits differentiation of RA CD4 + T cells into Th17 cells, decreases the percentage of IL-17A+T cells, inhibits the Th17 cell specific transcription factor RORc, decreases IL–17A levels, but had no effect on the proliferation of the IL-17A+T cells. These results illustrate the inhibitory effects of nicotine on the differentiation of RA Th17 cells. Further, these results imply that stimulation of the cholinergic anti-inflammatory pathway may change the distribution of Th17 cells in CIA by inhibiting Th17 cell differentiation.

αBgt is an acetylcholine receptor antagonist for α7nAch receptor, α9nAch receptor, and the mAch receptors. Previous studies have shown T cells do not express the mRNA of α9nAch receptor and muscle-type nAch receptor(Sato et al., 1999). Our study shows that the inhibitory effects of nicotine on Th17 cell differentiation can be reversed by αBgt, suggesting that nicotine mainly affects Th17 cell differentiation by activating the α7nAch receptor.

At present, the intracellular signal pathways involved in regulating Th17 cells differentiation have not been fully elucidated. Mitogen-activated protein kinase (MAPK) signaling pathways play an important role in cell proliferation, differentiation and cell death. MAPK superfamily members such as p38, MAPK, ERK and JNK have been reported to participate in the differentiation of CD4+T cell. Recent studies have shown that the MAPK signal pathways are also associated with Th17 cell differentiation. The activation of ERK1/2 in mice and humans negatively regulates Th17 cell differentiation, and α7nAch receptor is related to MAPK signaling(Cui et al., 2009; Sun et al.,

2013), and participates in Th17 cell differentiation. Based on the relationships among α7nAch receptor, ERK1/2 and Th17 cells, we evaluated the effects of nicotine on MEK1/2-ER1/2 signaling pathways during Th17 differentiation. Results showed that nicotine promoted the phosphorylation of ERK1/2, and that blocking MEK1/2 with U1026 reversed the activation of nicotine by ERK1/2. The inhibitory effects of nicotine on Th17 cell differentiation may be related to the activation of AP-1 and the Ets family, which are downstream transcription factors of ERK1/2 activation.

Our previous study found that nicotine modifies the Th1/Th2 ratio in CIA mice by increasing the distribution of Th2 cells in the spleen and peripheral blood(Wu et al., 2014). The specific mechanism was unclear. Further, we compared IFN-γ levels in RA PBMCs and in normal control individuals and found RA PBMCs to have higher IFN-γ levels, suggesting an elevation in RA Th1 cells, consistent with previous studies (Miltenburg et al., 1992; Scarsi et al., 2014). Nicotine had no effect on PBMC IFN-γ production and no effect on Th1 cell differentiation, which is consistent with our previous study in CIA (Wu et al., 2014). IL-4 production by RA PBMCs was significantly lower than control PBMCs, which is also consistent with a previous study(Kokkonen et al., 2010). Nicotine promoted Th2 differentiation and αBgt reversed the effect of nicotine on Th2 cell differentiation, demonstrating that nicotine promotes the differentiation of Th2 cells mainly by acting on the α7nAch receptor.

Based on the foregoing, nicotine does not effect the differentiation of RA Th1 cells (no promotion and no inhibition), but does stimulate Th2 cell differentiation, resulting in an improved Th1/Th2 ratio in RA.

5. Conclusions

Nicotine inhibits Th17 differentiation by acting on the α7nAch receptor and by activating the MEK1/2-ERK1/2 pathway. Further, nicotine promotes Th2 differentiation and improves the Th1/Th2 imbalance in RA.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China [81501854] and [81571602], the clinical medicine research special funds of Chinese medical association [12040650365]. The authors declare no conflict of interest.

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