Int J Clin Exp Med 2016;9(10):19574-19580
www.ijcem.com /ISSN:1940-5901/IJCEM0028190

Original Article

Interleukin-22 promotes macrophage M2 polarization via STAT3 pathway

Wei Luo*, Bing Yu*, Qin-Yi Qin, Jiong-Min Lu, Shan-Yu Qin, Hai-Xing Jiang

*Department of Gastroenterology, First Affiliated Hospital of Guangxi Medical University, Shuangyong Road 6, Nanning 530021, Guangxi, China. *Co-first authors.*

Received March 14, 2016; Accepted August 22, 2016; Epub October 15, 2016; Published October 30, 2016

Abstract: Background: Interleukin-22 (IL-22) plays a critical role in the immune system, but the effect on macrophages remains unknown. This study was aimed to investigate the role of IL-22 in the polarization of macrophages and the related pathway. Methods: RAW 264.7 cells (M0 macrophages) were treated with or without IL-22, and cells treated with resveratrol used as positive control. The M1, M2 macrophages makers were tested by RT-PCR, the TNF-α and IL-10 concentration in cell culture supernatant was measured by ELISA. The cells were also pretreated with signal transducer and activator of transcription 3 (STAT3) specific inhibitor AG490 before IL-22 treatment, then the expression of STAT3, p-STAT3 was detected by western-blot. Results: The markers of M2 macrophages (Arg1, CD163) were increased in a dose and time- dependent manner after IL-22 treatment, but the M1 macrophages markers (iNOS, TNF-α) did not change significantly when compared to those without IL-22 treatment. The expression of IL-10 in the supernatant was increased after IL-22 and resveratrol treatment compared with that without IL-22 treatment. However, no significantly changed was found in the expression of TNF-α after IL-22 treatment. After treatment with IL-22 on M0 macrophages, the expression of p-STAT3 protein was increased significantly, but STAT3 did not change greatly. Using AG490 to pretreat the M0 macrophages, the expression of protein of STAT3 and p-STAT3 did not change significantly after the treatment of IL-22, the expression of Arg1, CD163 and CD206 in AG490+IL-22 group were greatly decreased compared with the IL-22 group. Conclusion: This study indicates that IL-22 could induce M0 macrophages polarize into M2 macrophages in a dose and time- dependent manner, and this effect may via STAT3 pathway.

Keywords: Interleukin-22, macrophage, polarization, STAT3 pathway

Introduction

Interleukin-22 (IL-22) belongs to the IL-10 family, and can be produced by several immune cells, including CD4+ T cells (Th1, Th17 and Th22 cells) and NK cells [1]. IL-22 plays a pivotal role in promoting antimicrobial immunity and tissue repair by binding to the receptors, IL-22R1 and IL-10R2, which are widely expressed in the membrane of many cells. Evidences confirm that downstream of IL-22 signaling leads to increased of antimicrobial defense, activation of proliferative genes and up-regulated anti-apoptotic proteins [2]. IL-22 is also able to ameliorate liver fibrosis in mouse models by inhibiting hepatic stellate cells (HSCs) activity. Treatment with IL-22 can reduce liver fibrogenesis and accelerated the resolution of liver fibrosis during recovery [3, 4], and the anti-fibrotic effects of IL-22 are mediated via the activation of STAT3 pathway in HSCs [5].

Macrophages originate from blood monocytes, differentiate into distinct macrophages, which are identified by classically activated (M1) or alternatively activated (M2) phenotypes [6]. Macrophages are the principal immune cells in the liver, also known as Kupffer cells, located in the walls of the sinusoids of liver lining. The biological function of macrophages is varied depending on location of the affected tissues and on local cytokine context [7]. Generally, M1 macrophages are induced by interferon (IFN)-γ and secret IL-6, TNF-α and promote inflammatory reaction, whereas M2 macrophages are induced by IL-4, IL-10, IL-13 and exhibit an anti-inflammatory reaction [8]. During the liver fibrosis process, monocytes in peripheral blood

