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Angiotensin-(1–7) enhances angiotensin II induced phosphorylation of ERK1/2 in mouse bone marrow-derived dendritic cells

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ABSTRACT

It is well known that angiotensin-(1–7) (Ang-(1–7)) counterbalances vasoconstrictive and proliferative functions of angiotensin II (Ang II), some of those actions are via inhibition of Ang II induced activation of mitogen-activated protein kinases(MAPK). This study investigated the effects of Ang-(1–7) on Ang II-mediated cell signaling pathways in mouse bone marrow-derived dendritic cells (DC). The expression of receptor Mas and angiotensin-converting enzyme-related carboxypeptidase (ACE2) mRNA was examined by reverse transcription-polymerase chain reaction (RT-PCR); activation of MAPK was detected by immunoblotting after incubation of dendritic cells with Ang II in the presence or absence of Ang-(1–7), valsartan, PD123319, and D-Ala^7-Ang-(1–7). Ang II rapidly (5 min, 10^{-7} mol/L) stimulated phosphorylation of extracellular signal-related kinase (ERK1/2); this effect was partially inhibited by Ang II type 1 (AT1) receptor antagonist valsartan and significantly attenuated by Ang II type 2 (AT2) receptor antagonist PD123319. Ang-(1–7) alone also induced phosphorylation of ERK1/2; co-treatment of Ang-(1–7) and Ang II markedly enhanced ERK1/2 phosphorylation, the enhancement was eliminated by the Ang-(1–7) receptor antagonist D-Ala^7-Ang-(1–7). Both Ang-(1–7) and Ang II had no effect on p38 and c-Jun N-terminal kinase (JNK) phosphorylation. In conclusion, Ang II stimulates ERK1/2 phosphorylation via AT2 receptor in mouse DC, Ang-(1–7) enhances this effect. Generation of Ang-(1–7) by DC could thereby counteract on the pro-inflammatory function of locally generated Ang II.

1. Introduction

Dendritic cells (DC) are potent antigen-presenting cells, which play a central role in the innate and acquired immunity. Whether in human or mouse, after differentiated from progenitors, DC home to various tissues, where they reside in immature state and serve as sentinel screening foreign antigens or signals that indicate infection or inflammation (Stockwin et al., 2000; Banchereau and Steinman, 1998; Banchereau et al., 2000; Théry and Amigorena, 2001). Immature DC actively capture and process antigens, and in response to maturation-inducing stimuli such as bacterial products or inflammatory cytokines, they enter a maturation/activation program. During this process, DC leave peripheral tissues and migrate to the secondary lymphoid organs, activate the growth and differentiation of naive T cells, thereby initiating immune responses and inflammatory processes in human and mouse (Lanzavecchia and Sallusto, 2001; Guermonprez et al., 2002).

There is now a novel concept about the rennin-angiotensin system (RAS), which has been established in human, mouse, and rat. The concept says that the RAS has two axes, the angiotensin-converting enzyme (ACE)-angiotensin (Ang) II-Ang II type (AT) 1 receptor axis and the angiotensin-converting enzyme-related carboxypeptidase (ACE2)-Ang-(1–7)-Mas axis (Santos et al., 2000, 2003; Ferrario, 2002; Roks et al., 1999; Donoghue et al., 2000). The former axis performs the vasoconstrictive, proliferative and pro-inflammatory functions through the major player Ang II, the latter axis always counteracts on the effects of the former through the major effector, a heptapeptide Ang-(1–7). ACE2 actively converts Ang-(1–10) to Ang-(1–9), which is directly transformed to Ang-(1–7) by ACE; ACE2 also directly cleaves Ang II to form Ang-(1–7) (Donoghue et al., 2000). Ang-(1–7) potentiates the hypotensive effects of bradykinin, and increases the release of prostacyclin and nitric oxide directly or indirectly through the unique G protein-coupled receptor Mas, thereby elicits vasodilator, antiproliferative, natriuretic, diuretic actions and improves cardiac function (Santos et al., 2000, 2003; Ferrario, 2002). With the

Abbreviations: Ang, angiotensin; MAPK, mitogen-activated protein kinases; ACE2, angiotensin-converting enzyme-related carboxypeptidase; RT-PCR, reverse transcription-polymerase chain reaction; ERK1/2, extracellular signal-related kinase; AT1 receptor, Ang II type 1 receptor; AT2 receptor, Ang II type 2 receptor; JNK, c-Jun N-terminal kinase; DC, dendritic cells; Val, valsartan; PD, PD123319; D-Ala^7, D-Ala^7-Ang-(1–7); RAS, rennin-angiotensin system; ACE, angiotensin-converting enzyme.

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counteractions, Ang-(1–7) balances the two axes and restores the physiological homeostasis systemically and locally.

