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Priming of Toll-like receptor 4 pathway in mesenchymal stem cells increases expression of B cell activating factor

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ABSTRACT

Mesenchymal stem cells (MSCs) can be polarized into two distinct populations, MSC1 and MSC2, by activation of different Toll-like receptors (TLRs). TLR4-primed MSC1 expressed proinflammatory factors, whereas TLR3-primed MSC2 expressed suppressive factors. However, little is known about the function of TLRs on B lymphocyte-related immune modulation. In this study, we investigated the expression of B cell activating factor (BAFF), a member of the tumor necrosis factor ligand superfamily with notable stimulating activity on B cells, in human MSCs (hMSCs) and in murine MSCs (mMSCs) after activation of TLRs. BAFF was increasingly expressed in presence of TLR4 agonist (lipopolysaccharide, LPS), while TLR2 agonist (Zymosan) and TLR3-agonist (polyinocinic–polycytidykic acid, poly I:C) had no effect on BAFF expression. In addition, we demonstrated that signaling pathways of NF-κB, p38 MAPK, and JNK were involved in TLR4-primed BAFF expression. Our results suggested that TLR4 and downstream pathways in MSCs exert an important function in B lymphocyte-related immune regulation. Further defining a homogeneous population of MSCs should provide insight into MSC-based immune-modulating therapy.

1. Introduction

Bone marrow (BM) mesenchymal stem cells (MSCs) are multipotent non-hematopoietic stem cells that can differentiate into BM stromal cells, osteoblasts, adipocytes, chondrocytes, tenocytes, skeletal myocytes, neurons, and cells of visceral mesoderm [1]. MSCs have been studied for their immune-suppressive effect and have potentials for clinical applications, such as treating immune disorders. The immune-suppressive effect of MSCs is mediated by the secretion of soluble factors and by cell–cell contact-dependent regulation. After stimulated by IFN-γ, TNF-α and agonist Toll-like receptor 3 (TLR3), MSCs were able to produce indoleamine 2,3-dioxygenase (IDO) [2,3], prostaglandin E2 (PGE2) [4,5], tumor necrosis factor alpha stimulated gene-6 (TSG-6) [6], and nitricoxide (NO) [7] to inhibit T lymphocytes. Cell–cell contact-dependent mechanisms, including FAS/FASL [8], programmed death-1/programmed death ligand-1 [9], galectins [10], CD39-induced adenosine and Notch signaling [11] have been reported to be critical in MSC-related immune modulation.

TLRs play an important role in MSC-mediated immune-regulatory functions. They are recognized as a wide variety of pathogen-associated molecular patterns (PAMP) in bacteria, viruses, fungi, and some host-derived molecules [12]. Thirteen mammalian TLR analogs, including 10 in humans and 13 in mice, have been identified. TLR 1-6 are expressed at a higher level in adipose-derived mesenchymal stem cells (AD-MSCs) and BM-MSCs in humans and in mice [13]. Among them, the agonists of TLR3 and TLR4 in human MSCs (hMSCs) induce down-regulation of the of Notch ligand Jagged-1, and thus decrease the ability of MSCs to suppress allogeneic T cell proliferation [14]. In murine MSCs (mMSCs), an activation of TLR2 and TLR4 increased IL-6 secretion via NF-κB pathway [15]. On the other hand, TLR agonists-activated MSCs are able to increase B lymphocytes proliferation [16] and switch IgG production [17,18], suggesting a critical role in progression of B lymphocytes-relating immune disorders.

B cell-activating factor, BAFF, is a vital survival factor that supports generation, differentiation, maturation of B lymphocytes to maintain a functional B lymphocyte pool [18]. We previously reported that adipocytic differentiation of mMSCs promotes B lymphocytes proliferation by secretion of BAFF [19]. However,

Abbreviations: MSC, mesenchymal stem cells; BAFF, B cell activating factor; TLRs, Toll-like receptors; PAMP, pathogen-associated molecular patterns; Poly(I:C), polyinocinic–polycytidykic acid; LPS, lipopolysaccharide.

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little is known which TLR and related signaling pathway exert major function on BAFF secretion in MSCs.

In this study, we investigated the effect of TLR priming on BAFF expression in mMSCs and in hMSCs. We found that BAFF in hMSCs or in mMSCs was expressed at a higher level after TLR4-priming, indicating that TLR4 and a downstream pathway play a role in BAFF secretion and thus exert an important function in B lymphocyte-related immune regulation.

2. Materials and methods

2.1. Culture and differentiation of MSCs from human and mouse

Healthy human bone marrow samples were collected from eight patients (33–55 years old) from Huashan Hospital, Fudan University, who had subscribed a consent form. The patients were diagnosed as NK/T nasal lymphoma, hypersplenism, whose bone marrow examination excluded infiltration of malignant cells and other abnormalities. Human BM-MSCs were purified by the Percoll density gradient centrifugation method and were cultured in Dulbecco's modified Eagle's medium containing 4.0 mM of glucose (DMEM-LG, Hyclone, Beijing, China) supplemented with 10% fetal bovine serum (FBS, Hyclone, Beijing, China), 100 U/ml penicillin, and 100 U/ml streptomycin (Hyclone, Beijing, China), in a humidified atmosphere of 5% CO_2.