Table 1. The sequences of the primers used for the RT-PCR assays

	Forward	Reverse
GAPDH	5'-TGAAGAAGAGCCCATCCTCTG-3'	5'-GAGCTTCACAAAGTTGTCATTGAG-3'
iNOS	5'-AATCTTGGAGCGAGTTGTGG-3'	5'-CAGGAAGTAGGTGAGGGCTTG-3'
TNF-αA	5'-AGGCTCTGGAGAACAGCACAT-3'	5'-TGGCTTCTCTTCCTGCACCAAA-3'
ARG1	5'-CTCCAAGCCAAAGTCCTTAGAG-3'	5'-AGGAGCTGTCATTAGGGACATC-3'
CD163	5'-TGGGTGGGGAAAGCATAACT-3'	5'-AAGTTGTCGTCACACACCGT-3'
CD206	5'-TGGAATCAAGGGCACAGAAGT-3'	5'-CTCCATTTGCATTGCCCAGT-3'

are recruited into the liver by some chemokines and polarized into M1 or M2 macrophages depending upon the liver condition [9].

Since IL-22 treatment could alleviate liver fibrosis, however, the mechanisms underlying macrophages polarization during IL-22 treatment on liver fibrosis remained unknown. Therefore, in this study, we aimed to investigate the effect of IL-22 on the polarization of macrophages and the related pathway. Our results will provide a new evidence of IL-22 in ameliorating liver fibrosis through the regulation of macrophages.

Materials and methods

Reagents source

Recombinant human IL-22 was obtained from BD biosciences (San Diego, CA). The STAT3 antibody and phospho-STAT3 (p-STAT3) antibody were purchased from Cell Signal Technology, Inc. (Danvers, MA). The CD206 rabbit antibody was obtained from Abcam, Inc. (Cambridge, MA). The STAT3 inhibitor (AG490) was purchased from Tocris Bioscience (Ellis-ville, MO). Mouse TNF-α and IL-10 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Dakewe (Beijing, China). Resveratrol was purchased from Tokyo Chemical Industry (Shanghai, China).

Cell culture and treatment protocol

RAW 264.7 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD). The cells were seeded at a density of 5×10^4 cells/ml in 6-well plates and grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 4.5 g/L D-glucose (Gibco USA), 1× PBS, 100 U/ml penicillin (Gibco USA), 100 mg/ml streptomycin (Gibco USA), which in an 37°C, 5% CO_2 atmosphere. The cells were serum-starved for 12 h after confluent to 60%. Then the cells were washed softly with PBS for two times, and incubated in DMEM with 4.5 g/L D-glucose. The cells were treated with IL-22 (treatment group) or without IL-22 (negative control group), and resveratrol treatment (30 μmol/L) used as a positive control group. AG-490 (50 μmol/L) (inhibition group) was used to investigate the change of STAT3 pathway. The cells were collected in 12, 24 and 36 h. The expression of M1 (iNOS, TNF-α) markers, M2 markers (Arg1, CD163 and CD206) in the cells were measured by RT-PCR, the expression of TNF-α and IL-10 in the cell culture supernatant were tested by ELISA. The STAT3 and p-STAT3 were detected by western-blot.

RNA extraction and RT-PCR test

Cells were collected and the total RNA was extracted using Trizol reagent (Qiagen, USA), and treated with DNAse I (Fermentas, USA) for 30 minutes in order to digest the genomic DNA. The quality of RNA samples was monitored by spectrophotometer (Thermo NanoDrop 2000, USA). A total of 1 μg of RNA was reverse transcribed with a cDNA synthesis kit (Takara, Japan) and random hexamer primers according to the manufacturer's instructions. Transcript levels were determined using the SYBR Green master mix (Takara, Japan) and StepOnePlus Real-Time PCR System. The cycling conditions for the detection system were 95°C for 30 sec, 40 cycles of 95°C, 5 sec, 60°C, 30 sec. The relative gene expressions were normalized to the level of GAPDH housekeeping gene and quantified by the $2^{-\Delta\Delta CT}$ method. Primer sequences for real-time polymerase chain reaction (RT-PCR) are listed in **Table 1**.