The mitogen-activated protein kinase (MAPK) signaling pathway is a highly conserved pathway of serine/threonine kinases which consists of at least three broad families (p38, extracellular signal-related kinase (ERK1/2), and c-Jun N-terminal kinase (JNK)). By transducing signals from the cell surface to the nucleus, the MAPK family regulates diverse cellular functions, including cell proliferation, differentiation, and survival. In the maturation process of human or mouse DC, activation of MAPK plays a crucial role (Nakahara et al., 2004; Arrighi et al., 2001; Yanagawa et al., 2002). As a well-known activator of this signaling pathway, Ang II stimulates several cellular responses by activating p38, ERK1/2, and JNK in many species (including human and mouse) cell types, such as cardiac fibroblasts, peritoneal mesothelial cells, mesangial cells, neurite cells and macrophages (Scheuren et al., 2002; Kiribayashi et al., 2005; Zhang et al., 2005; Gendron et al., 1999; Wang et al., 2008). Although it was reported that Ang-(1–7) can inhibit the activation of MAPK stimulated by Ang II in several rat cell types and human and mouse DC express ACE, Ang II, AT1 and AT2 receptors (Su et al., 2006; Tallant et al., 2005; Lapteva et al., 2001; Nahmod et al., 2003), whether DC have Mas receptor and Ang-(1–7) can attenuate the Ang II-induced MAPK phosphorylation in DC remains to be elucidated. In this study, we examined the effects of Ang-(1–7) on Ang II-mediated MAPK signaling pathways in mouse bone marrow-derived dendritic cells.

2. Materials and methods

2.1. Animals

C57BL/6 mice (6–8-week-old females) were purchased from animal center of Zhejiang University and kept in a pathogen free laboratory in the first affiliated hospital. All animal experiments were approved by the Committee on Ethics of Animal Experiments Zhejiang University and were conducted according to the guideline for animal experiments of Zhejiang University.

2.2. Reagents and antibodies

All culture media including RPMI1640 containing L-glutamine, fetal bovine serum, and nonessential amino acids were purchased from Invitrogen, Gibco. Recombinant mouse GM-CSF (rmGM-CSF) and IL-4 (rmIL-4) were obtained from PeproTechAsia. Phycoerythrin (PE)-labeled monoclonal antibody for mouse CD11c and relative isotype were obtained from eBioscience. M-MLV Reverse Transcriptase cDNA synthesis kit was from Promega. Ang II, Ang-(1–7), and PD123319 were obtained from Sigma–Aldrich. Valsartan was a kindly present from MSD China. D-Ala^7-Ang-(1–7) was purchased from GenScript Corp. Cell lysis buffer kit was obtained from Beyotime Institute of Biotechnology. Antiphosphospecific antibodies to p38, ERK1/2 and antibodies to total p38, ERK1/2 were from Cell Signaling Technology. Antibodies to phosphorylated and total JNK were from Santa Cruz Biotechnology. Enhanced chemiluminescence kit was obtained from Biological Industries.

2.3. Cell preparation

Bone marrow-derived dendritic cells were generated from C57BL/6 mice as described previously (Inaba et al., 1992; Lutz et al., 1999; Son et al., 2002), with minor modifications. Briefly, bone marrow mononuclear cells were prepared from mouse femur and tibia bone marrow suspensions by depletion of red cells and allowed to adhere for 2 h in a density of 1×10^6 mL^{-1}, 2 mL/well in 6-well plates in RPMI1640 at 37 °C, 5% CO_2, then the RPMI1640 with suspended cells were drained and the complete media (CM, RPMI 1640 containing 2 mM L-glutamine, supplemented with 10% fetal bovine serum, 1% of nonessential amino acids, 20 ng/mL rmGM-CSF and 10 ng/mL rmIL-4 were added in 2 mL/well. The cultures were usually fed every 2 d by adding fresh complete media in 1 mL/well. To isolate the DC population, the cells were collected and suspended in 2–4 mL of warm CM, the same volume of 30% (v/v) iopamidol in CM was underlaid and centrifugated at $1200 \times g$ for 20 min at room temperature. After centrifugation, cells in the interface were collected and washed with CM three times and were subjected to phenotypic analysis by flow cytometry, the resulting population containing ≥90% CD11c$^+$ cells was used on days 8–10 without additional purification.