BALB/c mice at 6–8 weeks of age were purchased from the Slack Animal Center of Science Academy of China (Shanghai, China). All animals were bred and maintained under specific pathogen free conditions. Murine BM cells were flushed out of the tibia and femur by phosphate-buffered saline (PBS). The cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 3 days to allow attachment of adherent cells. Thereafter, medium was changed every other day. Passages were performed when cells obtained 80% confluence. After three passages, phenotype and differentiation assays were performed as previous report [19] and the cells were used for further experiments.

To stimulate adipogenic differentiation, MSCs were cultured in the adipogenic medium, DMEM-LG containing 10% FBS, 10^{-7} mol/l dexamethasone (Sigma), 50 mg/ml indomethacin (Sigma), 0.5 mM of 3-isobutyl-L-methyl-xanthine (Sigma), and 0.01 mg/ml of insulin (Sigma) for 14 days. Adipogenesis was detected by oil red O staining, and was confirmed by phase contrast microscopic observation.

To stimulate osteogenesis, 10^{-8} mM dexamethasone, 0.2 mM ascorbic acid-2-phosphate, and 10 mM beta-glycerophosphate were added into the basic medium, and the medium was changed twice weekly. Alkaline phosphatase (ALP) expression was examined at day 21. ALP staining was carried out using an ALP kit according to the manufacturer's instructions (Beyotime).

2.2. Flow cytometry analysis

Murine and human MSCs were harvested and analyzed by flow cytometry with a BD-FACS Calibur flow cytometer (BD Biosciences, San Jose, CA). Murine MSCs were stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD44, CD31, CD45, Scal-1 (stem cell antigen-1), phycoerythrin (PE)-conjugated anti-CD106 and APC-conjugated anti-CD34. Human MSCs were stained with FITC-conjugated anti-CD44, CD31, CD45, CD90, PE-conjugated anti-CD105, CD34, and APC-conjugated anti-CD90 (all from Biolegend, San Diego, CA, USA).

2.3. TLR priming

Zymosan (1 mg/ml, Sigma–Aldrich, MO), polyinocinic–polycytidykic acid (Poly(I:C), 1 mg/ml , InvivoGen, CA) and lipopolysaccharide (LPS, Sigma–Aldrich, MO) were used as the agonists for TLR2, TLR3 and TLR4, respectively. TLR-agonists were added to fresh growth medium and incubated with the cells for 48 h.

2.4. Real-time quantitative PCR analysis

Total RNA of mMSCs or hMSCs was isolated by Trizol reagent (invitrogen) according to the manufacturer's instructions. Amplification and detection of specific products were carried out in a StepOnePlus™ Real-Time PCR System (Life Technologies). Specific primers were used to amplify cDNA, for murine BAFF (5′-ACGGAGACGACACCTTCTTT-3′; 5′-GGCTTTCCCCATCTTTTAGTT-3′) and for human BAFF (5′-CAATCCAATCGGAGGGTA-3′; 5′-TGTTTGTTGATG TCCTGC-3′).

2.5. Blocking of TLR2 and TLR4

MSCs were seeded in basic medium in 6-well plates at a concentration of $3 \times 10^4/cm^2$, the medium was then added with 2 μg/ml of anti-TLR2 (functional grade purified, MTS510), anti-TLR4 (functional grade purified, 6C2) or isotype control antibody (all purchased from eBioscience) respectively for 30 min at 37 °C.

2.6. Western blotting analysis of BAFF expression

Western blot analysis was executed as the previous report [19]. Immunoreactivity was detected using the ImageQuant LAS 4000 mini (GE). Antibody against BAFF was purchased from Sigma–Aldrich (St. Louis, MO, USA). Antibody against β-Actin was purchased from Santa Cruz (San Diego, CA, USA). The results were analyzed using ImageJ software.

2.7. Immunocytochemistry analysis of NF-κB nuclear translocation in mMSCs

Murine MSCs were seeded on the slides, and stimulated by LPS for 4 h. The cells were fixed, permeabilized, and then incubated with the primary antibody to NF-κB (Cell Signaling Technology, Danvers, MA) overnight. The secondary antibody conjugated with Dylight was counterstained for 30 min at 37 °C. The slides were examined after stained with 6-diamidino-2-phenylindole (DAPI) and photographed using a confocal microscope (TCS SP5 AOBS, Leica Microsystems CMS GmbH, Mannheim, Germany).