Enzyme linked immunosorbent assay (ELISA)

The cell culture supernatant was collected, and TNF-α and IL-10 concentration was measured using a commercially available ELISA kit (R&D System) according to the instructions described by the manufacturer. The optical density (OD) of the microplate was read at 450 nm.

Western blot assay

After treatment with IL-22 for 36 h, the protein was obtained from 2×10^6 cells by lysis buffer

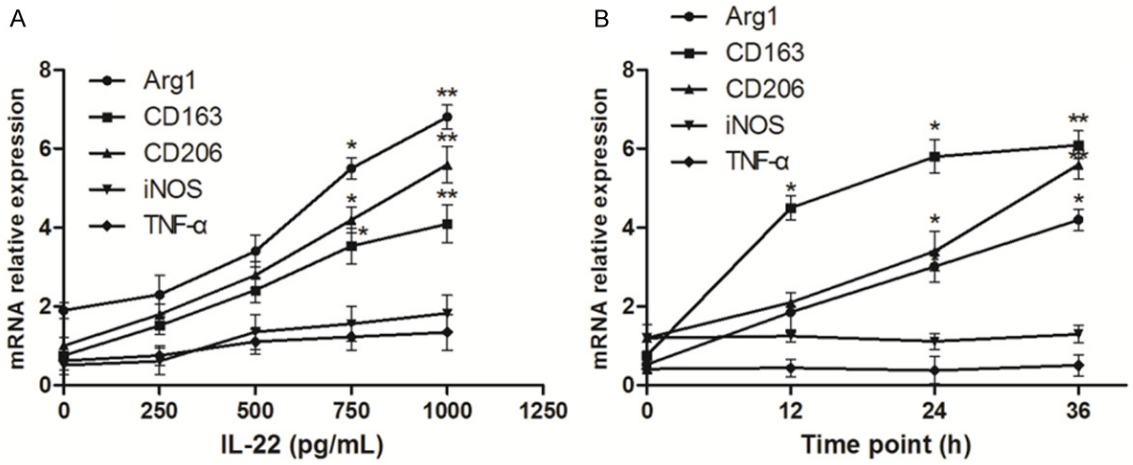

Figure 1. A: The mRNA expression of M1, M2 markers after IL-22 treatment at different concentration; B: The mRNA expression of M1, M2 markers after IL-22 treatment at different time point. Data was expressed as mean ± SD. The differences among multiple groups were analyzed using one-way ANOVA followed by LSD post-hoc test. **P*<0.05, ***P*<0.01 compared with 0 concentration or 0 h.

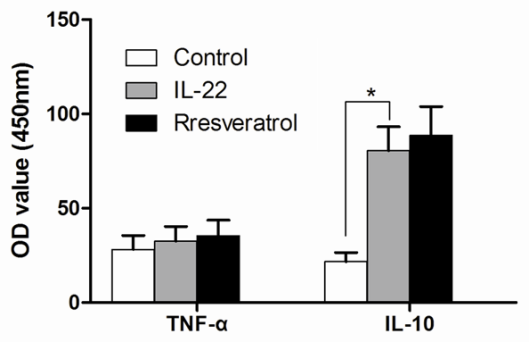

Figure 2. The expression of IL-10 and TNF-α after IL-22 treatment. Data was expressed as mean ± SD. one-way ANOVA test showed that no significant differences in the expression of TNF among IL-22 treatment, resveratrol treatment group and control group (*P*>0.05); one-way ANOVA/LSD post-hoc test showed that the expression of IL-10 was significantly elevated in IL-22, resveratrol group compared with control group. **P*<0.05 compared with control group.