2.4. Reverse transcription-polymerase chain reaction

Reverse transcription-polymerase chain reaction (RT-PCR) was performed to demonstrate the presence of mRNA for the putative receptor for Ang-(1–7), the *mas*1 oncogene, and the Ang-(1–7) forming enzyme ACE2 in mouse bone marrow-derived dendritic cells. Briefly, RNA (2 μg) was isolated from DC and the same mouse cardiac myocytes. The RNA was reverse transcribed, denatured at 94 °C for 4 min, and then subject to 35 cycles of PCR at 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s (Ang-(1–7) receptor), 35 cycles of PCR at 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s (ACE2), followed by extension at 72 °C for 7 min, respectively. The selected primers designed for mouse Ang-(1–7) receptor and ACE2 were as follows, upstream 5′-AGGGTGACTGACTGAGTTTGG-3′ and downstream 5′-GAAGGTAAGAGGACAGGAGC-3′ for Ang-(1–7) receptor, upstream 5′-TGGGCAAACTCTATGCTGA-3′ and downstream 5′-TTCATTGGCTCCGTTTCTTA-3′ for ACE2. Products were identified by running samples on agarose gels stained with ethidium bromide.

2.5. Western blot analysis

Dendritic cells were incubated for various times (up to 60 min) and various concentrations (up to 10^{-6} mol/L) with Ang II, followed by immunoblot assays for phosphorylated MAPK. In certain experiments, cells were pretreated with either 10^{-7} mol/L Ang-(1–7), 10^{-6} mol/L valsartan, 10^{-6} mol/L PD123319 or 10^{-5} mol/L D-Ala^7-Ang-(1–7) for 30 min prior to application of Ang II. The phosphorylation state of the MAPK p38, ERK 1/2 and JNK was measured by Western blotting as described (Su et al., 2006; Zimpelmann and Burns, 2001). Briefly, cells were lysed using a commercial lysis buffer kit according to the manufacturer's instructions. After quantification of proteins, equal amounts of protein lysates (40 μg) were mixed with loading buffer consisting of 10% glycerol, 50 mM DTT, and 0.1% (w/v) bromophenol blue, boiled for 5 min, followed by centrifugation at $12{,}000 \times g$ for 5 min to remove insoluble debris, and then run on 10% sodium dodecyl sulfate-polyacrylamide gels and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat milk in PBS containing 0.1% Tween-20 (PBS-T) for 1 h at room temperature. The membranes were then incubated overnight at 4 °C with a 1:1000 dilution of antiphosphospecific antibodies to p38, ERK1/2, and JNK. Membranes were then incubated with 1:2000 dilution of horseradish peroxidase-conjugated secondary antibody, and then washed. Phosphorylated proteins were detected by enhanced chemiluminescence on film. Prestained standards were used as molecular weight markers. To control for protein loading, all membranes were stripped and re-probed with antibodies to unphosphorylated p38, ERK1/2, and JNK. Signals for phosphorylated MAPK proteins on Western blots were quantified by densitometry and corrected for unphosphorylated protein lev-