2.8. Statistical analysis

Data were represented as mean ± SD. Multiple group comparisons were performed by one-way analysis of variance (ANOVA) followed by the Bonferroni procedure for comparison of means. Comparison between any two groups was analyzed by the two-tailed Student's *t*-test with GraphPad Prism4 Software. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Characterization and differentiation of MSCs

Non-hematopoietic BM stromal cells were separated based on plastic adhesion, and characterized by immunophenotypes and their differentiation potential. Murine MSCs were negative for CD45, CD31, or CD34, but mild positive for CD106 and strong positive for Sca-1 and CD44 (Fig. 1A). While human MSCs were also negative for CD45, CD31, or CD34, they were positive for CD105, CD90, and CD44 (Fig. 1B). The adherent cells grew as a spindle-shaped fibroblastic morphology after 2–3 passages. To determine

their multi-lineage potency, we induced MSC differentiation to osteogenic and adipogenic lineages. After culturing in osteogenic induction medium for 3 weeks, the cells became positive for Alkaline Phosphatase staining, indicating that our cultured MSCs had osteogenic differentiation potential (Fig. 1C). The adipogenic differentiation of MSCs was confirmed by oil red O staining after induction in adipogenic medium for 14 days (Fig. 1C). These data suggested that the cultured BM stromal cells possessed typical characteristics of MSCs.

Investigations on the effect of TLR activation on MSC differentiation raise a controversial issue, in which one report indicated that priming of TLR3 and TLR4 promotes the osteoblast differentiation [20], while the other report indicated that TLR2 activation decreases the multi-lineage differentiation capability of mMSCs [21]. To investigate the effects of TLR2 and TLR4 on mMSC differentiation, we induced MSC differentiation in presence of TLR2 agonist, zymosan (1 μg/mL), and TLR4 agonist, LPS (1 μg/ml), respectively. With the treatment of LPS or zymosan, the cells were positively stained oil red O and Alkaline Phosphatase, indicating that mMSCs retained their potential to differentiate into osteoclasts and adipocytes when TLR2 and TLR4 were activated (Fig. 1D and E).

3.2. TLR4 activation increased BAFF production in mMSCs and in hMSCs

To investigate whether TLR stimulation alters BAFF expression in MSCs, the expression of BAFF in mMSCs in response to different concentration of LPS was determined by real-time PCR analysis. BAFF expression was increased in a dose-dependent manner and peaked at the concentration of 1 μg/ml LPS (Fig. 2A). Compared with the result of TLR2 stimulation by 1 μg/ml zymosan, TLR4-activation increased BAFF expression by 2–3 folds, whereas TLR2-activation increased BAFF expression modestly (Fig. 2B and C).

To determine whether the agonists of TLRs influence the BAFF expression in hMSCs, hMSCs were treated with TLR2 agonist (zymosan), TLR3 agonist (poly(I:C)), or TLR4 agonist (LPS) for 48 h. BAFF expression in TLR4-stimulated hMSCs, at mRNA level and at protein level, was higher than that in TLR2-stimulated and TLR3-stimulated hMSCs (Fig. 2D and E). Taken together, these results indicated that an activation of TLR4, rather than TLR2 or TLR3, increased BAFF expression.

3.3. Blockade of TLR4 decreased BAFF expression

To further elucidate whether TLRs influence BAFF expression in a direct or indirect way, mMSCs were treated with anti-TLR4 or anti-TLR2 mAb to block the receptors before addition of LPS or zymosan. After the blockage of TLR4, BAFF expression was decreased by 53.2 ± 12.2% ($p < 0.05$), whereas TLR2 blockage only decreased BAFF expression by 23.5 ± 19.1% that had no significant difference compared to addition of zymosan only (Fig. 3). This indicated that TLR4, but not TLR2, regulated BAFF expression.