that contained protease inhibitors and phosphatase inhibitors (Sigma, USA). Cell lysates were centrifuged at 14,000 rpm at 4°C for 15 min. The concentration of cellular protein was determined by the Pierce BCA assay. Four parts of equal concentrations of protein and one part of 5× sample buffer were mixed and heated at 95°C for 10 min, then the samples were separated on 10% sodium dodecyl sulfate (SDS) -polyacrylamide gels. After electrophoresis for 90 min, the proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane (Merck Millipore, USA). The membranes were blocked for 1 h at room temperature with 5% non-fat milk in 0.1% TBS-Tween 20 and incubated with the dilution of polyclonal rabbit anti-STAT3 (1:3000, Abcam, UK) and anti-p-STAT3 primary antibody (1:3000, Abcam, UK) at 4°C overnight, followed by the LI-COR IRDye 680-labeled secondary antibodies (Rockland Immunochemical, Gilbertsville, PA) for 1 h at room temperature. The signals were detected with Odyssey Infrared Imaging System (Li-COR Biosciences, Lincoln, NE) and quantitated with Fluorchem 8900 system (Alpha Innotech, San Leandro, CA).

Statistical analysis

All data were analyzed with SPSS 16.0 software (SPSS Inc, Chicago, IL, USA). Data was expressed as mean ± standard deviation (SD), and comparison between two groups were using Student's t test. The differences among multiple groups were analyzed using one-way analysis of variance (ANOVA) followed by LSD post-hoc test. A value of *P*<0.05 was considered statistically significant.

Results

IL-22 increased the expression markers of M2 macrophages

The Raw 264.7 cells (M0 macrophages) were treated with IL-22 at the concentration of 250, 500, 750, 1000 pg/mL, respectively, and the

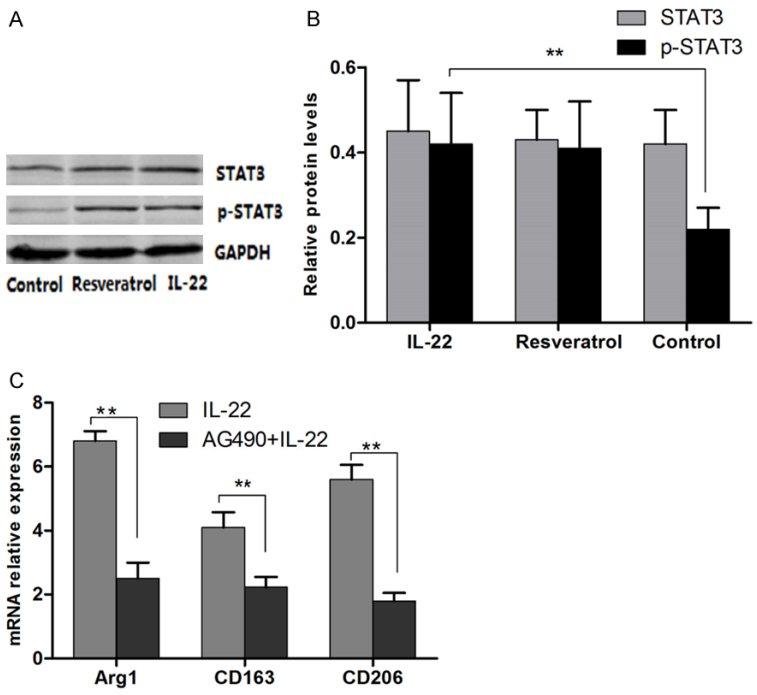

Figure 3. STAT3 protein expression and macrophage markers changes. A: West-blot showed the expression of STAT3, p-STAT3 protein and GAPDH in the Raw 264.7 cells; B: The relative protein level of p-STAT3. one-way ANOVA/LSD post-hoc test showed that the expression of IL-10 was significantly elevated in IL-22, resveratrol group compared with control group, $^{**}P<0.01$ compared with control; C: Expression of Arg1, CD163 and CD206 after IL-22 treatment or IL-22+AG490 treatment. Student's t-test showed that expression of Arg1, CD163 and CD206 in IL-22+AG490 group were greatly decreased compared with IL-22 group ($^{**}P<0.01$).

expression of macrophages markers was detected at the time of 12 h, 24 h and 36 h, respectively. One-way ANOVA showed significant differences in the expression of macrophages markers among different concentration and time points, and LSD post-hoc test showed that the markers of M2 macrophages, Arg1, CD163 and CD206 were increased greatly, which were peaked at the time of 36 h when the concentration of IL-22 was 1000 pg/mL. Similar results were observed in the group of resveratrol treatment. However, the M1 macrophages markers (iNOS, TNF-α) did not change significantly. See **Figure 1**.