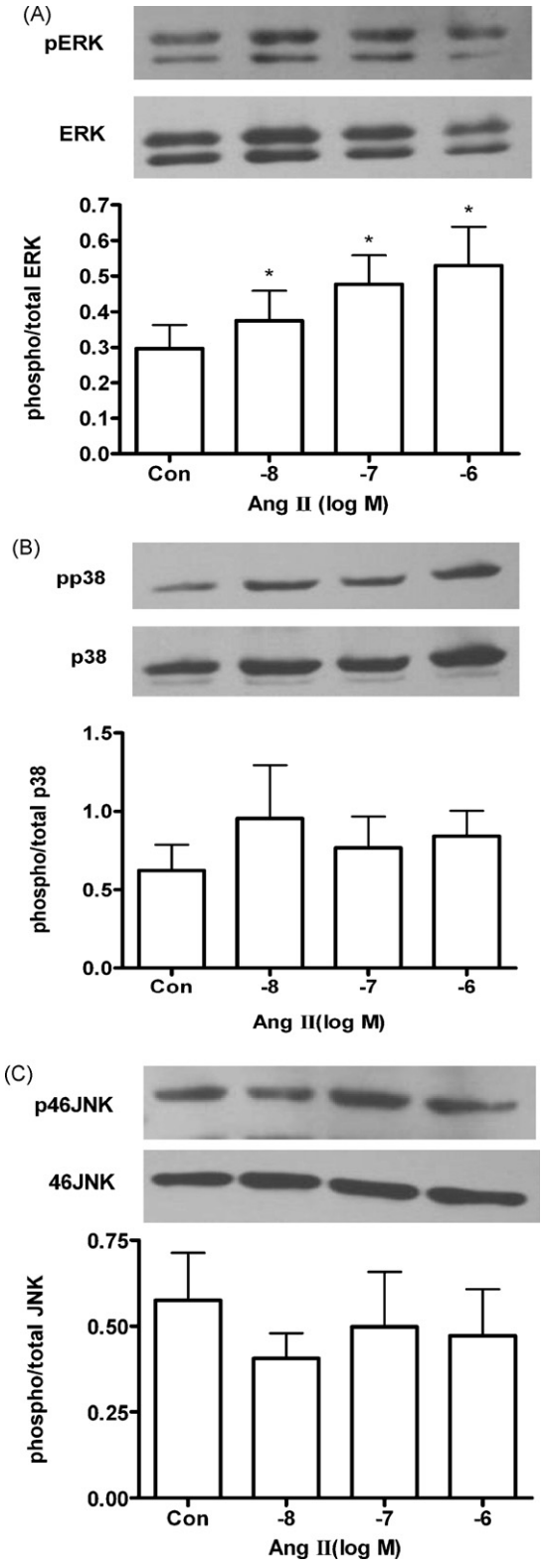

Fig. 1. Ang II induces concentration-dependent phosphorylation of ERK1/2 in mouse bone marrow-derived DC. The effect of Ang II (10^{-8} to 10^{-6} mol/L) on ERK1/2, p38 and JNK phosphorylation is shown, with a representative Western blot depicted above, respectively. Cells were treated with Ang II at the indicated concentrations for 5 min, and Western analysis was performed for phosphorylated MAPK and total MAPK proteins. Ang II had no significant effect on the amount of total MAPK. Values are means ± S.E.M. (A) (*) denotes $P < 0.05$ compared with control (Con) ($n = 4$); (B and C) P = NS ($n = 4$).

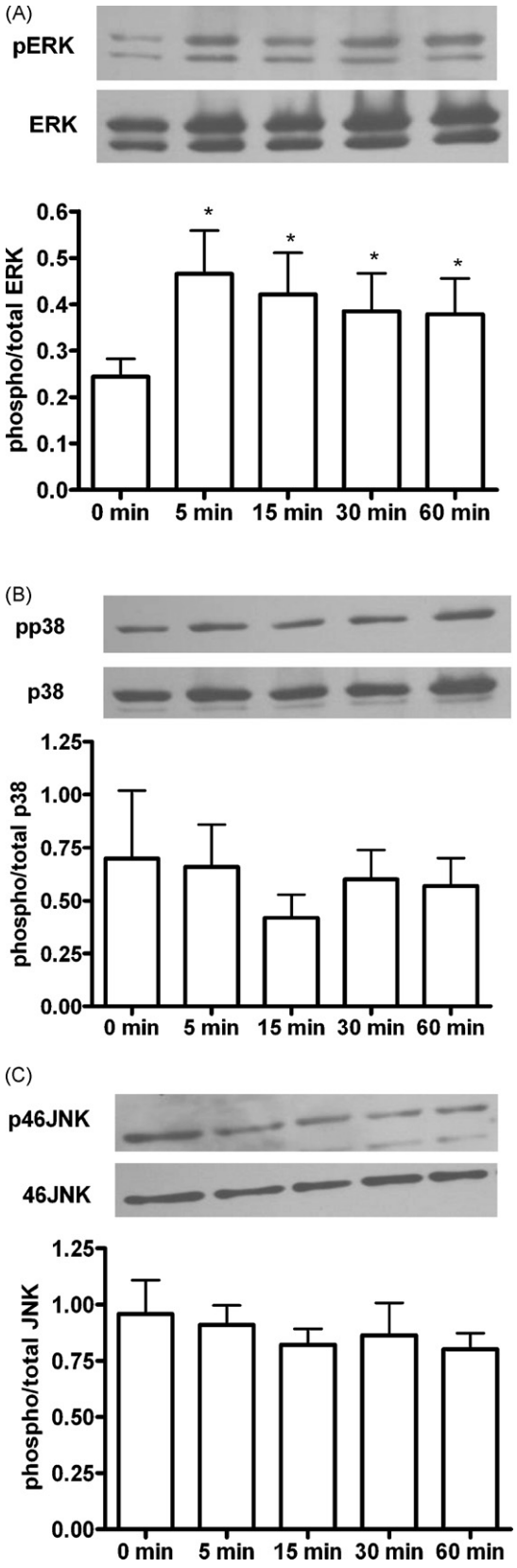

Fig. 2. Ang II rapidly induces phosphorylation of ERK1/2 in mouse bone marrow-derived DC. The effect of Ang II on MAPK phosphorylation is shown, with a representative Western blot depicted above, respectively. Cells were treated with Ang II at 10^{-7} mol/L for 0, 5, 15, 30, 60 min, and Western analysis was performed for phosphorylated MAPK and total MAPK protein. Values are means ± S.E.M. (A) (*) denotes $P < 0.05$ compared with 0 min ($n = 4$); (B and C) P = NS ($n = 4$).