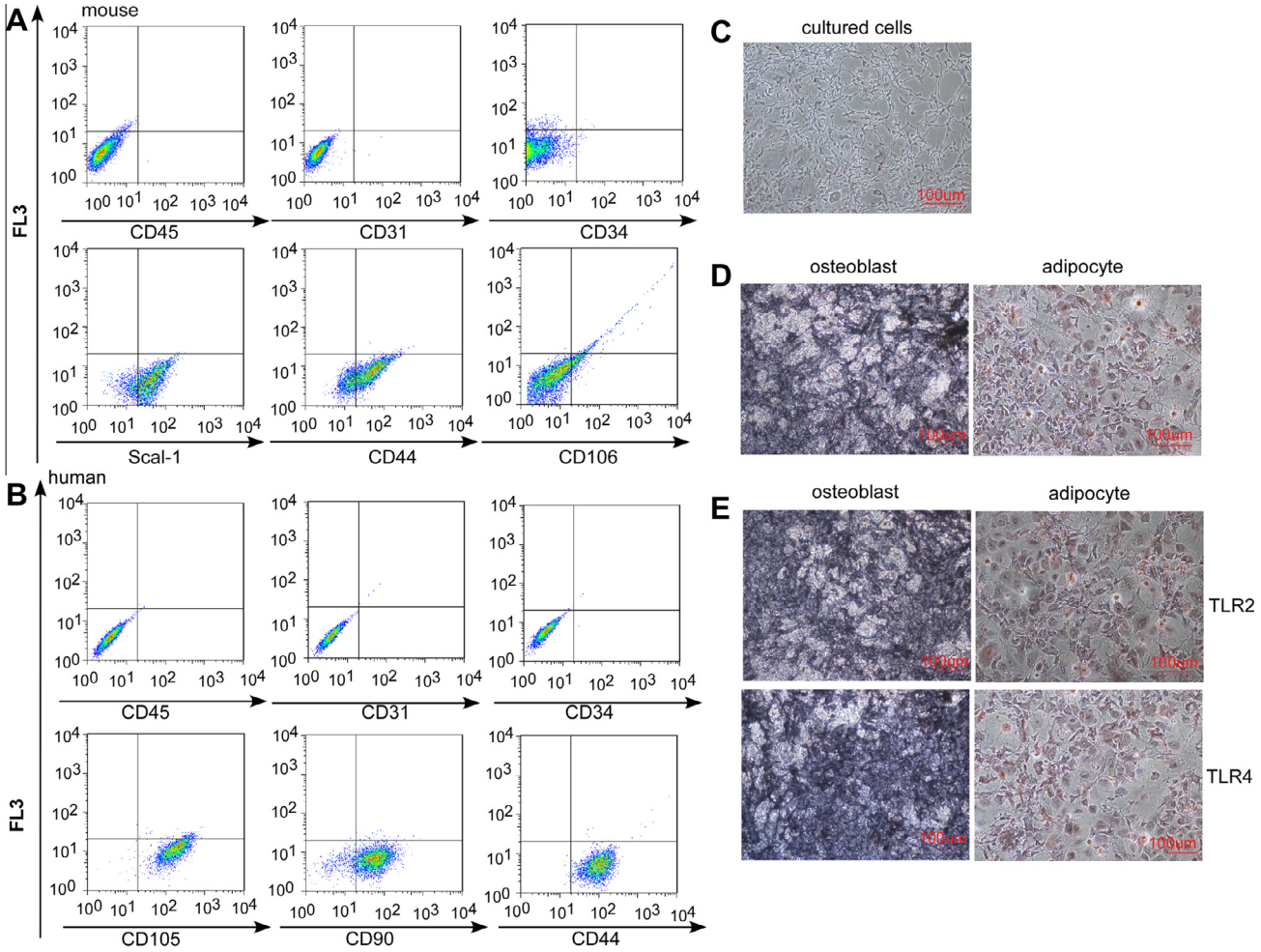

Fig. 1. Characterization of BM-MSCs. Immunophenotype of bone marrow-derived MSCs from mice (A) and humans (B) were analyzed by flow cytometry. (C) Cultured mMSCs showed a spindle-shaped fibroblastic morphology, Scale bar = 100 μm. (D) For osteogenic induction, the cells were stained for alkaline phosphatase at day 21. Scale bar = 100 μm. For adipogenic induction, the accumulated lipid vacuoles were stained with oil red O at day 14. Scale bar = 100 μm. (E) Murine MSCs were differentiated in presence of agonists of TLR2 or TLR4 for two weeks, and were stained by alkaline phosphatase and oil red O ($n = 3$).