IL-22 elevated the expression of cytokine of M2 macrophages

After treated with IL-22 at a concentration of 1000 pg/mL for 36 h, the expression of IL-10 in the cell culture supernatant was increased, and one-way ANOVA/LSD post-hoc test showed that IL-10 was substantially elevated in IL-22 and resveratrol group compared with control group ($P<0.05$), but no significantly differ-ence between IL-22 and resveratrol group ($P>0.05$). In addition, no significantly changed in the expression of TNF-α after IL-22 treatment compared with controls ($P>0.05$). See **Figure 2**.

STAT3 pathway was activated during IL-22 treated on macrophages

After treatment with IL-22 on M0 macrophages, western-blot assay showed that the expression of STAT3 and p-STAT3 protein was increased. One-way ANOVA/LSD post-hoc test showed that p-STAT3 were significantly increased in both IL-22 and resveratrol group, and there was difference compared with control group ($P<0.05$), but STAT3 did not elevate significantly ($P>0.05$). See **Figure 3A**, **3B**.

Using STAT3 pathway inhibitor, AG490, to pretreat the M0 macrophages for 24 h, and then IL-22 was added in the medium for 36 h, we observed that the expression of STAT3 and p-STAT3 protein was little change after the treatment of AG490+IL-22. The Student's t-test showed the M2 macrophages markers (Arg1, CD163 and CD206) in AG490+IL-22 group were greatly decreased compared with IL-22 group ($P<0.01$). See **Figure 3C**.

Discussion

Macrophages are crucial immune cells in the regulation of both initiation and resolution of immune responses, and play a protective or pathogenic role under both homeostatic and pathological conditions [10-12]. Due to their highly plasticity and diversity, macrophages exhibit a broad spectrum of diverse activities in different conditions [13]. Despite human macrophages are largely bone marrow -independent origin, tissue-resident macrophages, such as Kupffer cells in liver, microglia in brain, show distinct transcriptional and epigenetic signa-

tures [14, 15], suggesting that macrophages are shaped by local microenvironment to express organ-specific functions.

M1 macrophages produce amount of inflammatory molecules, including TNF-α, IL-1β, IL-6, and promote cytotoxic adaptive immunity by up-regulating MHCII in conjunction with co-stimulatory molecules (CD40, CD80, CD86), Th1- and Th17-orienting cytokines (IL-12, IL-27, IL-23) and Th1-recruiting chemokines CXCL9, CXCL10, CXCL11 [16]. In contrast, M2 macrophages secret anti-inflammatory cytokines, such as IL-10, TGF-β, and express endocytic receptors (CD163, Stabilin-1) and c-type lectin (CD206, CD301, dectin-1) receptors [16], finally lead to the resolution of inflammation. Furthermore, by secreting chemokines CCL17, CCL18, CCL22 or CCL24, M2 macrophages could recruit Th2, Tregs cells, eosinophils and basophils to the local environment and play a synergic anti-inflammatory effect [17].

In the present study, using resveratrol as positive control, which is considered as the classic agent induces M0 macrophages polarized into M2 macrophages [18], we observed that, after IL-22 treated on M0 macrophages, the markers of M2 macrophages (Arg1, CD163 and CD206) and the cytokines (IL-10) secreted by M2 macrophages were remarkably increased, and these results were similar to those of resveratrol treatment, suggesting IL-22 could induce M0 macrophages polarized into M2 macrophages, and this effect was in a dose- dependent manner and time- dependent manner. However, we failed to observe the significant changes of M1 macrophages markers after IL-22 treatment, suggesting that IL-22 may not induce M0 macrophages polarize into M1 macrophages.