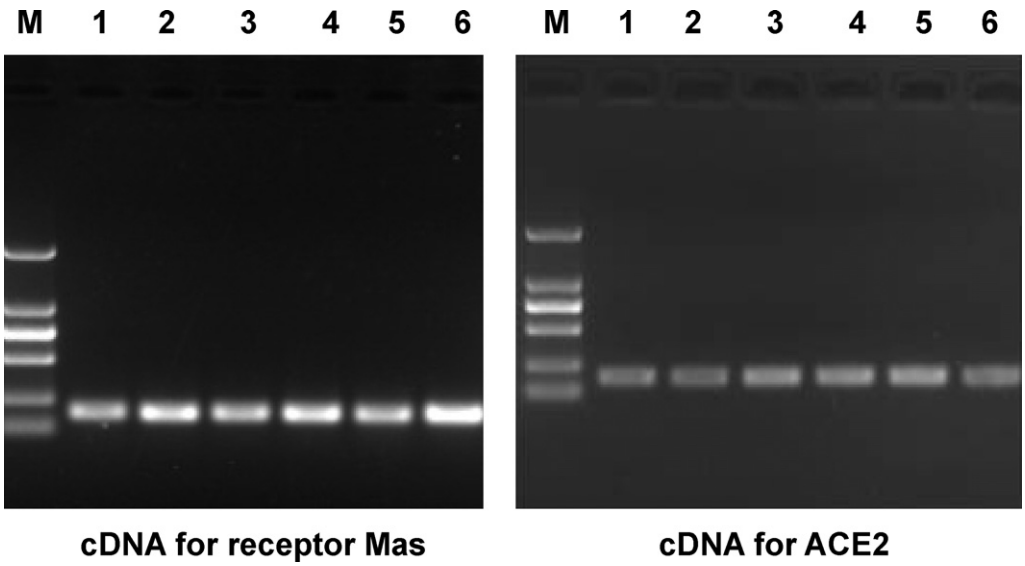

Fig. 3. Expression of Mas receptor and ACE2 in mouse bone marrow-derived DC. DC from different mouse express Mas receptor and ACE2 mRNA. M: DL2000 DNA marker; the bands indicate 100, 250, 500, 750, 1000, 2000 bp from lower to upper. Bands 1–3 represent the cDNA from cardiac myocytes; bands 4–6 represent the cDNA from DC. Data represent one of three experiments with similar results.

els, using an image-analysis software program. For ERK, graphic data show the p42 signal, while for JNK, graphic data indicate the p46 signal. Similar effects were observed on p44 ERK.

2.6. Statistical analysis

Results are presented as mean ± S.E.M. Comparisons between two groups were performed using Student's *t*-test of SPSS 15.0. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Ang II phosphorylates p42/p44 MAPK but not p38 and JNK

In primary cultures of mouse bone marrow-derived DC incubated with Ang II for 5–60 min, significant concentration-dependent increased phosphorylation of ERK1/2 was detected, with peak stimulation at 5-min incubation with Ang II 10^{-7} mol/L (relative expression: Fig. 2: pERK1/2: 0.46 ± 0.09, $P < 0.05$ versus 0 min 0.24 ± 0.04); there were no effects of Ang II on nonphosphorylated MAPK and phosphorylation of p38 and JNK observed (Figs. 1 and 2, $n = 4$).

3.2. Mouse bone marrow-derived dendritic cells express mRNA for the Mas receptor and ACE2

To determine if DC express mRNA for the Mas receptor and Ang-(1–7) forming enzyme ACE2, RT-PCR was performed on RNA isolated from these cells, the RNA from the same mouse cardiac myocytes served as positive control. In three separate experiments, RT-PCR generated a single band corresponding to the expected 175 bp product for the Mas receptor cDNA and a 213 bp product for ACE2 cDNA in mouse bone marrow-derived DC, respectively (Fig. 3).

3.3. Ang-(1–7) enhances Ang II-stimulated phosphorylation of ERK 1/2

Pretreatment with 10^{-7} mol/L Ang-(1–7) for 30 min significantly promoted phosphorylation of ERK 1/2 induced by Ang II (10^{-7} mol/L) (relative expression: pERK1/2: 1.20 ± 0.20, $P < 0.05$ versus Ang II 0.91 ± 0.13); more interestingly, pre-incubation with AT2 receptor antagonist PD123319 (10^{-6} mol/L) almost completely eliminated Ang II-stimulated phosphorylation of ERK 1/2 (relative expression: 0.52 ± 0.08, $P < 0.01$ versus Ang II 0.91 ± 0.13), yet pre-incubation with AT1 receptor antagonist valsartan (10^{-6} mol/L) only partially inhibited the stimulation (relative expression: 0.72 ± 0.05, P = NS versus Ang II 0.91 ± 0.13); no effect of Ang-(1–7) plus Ang II on p38 and JNK phosphorylation were observed (Fig. 4, $n = 6$).