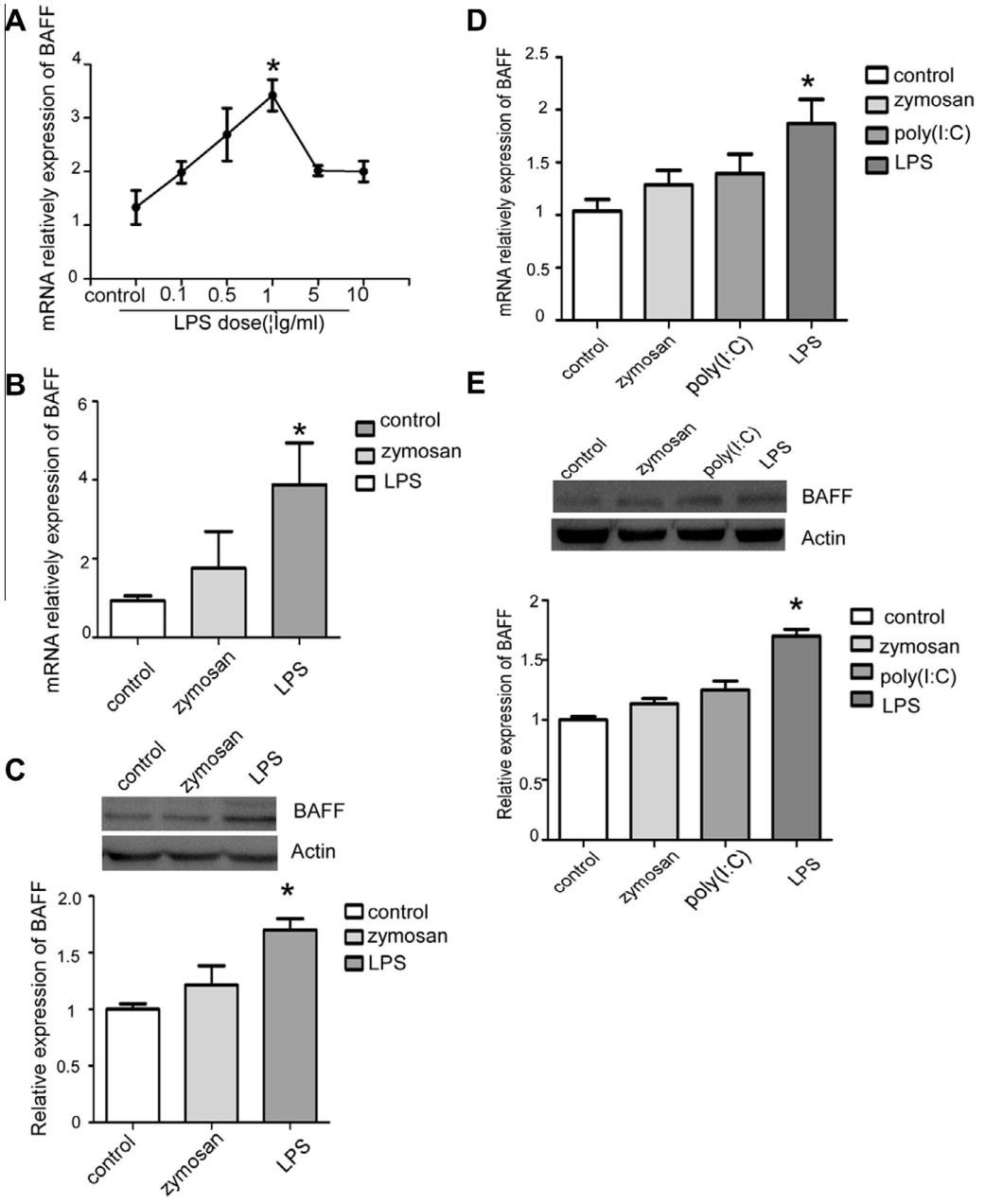

Fig. 2. TLR4-activation increased BAFF production in mMSCs and in hMSCs. (A) Murine MSCs were treated with LPS at the concentration of 0.1 μg/ml, 0.5 μg/ml, 1 μg/ml, 5 μg/ml, and 10 μg/ml for 48 h. The expression of BAFF was determined by real-time RT-PCR. (B) Murine MSCs were stimulated with zymosan (1 μg/ml) or LPS (1 μg/ml) for 48 h. The expression of BAFF was determined by real-time RT-PCR. (C) The BAFF protein expression in mMSCs was detected by western blot, and analyzed by ImageJ software. (D) Human MSC was stimulated with zymosan (1 μg/ml), poly(I:C) (1 μg/ml) or LPS (1 μg/ml) for 48 h. The expression of BAFF was determined by real-time RT-PCR. (E) The BAFF protein expression in hMSCs was detected by western blot, and analyzed by ImageJ software. Data were presented as the mean ± SD, and were compared to the control (*$p < 0.05$, $n = 5$).

3.4. NF-κB, JNK and p38 MAPK pathways regulated TLR4-related BAFF expression

It had been reported that NF-κB, JNK and p38 MAPK are related to TLRs and their downstream signaling pathways to trigger induction and secretion of various cytokines, chemokines and other inflammatory mediators. To determine whether NF-κB, JNK and p38 MAPK were involved in TLR4-related BAFF expression, specific inhibitors to NF-κB pathway (pyrrolidinedithiocarbamic acid, PDTC), JNK pathway (SP600125) and p38 MAPK pathway (SB203580) were added into cultured media of mMSCs before LPS stimulation. An addition of PDTC, SP600125 or SB203580 decreased BAFF expression by 90.4 ± 2.4%, 87.0 ± 7.2% and 97.9 ± 4.8%, respectively ($p < 0.05$) (Fig. 4A), indicating that these signaling pathways were involved in TLR4-regulated BAFF expression. In addition, we showed that NF-κB translocated into the nuclear after LPS stimulation by immunofluorescence analysis of NF-κB. The specific inhibitor to NF-κB signaling, PDTC, abrogated the p-NF-κB p65 transloaction (Fig. 4B).