By activating certain signaling pathways, such as STAT3, ERK1/2 and PI3K/Akt pathway, IL-22 was reported to exert its biological function in many cells or diseases. Among these signaling pathways, the STAT3 pathway was widely studied. Zhu et al [19] reported that STAT3 was an essential pathway in mediating the effects of IL-22 secreted by NKp44+NK cells on the proliferation of fibroblast-like synoviocytes in patients with rheumatoid arthritis. IL-22 also up-regulates β-defensin-2 expression in human alveolar epithelium via STAT3 but not NF-κB pathway [20]. The IL-22-STAT3 signal pathway could also revert IFN-γ-induced epithelial-mesenchymal transition of pleural mesothelial cells, and might play a protective role in anti-pleural fibrosis in tuberculous pleurisy [21].

Several signaling pathways are found to participate in the polarization and function of M1 or M2 macrophages. Yang et al [22] showed that TSP50 expressed in cells could activate surrounding macrophages and induce M2b polarization, partly through inducing TNF-α/IL-1β secretion and subsequent NF-κB pathway activation. IL-4 regulates macrophages polarization via the MAPK signaling pathway to protect against atherosclerosis [23]. Downstream STAT6, activation of PGC-1 is crucial for the M2 switch [24]; whereas PPAR and PPAR [25] have a key role in maintaining the M2 phenotype by inducing distinct genes associated with anti-inflammatory functions. In the present studies, we found that although the protein levels of STAT3 did not change substantially after IL-22 treatment, however, the phosphorylation of STAT3 was elevated greatly. In addition, the STAT3 specific inhibitor could greatly inhibit the effect of IL-22 on the polarization of M2 macrophages, suggesting that the STAT3 pathway may involve in this process.

In conclusion, this study reveals that IL-22 could induce M0 macrophages polarize into M2 macrophages in a dose and time- dependent manner, and this effect may be through the regulation of STAT3 pathway, especially through up-regulating p-STAT3 protein.

Acknowledgements

This study was partially supported by research funding from the National Natural Science Foundation (No. 81560103; 81660107; Guangxi Natural Science Foundation (2016GXNSFAA380259; 2015GXNSFBA139173).

Disclosure of conflict of interest

None.

Authors' contribution

Conceived and designed the experiments: JHX and LW. Performed the experiments: YB, QQY, LJM. Analyzed the data: YB, QQY, LJM. Wrote the paper: LW, QSY and JHX.

Address correspondence to: Dr. Shan-Yu Qin, Department of Gastroenterology, First Affiliated Hospital of Guangxi Medical University, Shuangyong Road 6, Nanning 530021, Guangxi, China. Tel: +86-0771-5356725; Fax: +86-0771-5356725; E-mail: gxjianghx@163.com

References

[1] Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J and Ouyang W. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 2007; 445: 648-651.

[2] Cobleigh MA and Robek MD. Protective and pathological properties of IL-22 in liver disease: implications for viral hepatitis. Am J Pathol 2013; 182: 21-28.

[3] Lu DH, Guo XY, Qin SY, Luo W, Huang XL, Chen M, Wang JX, Ma SJ, Yang XW and Jiang HX. Interleukin-22 ameliorates liver fibrogenesis by attenuating hepatic stellate cell activation and downregulating the levels of inflammatory cytokines. World J Gastroenterol 2015; 21: 1531-1545.

[4] Kong X, Feng D, Wang H, Hong F, Bertola A, Wang FS and Gao B. Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis in mice. Hepatology 2012; 56: 1150-1159.

[5] Kong X, Feng D, Mathews S and Gao B. Hepatoprotective and anti-fibrotic functions of interleukin-22: therapeutic potential for the treatment of alcoholic liver disease. J Gastroenterol Hepatol 2013; 28 Suppl 1: 56-60.

[6] Hanahan D and Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 2012; 21: 309-322.

[7] Malyshev I and Malyshev Y. Current Concept and Update of the Macrophage Plasticity Concept: Intracellular Mechanisms of Reprogramming and M3 Macrophage "Switch" Phenotype. Biomed Res Int 2015; 2015: 341308.