3.4. D-Ala^7-Ang-(1–7) reverses the enhancing effect of Ang-(1–7) on Ang II-stimulated ERK1/2 phosphorylation

Incubation of mouse bone marrow-derived DC with Ang-(1–7) (10^{-7} mol/L) alone also induced ERK1/2 phosphorylation (relative expression: pERK1/2: 0.57 ± 0.05, $P < 0.01$ versus control 0.35 ± 0.04); the selective Ang-(1–7) receptor antagonist, D-Ala^7-Ang-(1–7) (10^{-5} mol/L), reversed the promoting effect of Ang-(1–7) on Ang II-stimulated ERK1/2 phosphorylation (0.63 ± 0.07, $P < 0.01$ versus Ang II + Ang-(1–7) 0.95 ± 0.08); D-Ala^7-Ang-(1–7) (10^{-5} mol/L) alone had no effect on ERK1/2 phosphorylation; neither Ang-(1–7) nor D-Ala^7-Ang-(1–7) had effect on phosphorylation of p38 and JNK (Fig. 5, $n = 6$).

4. Discussion

The present study demonstrated that Ang II rapidly induced phosphorylation of ERK1/2, but had no effect on phosphorylation of p38 and JNK in mouse bone marrow-derived DC. This effect was completely prevented by incubation with AT2 receptor antagonist PD123319 and partially inhibited by AT1 receptor antagonist valsartan, suggesting that AT2 receptor is the major role in mediating this response in DC. Since ERK1/2 signaling pathway positively regulates inflammatory cytokine production by DC, and does not affect or negatively regulates phenotypic and functional maturation of DC in human and mouse (Nakahara et al., 2006; Agrawal et al., 2006), this result may partially explain why pre-incubation of human monocyte DC with Ang II can only increase the production of TNF-α and IL-6 in the experiment performed by Lapteva et

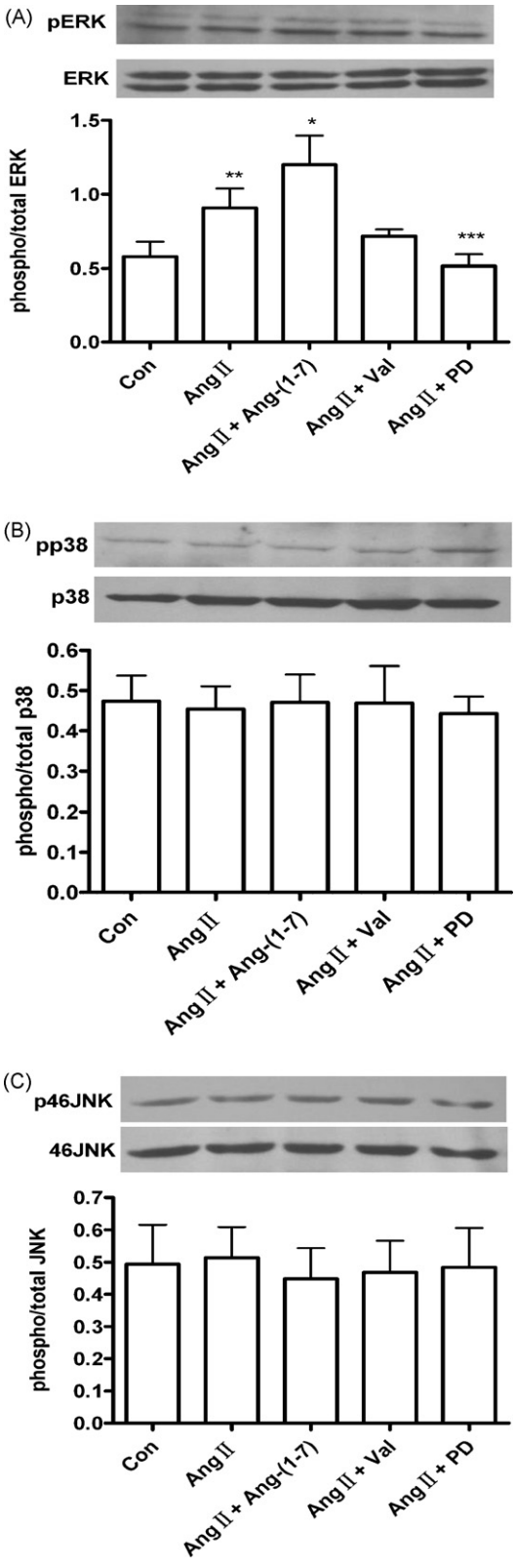

Fig. 4. Ang-(1–7) enhances Ang II-stimulated phosphorylation of ERK 1/2. Shown is the effect of pretreatment of mouse bone marrow-derived DC for 30 min with Ang-(1–7) (10^{-7} mol/L), the AT1 receptor antagonist valsartan (Val, 10^{-6} mol/L), or the AT2 receptor antagonist PD123319 (PD, 10^{-6} mol/L), followed by administration of Ang II (10^{-7} mol/L) for 5 min, on phosphorylation of MAPK. Representative Western blots are shown above the graph, respectively. Values are means ± S.E.M. (A) (*) denotes $P < 0.05$ vs. Ang II, (**) denotes $P < 0.01$ vs. control (Con), (***) denotes $P < 0.01$ vs. Ang II ($n = 6$); (B and C) P = NS ($n = 6$).