4. Discussion

Two homogenous phenotypes of MSCs, MSC1 and MSC2, have been classified recently. The polarization of MSC1 or MSC2 depends on priming of TLRs on MSC surface [22]. After TLR3 activation, MSCs are induced into MSC2 phenotype and produce

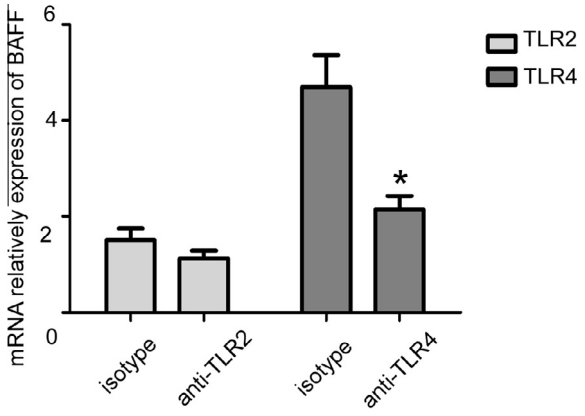

Fig. 3. Blockade of TLR4 decreased the BAFF expression. Murine MSCs were treated with anti-TLR2 or anti-TLR4 antibodies for 30 min at 37 °C before stimulated with zymosan or LPS, respectively. After cultured in presence of anti-TLR2 + zymosan or anti-TLR4 + LPS for 48 h, BAFF expression was determined by real-time RT-PCR. Data were presented as the mean ± SD, and were compared to the isotype control ($^*p < 0.05$, $n = 5$).

anti-inflammatory factors, including IDO, PGE2, NO, TGF-β, hepatocyte growth factor (HGF) and hemoxygenase (HO), to suppress T cell proliferation and favor proliferation of regulatory T cells (Tregs) [22]. However, the activation of TLR4 polarizes MSCs into a proinflammatory MSC1 phenotype to secret chemokines, including MIP-1a and MIP-1b, RANTES, CXCL9 and CXCL10, that recruit lymphocytes to the sites of inflammation and enhance T cell-mediated immune responses [23].

In addition, some sub-populations of MSCs have been characterized to respond differently to TLRs. For example, Nestin$^+$ MSCs respond to TLR4-activation by secretion of monocyte chemotactic protein-1 (MCP1) that promotes neutrophil migrating into tissues [24]. However, little is known about the relation of TLRs to B cell-mediated immune modulation.

BAFF has been proved to be a critical factor for the proliferation of B lymphocytes. In our previous study, we found that MSCs underwent adipogenic differentiation not only stimulate T lymphocytes, but also enhance B-lymphocyte proliferation by secreting BAFF [19]. In this study, we distinguished MSC sub-population types in BAFF expression to assess the effect of MSCs on immune modulating properties resulted from their heterogeneity. It had been demonstrated that TLR2, TLR3 and TLR4 are expressed at a higher level in hMSC [15], and TLR1-8 is expressed at a higher level in mMSCs [21]. TLR2 and TLR4 have been proved to affect the immune-modulation effects of mMSCs, whereas other TLRs have no effect on the immune-modulation functions of MSCs [25]. Therefore, we investigated the effects of TLR2, TLR3 and TLR4 on BAFF expression. Our data showed that TLR4-priming in hMSCs and in mMSCs increased BAFF expression, whereas a stimulation of TLR2 or TLR3 had no effect on BAFF expression. Our study of TLR blockage also indicated that TLR4-priming, rather than TLR2- or TLR3-priming, played an important role in BAFF expression, and thus regulated B-lymphocyte proliferation.

MSCs have been used for the treatment of graft-versus-host disease (GVHD) and other autoimmune diseases based on their immune-suppressive function [26]. Specific biomarkers of MSCs regarding the efficacy of treatment are different. TLR3-primed MSCs give rise to MSC2 phonotype, which produces anti-inflammatory factors, but not pro-inflammatory, and which has a potential in immune-suppressive treatment [22].

Compared to immune-suppressive functions of MSCs, the proinflammatory functions of MSCs are not extensively investigated and the results are often contradictory. An activation of TLR2 increases IL-6 production and the number of CD4$^+$ T cells, promoting the inflammatory reaction [21,25]. Some studies indicated that the stimulation of either TLR3-agonist or TLR4-agonist decreases the suppressive ability of MSCs on allogenic T cell proliferation, indicating that MSC1 and MSC2 all have pro-inflammatory function [14]. The inconsistence may due to the differences of MSC sources, MSC numbers, and incubating time of TLR-agonists during the induction period.

The proinflammatory capability under the stimulation of certain infectious molecules arouses potential dangers in clinical applications of MSCs [22]. In our study, TLR3-activation had no effect on the BAFF expression in hMSCs. However, TLR4-activation increased BAFF expression in MSCs and blockage of TLR4 pathway significantly abolished TLR4-induced BAFF expression, indicating that TLR4-primed MSC1 phenotype impacted on B cell proliferation. Our study elucidated potential mechanisms that NF-κB, JNK, P38 MAPM signaling pathways were involved in TLR4-related BAFF expression. Our results were consistent with previous the studies, in which TLR4-primed MSC1 promote T lymphocyte proliferation and produce proinflammatory factors [22].