[8] Nikolic-Paterson DJ, Wang S and Lan HY. Macrophages promote renal fibrosis through direct and indirect mechanisms. Kidney Int Suppl (2011) 2014; 4: 34-38.

[9] Ohtsuki T, Kimura K, Tokunaga Y, Tsukiyama-Kohara K, Tateno C, Hayashi Y, Hishima T and Kohara M. M2 Macrophages Play Critical Roles in Progression of Inflammatory Liver Disease in Hepatitis C Virus Transgenic Mice. J Virol 2015; 90: 300-307.

[10] Wynn TA, Chawla A and Pollard JW. Macrophage biology in development, homeostasis and disease. Nature 2013; 496: 445-455.

[11] Murray PJ and Wynn TA. Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 2011; 11: 723-737.

[12] Gordon S, Pluddemann A and Martinez Estrada F. Macrophage heterogeneity in tissues: phenotypic diversity and functions. Immunol Rev 2014; 262: 36-55.

[13] Davies LC, Jenkins SJ, Allen JE and Taylor PR. Tissue-resident macrophages. Nat Immunol 2013; 14: 986-995.

[14] Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, Stender JD, Chun HB, Garner H, Geissmann F and Glass CK. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell 2014; 159: 1327-1340.

[15] Gibbings SL, Goyal R, Desch AN, Leach SM, Prabagar M, Atif SM, Bratton DL, Janssen W and Jakubzick CV. Transcriptome analysis highlights the conserved difference between embryonic and postnatal-derived alveolar macrophages. Blood 2015; 126: 1357-1366.

[16] Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN and Wynn TA. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 2014; 41: 14-20.

[17] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A and Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004; 25: 677-686.

[18] Buttari B, Profumo E, Segoni L, D'Arcangelo D, Rossi S, Facchiano F, Saso L, Businaro R, Iuliano L and Rigano R. Resveratrol counteracts inflammation in human M1 and M2 macrophages upon challenge with 7-oxo-cholesterol: potential therapeutic implications in atherosclerosis. Oxid Med Cell Longev 2014; 2014: 257543.

[19] Zhu J, Jia E, Zhou Y, Xu J, Feng Z, Wang H, Chen X and Li J. Interleukin-22 Secreted by NKp44+ Natural Killer Cells Promotes Proliferation of Fibroblast-Like Synoviocytes in Rheumatoid Arthritis. Medicine (Baltimore) 2015; 94: e2137.

[20] Li A, Gan Y, Wang R, Liu Y, Ma T, Huang M and Cui X. IL-22 Up-Regulates beta-Defensin-2 Expression in Human Alveolar Epithelium via STAT3 but Not NF-kappaB Signaling Pathway. Inflammation 2015; 38: 1191-1200.

[21] Ye Z, Li M, Mei Z, Zhen G and Zhang P. [The effects and mechanisms of interleukin-22 and interferon-gamma on epithelial-mesenchymal transition of pleural mesothelial cells].

Zhonghua Jie He He Hu Xi Za Zhi 2015; 38: 501-506.

[22] Yang C, Zhang DM, Song ZB, Hou YQ, Bao YL, Sun LG, Yu CL and Li YX. Protumoral TSP50 Regulates Macrophage Activities and Polarization via Production of TNF-alpha and IL-1beta, and Activation of the NF-kappaB Signaling Pathway. PLoS One 2015; 10: e0145095.

[23] Zhao XN, Li YN and Wang YT. Interleukin-4 regulates macrophage polarization via the MAPK signaling pathway to protect against atherosclerosis. Genet Mol Res 2016; 15.

[24] Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, Wagner RA, Greaves DR, Murray PJ and Chawla A. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell Metab 2006; 4: 13-24.

[25] Odegaard JI, Ricardo-Gonzalez RR, Red Eagle A, Vats D, Morel CR, Goforth MH, Subramanian V, Mukundan L, Ferrante AW and Chawla A. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. Cell Metab 2008; 7: 496-507.