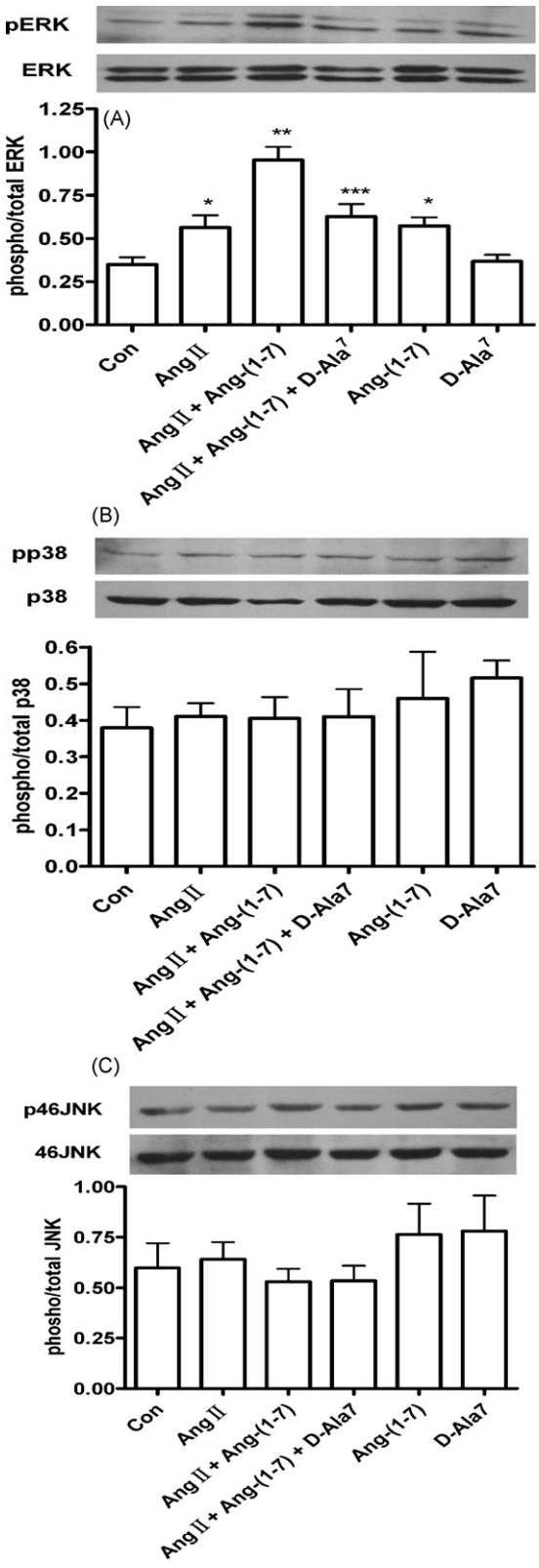

Fig. 5. D-Ala^7-Ang-(1–7) reverses the enhancing effect of Ang-(1–7) on Ang II-stimulated ERK phosphorylation. Mouse bone marrow-derived DC were pretreated with D-Ala^7-Ang-(1–7) (D-Ala^7, 10^{-5} mol/L) for 30 min prior to administration of Ang II, with or without Ang-(1–7) (10^{-7} mol/L). Shown are effects on phosphorylation of MAPK. Values are means ± S.E.M. (A) (*) denotes $P < 0.01$ vs. control (Con), (**) denotes $P < 0.01$ vs. Ang II, and (***) denotes $P < 0.01$ vs. Ang II + Ang-(1–7) ($n = 6$); (B and C) P = NS ($n = 6$).

al. (2002), and indicate that the activation of AT2 receptor in DC will produce pro-inflammatory effect to some extent. Although in most cell types, Ang II activates MAPK family via AT1 receptor, there does have some exceptions. In rabbit renal proximal tubule epithelial cells, NG108-15 neuronal cells, and calf pulmonary endothelial cells, investigators have demonstrated that AT2 receptor activation by Ang II leads to phosphorylation of ERK1/2 and subsequent physiologic or pathophysiologic functions (Gendron et al., 1999; Dulin et al., 1998; Li et al., 2007). In accordance with these previous studies, our results suggests that the mechanism by which Ang II elicits it's regulatory functions depends on cell type and activation of AT2 receptor does not always opposes the effects of AT1 receptor.