In conclusion, MSCs are gaining more and more attention due to their immune-modulating functions, such as involving in immune suppression. However, the heterogeneity of MSCs hinders further

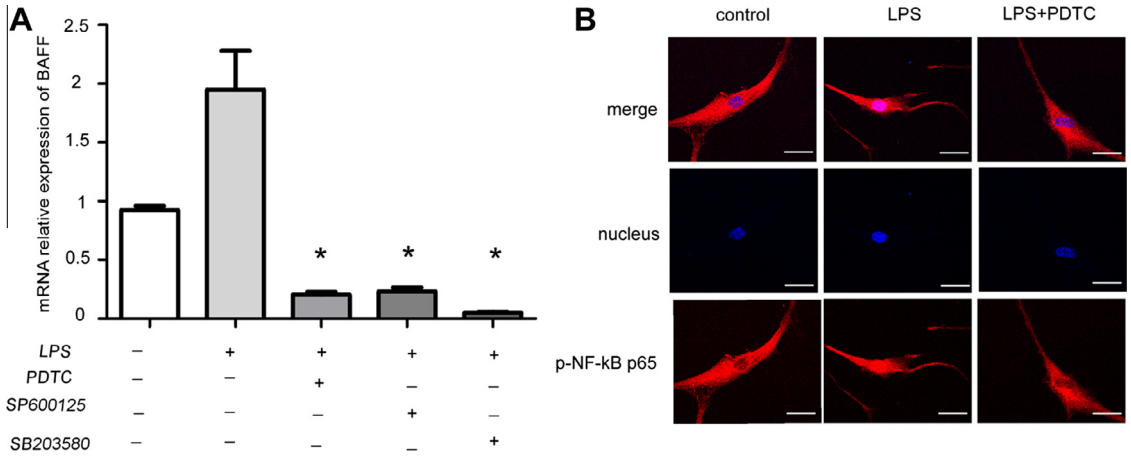

Fig. 4. NF-κB, JNK and p38 MAPK pathways were involved in the regulation of TLR4-related BAFF expression. (A) The MSCs were cultured in presence of PDTC (a NF-κB inhibitor), SP600125 (a JNK inhibitor), or SB203580 (a p38 MAPK inhibitor) at the concentration of 1 μM for 30 min. The cells were then incubated with LPS for additional 48 h. The expression of BAFF was determined by real-time RT-PCR. Data were presented as the mean ± SD, and were compared to the group treated only with LPS ($^*p < 0.05$, $n = 5$). (B) NF-κB nuclear translocation was analyzed by immunofluorescence on untreated mMSCs (left), mMSCs stimulated with LPS for 4 h (middle), and mMSCs treated with PDTC and LPS for 4 h (right), scale bar = 15 μm.

clarification of regulating mechanism, and results in some uncertainty during clinical treatment. Expression of proinflammatory factors under certain infectious status also elucidates more information about the immune-modulation capabilities of MSCs. Whether the proinflammatory function of MSCs aggravates the diseases needs to be further clarified. In this study, we found that TLR4-primed MSC1 enhanced BAFF expression, whereas TLR3-primed MSC2 exerted no influence on BAFF expression. It supports a growing evidence that a homogeneous population of MSCs will be important to their application in stem cell-based therapy, which should be improved by future investigations of identification and characterization of specific biomarkers related to different immune modulating function in MSCs.