The major finding of the present study was that Ang-(1–7) stimulated ERK1/2 phosphorylation alone and significantly enhanced Ang II-induced phosphorylation of ERK1/2 in mouse bone marrow-derived DC. Ang-(1–7) is a heptapeptide component of RAS. Studies in mammalian (including human, rat and mouse) cells demonstrated that Ang-(1–7) inhibit a lot of processes stimulated by Ang II, such as vasoconstriction, cell growth and proliferation, proarrhythmia, prothrombogenic actions, and fibrogenic responses as well as activation of the MAPK family(Su et al., 2006; Tallant et al., 1999, 2005; Iyer et al., 2000; Ferreira and Santos, 2005). All of those processes stimulated by Ang II are mediated by AT1 receptor, and Ang-(1–7) takes the inhibitory effects via a specific receptor, the G-protein-coupled receptor Mas, whose relationship with Ang-(1–7) was established in mouse kidney and Chinese hamster ovary cells (Santos et al., 2003). It is also reported that Ang-(1–7) alone can activate p38MAPK through Mas receptor in rat proximal tubular cells (Su et al., 2006). In this study, the RT-PCR results documented the existence of receptor Mas in mouse bone marrow-derived DC, together with the complete inhibitory effect of Mas receptor antagonist D-Ala^7-Ang-(1–7)on the actions of Ang-(1–7), we suggest that the induction of phosphorylation of ERK1/2 by Ang-(1–7) alone and the enhancement of Ang-(1–7) on Ang II-induced phosphorylation of ERK1/2 are mediated by Mas receptor. The Mas receptor is reported to antagonize AT1 receptor through the formation of a hetero-oligomeric complex in cultured mammalian cells (Kostenis et al., 2005), and there is evidence that Ang-(1–7) can down-regulate AT1 receptor in cultured rat vascular smooth muscle cells (Clark et al., 2001), these may facilitate the effect of AT2 receptor. Since the stimulatory effect of ERK1/2 phosphorylation by Ang II is via AT2 receptor in this present study, we can not rule out the contribution of the complicated interactions and cross-talk between these three receptors to the enhancement of ERK1/2 phosphorylation (Kostenis et al., 2005; Clark et al., 2001; Castro et al., 2005). Furthermore, as found in human and rat cells, ACE2 can directly cleave Ang II to form Ang-(1–7) (Donoghue et al., 2000), the coexistence of Mas receptor and ACE2 discovered in the present study suggests that there has an ACE2-Ang-(1–7)-Mas axis in mouse bone marrow-derived DC as well as ACE-Ang II-AT1 axis.

Lots of studies about human monocyte DC have demonstrated that in DC maturation processes, the p38MAPK signaling pathway positively regulates the phenotypic (surface antigen expression) and functional maturation (endocytotic activity and allostimulatory capacity) as well as cytokine production; the JNK signaling pathway has little effect on surface antigen expression, no effect on allostimulatory capacity and only positively regulates the secretion of TNF-α and IL-12p70; while the ERK1/2 signaling pathway does not affect or negatively regulates the phenotypic maturation, negatively regulates the functional maturation and IL-12 production, weakly up-regulates inflammatory cytokine production (Nakahara et al., 2004; Arrighi et al., 2001; Nakagawa et al., 2004; Yu et al., 2004; Aiba et al., 2003; Puig-Kröger et al., 2001). Increasing evidence in mouse showed that activation of ERK1/2 signaling pathway leads to the inhibition of Th1 cell polarization and promoted the production of anti-inflammatory cytokine IL-10 by DC and the induction of Th2 cells, hence the anti-inflammatory and immunosuppressive effect (Loscher et al., 2005; Agrawal et al., 2006; Yanagawa et al., 2002). In the present study, Ang-(1–7) enhanced the phosphorylation of ERK1/2 induced by Ang II in mouse bone marrow-derived DC, thereby may turn the Th1 immune response to Th2 immune response through inhibition of the production of Th1-promoting cytokine IL-12 and other inflammatory cytokines and increase of the production of anti-inflammatory cytokine IL-10, thus elicits anti-inflammatory effect to counterbalance the well established pro-inflammatory function of Ang II.

5. Conclusions

This is the first study of the effects of RAS on MAPK family in mouse bone marrow-derived DC. Our findings include: first, DC are fully equipped with RAS including the ACE-Ang II-AT1 axis and the ACE2-Ang-(1–7)-Mas axis; secondly, Ang II stimulates phosphorylation of ERK1/2 via AT2 receptor, Ang-(1–7) enhances the stimulatory effect by activating Mas receptor; thirdly, neither Ang II nor Ang-(1–7) has influence on phosphorylation of p38 and JNK. By enhancing the activation of ERK1/2, generation of Ang-(1–7) by DC might counterbalance the pro-inflammatory effects of locally generated Ang II. Although it remains to be established the exact entire signaling pathway, the novel regulatory mechanism of RAS in DC may have important pathophysiologic and therapeutic implications in inflammatory diseases.

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