Acknowledgments

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References

[1] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.
[2] M. Krampera, L. Cosmi, R. Angeli, A. Pasini, F. Liotta, A. Andreini, V. Santarlasci, B. Mazzinghi, G. Pizzolo, F. Vinante, P. Romagnani, E. Maggi, S. Romagnani, F. Annunziato, Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells, Stem Cells 24 (2006) 386–398.
[3] K. English, F.P. Barry, C.P. Field-Corbett, B.P. Mahon, IFN-gamma and TNF-alpha differentially regulate immunomodulation by murine mesenchymal stem cells, Immunol. Lett. 110 (2007) 91–100.
[4] J.M. Ryan, F. Barry, J.M. Murphy, B.P. Mahon, Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells, Clin. Exp. Immunol. 149 (2007) 353–363.
[5] S. Aggarwal, M.F. Pittenger, Human mesenchymal stem cells modulate allogeneic immune cell responses, Blood 105 (2005) 1815–1822.
[6] R.H. Lee, A.A. Pulin, M.J. Seo, D.J. Kota, J. Ylostalo, B.L. Larson, L. Semprun-Prieto, P. Delafontaine, D.J. Prockop, Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6, Cell Stem Cell 5 (2009) 54–63.
[7] K. Sato, K. Ozaki, I. Oh, A. Meguro, K. Hatanaka, T. Nagai, K. Muroi, K. Ozawa, Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells, Blood 109 (2007) 228–234.
[8] K. Akiyama, C. Chen, D. Wang, X. Xu, C. Qu, T. Yamaza, T. Cai, W. Chen, L. Sun, S. Shi, Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis, Cell Stem Cell 10 (2012) 544–555.
[9] A. Augello, R. Tasso, S.M. Negrini, A. Amateis, F. Indiveri, R. Cancedda, G. Pennesi, Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway, Eur. J. Immunol. 35 (2005) 1482–1490.
[10] M. Sioud, New insights into mesenchymal stromal cell-mediated T-cell suppression through galectins, Scand. J. Immunol. 73 (2011) 79–84.
[11] Y.P. Li, S. Paczesny, E. Lauret, S. Poirault, P. Bordigoni, F. Mekhloufi, O. Hequet, Y. Bertrand, J.P. Ou-Yang, J.F. Stoltz, P. Miossec, A. Eljaafari, Human mesenchymal stem cells license adult CD34+ hemopoietic progenitor cells to differentiate into regulatory dendritic cells through activation of the Notch pathway, J. Immunol. 180 (2008) 1598–1608.
[12] B. Beutler, Inferences, questions and possibilities in Toll-like receptor signalling, Nature 430 (2004) 257–263.
[13] O. DelaRosa, E. Lombardo, Modulation of adult mesenchymal stem cells activity by toll-like receptors: implications on therapeutic potential, Mediators Inflamm. 2010 (2010) 865601.
[14] F. Liotta, R. Angeli, L. Cosmi, L. Fili, C. Manuelli, F. Frosali, B. Mazzinghi, L. Maggi, A. Pasini, V. Lisi, V. Santarlasci, L. Consoloni, M.L. Angelotti, P. Romagnani, P. Parronchi, M. Krampera, E. Maggi, S. Romagnani, F. Annunziato, Toll-like receptors 3 and 4 are expressed by human bone marrow-derived mesenchymal stem cells and can inhibit their T-cell modulatory activity by impairing Notch signaling, Stem Cells 26 (2008) 279–289.
[15] S.L. Tomchuck, K.J. Zwezdaryk, S.B. Coffelt, R.S. Waterman, E.S. Danka, A.B. Scandurro, Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses, Stem Cells 26 (2008) 99–107.
[16] I. Rasmusson, K. Le Blanc, B. Sundberg, O. Ringden, Mesenchymal stem cells stimulate antibody secretion in human B cells, Scand. J. Immunol. 65 (2007) 336–343.
[17] C. Pontikoglou, M.C. Kastrinaki, M. Klaus, C. Kalpadakis, P. Katonis, K. Alpantaki, G.A. Pangalis, H.A. Papadaki, Study of the quantitative, functional, cytogenetic, and immunoregulatory properties of bone marrow mesenchymal stem cells in patients with B-cell chronic lymphocytic leukemia, Stem Cells Dev. 22 (2013) 1329–1341.
[18] F. Loder, B. Mutschler, R.J. Ray, C.J. Paige, P. Sideras, R. Torres, M.C. Lamers, R. Carsetti, B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals, J. Exp. Med. 190 (1999) 75–89.
[19] H. Wang, T. Chen, T. Ding, P. Zhu, X. Xu, L. Yu, Y. Xie, Adipogenic differentiation alters the immunoregulatory property of mesenchymal stem cells through BAFF secretion, Hematology 16 (2011) 313–323.
[20] E. Lombardo, O. DelaRosa, P. Mancheno-Corvo, R. Menta, C. Ramirez, D. Buscher, Toll-like receptor-mediated signaling in human adipose-derived stem cells: implications for immunogenicity and immunosuppressive potential, Tissue Eng. Part A 15 (2009) 1579–1589.
[21] M. Pevsner-Fischer, V. Morad, M. Cohen-Sfady, L. Rousso-Noori, A. Zanin-Zhorov, S. Cohen, I.R. Cohen, D. Zipori, Toll-like receptors and their ligands control mesenchymal stem cell functions, Blood 109 (2007) 1422–1432.
[22] R.S. Waterman, S.L. Tomchuck, S.L. Henkle, A.M. Betancourt, A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype, PLoS One 5 (2010) e10088.
[23] W. Li, G. Ren, Y. Huang, J. Su, Y. Han, J. Li, X. Chen, K. Cao, Q. Chen, P. Shou, L. Zhang, Z.R. Yuan, A.I. Roberts, S. Shi, A.D. Le, Y. Shi, Mesenchymal stem cells: a double-edged sword in regulating immune responses, Cell Death Differ. 19 (2012) 1505–1513.
[24] C. Shi, T. Jia, S. Mendez-Ferrer, T.M. Hohl, N.V. Serbina, L. Lipuma, I. Leiner, M.O. Li, P.S. Frenette, E.G. Pamer, Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands, Immunity 34 (2011) 590–601.
[25] J. Lei, Z. Wang, D. Hui, W. Yu, D. Zhou, W. Xia, C. Chen, Q. Zhang, Z. Wang, Q. Zhang, A.P. Xiang, Ligation of TLR2 and TLR4 on murine bone marrow-derived mesenchymal stem cells triggers differential effects on their immunosuppressive activity, Cell Immunol. 271 (2011) 147–156.
[26] F. Dazzi, M. Krampera, Mesenchymal stem cells and autoimmune diseases, Best Pract. Res. Clin. Haematol. 24 (2011) 49–57.