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Staphylococcal enterotoxin C2 stimulated the maturation of bone marrow derived dendritic cells via TLR-NFκB signaling pathway

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ABSTRACT

As a kind of superantigen, staphylococcal enterotoxin C2 (SEC2) is well known as a powerful immunomodulator. However, most previous studies about SEC2 focus on its T cell activating characters. But the direct effect of SEC2 on antigen-presenting cells (APCs) which are important for the T cell activation is not clearly. In this study, we investigated the effect of SEC2 on murine bone marrow-derived dendritic cells (BMDCs) which are known as the specialized professional APCs. Contrary to its effects on T cells, SEC2 could not induce proliferation or cytotoxicity to BMDCs even in high concentrations. While SEC2 could promote the mature of BMDCs with increased expression of co-stimulatory molecules on cell membrane such as CD80, CD86, and MHC II. The production of pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-6 were also increased in BMDCs treated with SEC2. We also found that SEC2 enhanced the genes expression of pattern recognition receptors including toll-like receptors 2 (TLR2) and TLR4 in BMDCs, and up-regulated the key signal molecule MyD88 in both mRNA and protein levels. In addition, SEC2 also caused IκBα degradating and NFκB p65 translocating from the cytoplasm to the nucleus in BMDCs. The siRNAs for both TLR2 and TLR4, as well as NFκB specific inhibitor BAY 11–7085 could inhibit the co-stimulatory molecules expression and pro-inflammatory cytokines releasing induced by SEC2. Moreover, TLR2/4 specific siRNAs inhibited p65 and MyD88 upregulation induced by SEC2. In summary, all our results indicated that SEC2 could stimulate BMDCs maturation through TLR-NFκB signaling pathways.

1. Introduction

Dendritic cells (DCs) are the most potent APCs (antigen-presenting cells) in mammalian immune system to respond against foreign invaders [1]. Their main function is to process antigen material and present it on the cell surface to the T cells of the immune system. They act as messengers between the innate and the adaptive immune systems. Immature DCs are characterized by high endocytic activity and low T-cell activation potential. Once contacted with foreign antigen, immature DCs become activated into mature dendritic cells presenting foreign antigen fragments at their cell surface by MHC molecules and up-regulating cell-surface co-receptors in T cell activation such as CD80 (B7.1) and CD86 (B7.2) [2].

The stimulus induce DC maturation through pattern recognition receptors (PRRs) and activate innate and adaptive immune responses through DC [3]. Toll-like receptors (TLRs) are a serial of the most important members of PRRs. Previously findings revealed the TLRs have key functions in the control of immune response [4–6]. TLRs can recognize a wide variety of microorganism antigens and elicit the mature of DC through NFκB signaling pathway with cytokines release and co-stimulatory molecules expressions up regulation [7,8].

Staphylococcal enterotoxin C2 (SEC2) belong to the family of Staphylococcal enterotoxins (SEs) which are secreted by *Staphylococcus aureus* and *Streptococcus aureus*[9]. SEs can directly activate about 2–20% T-cells at a very low concentration and release many kinds of cytokines [10]. To activate T cells, intact SEC2 binds with Vβ chain of T cell receptor (TCR) and MHC II molecules on APCs to form a trimer without being processed by APCs. Previous studies on the effect of SEC2 showed that SEC2 and its mutants have potent effects on T cell activation by recognition and contraction to TCR [11,12]. However, the direct effects of SEC2 on APCs were not reported. Previous studies of SEs such as SEA and SEB showed that they could upregulate the expression of factors which were related to Toll-like receptors (TLRs) signaling in DCs [13,14]. The stimulation of TLRs and MyD88 could

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induced the activation of T cells through NFκB pathway. In our previous study, we found that SEC2 and its mutants could activated the NFκB pathway in T-cell [15]. So we hypothesized that SEC2 might also have a direct effect on DCs through TLR-NFκB pathway.

So, the aim of this research is to study the relationship between SEC2 and murine bone marrow derived dendritic cell (BMDCs). Through our study, we confirmed that SEC2 can stimulate the maturation of BMDCs via TLR- NFκB signaling pathway.

2. Material and methods

2.1. Animals

Female BALB/c mice (4–6 weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) and maintained under specific pathogen free (SPF) conditions. Feed and water were supplied ad libitum. All animals were treated according to the regulations for the administration of affairs concerning experimental animals by the government of China.

2.2. Chemicals and reagents

SEC2 is prepared and preserved by our laboratory [16], and the endotoxin was removed by ToxinEraser™ Endotoxin Removal Kit (Genscript, China). RMPI-1640 was purchased from Thermo Fisher Scientific (Hyclone, Waltham, MA, USA). LPS was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Recombinant moues GM-CSF and IL-4 were obtained from PeproTech Inc. (Rocky Hill, NJ, USA). Anti-mouse antibodies FITC-CD80, PE-CD86, and PE-MHC II were obtained from Biolegend (San Diego, CA, USA). Cell Titer 96 Aqueous One Solution Cell Proliferation assay (MTS) was purchased from Promega (Madison, USA). RNAiso kit for total RNA extraction, PrimeScript™ RT Master Mix (Perfect Real Time) kit for RNA reverse transcription, and SYBR Premix Ex Taq™ Kit for real-time-PCR assay were purchased from Takara (Dalian, China). The ELISA kits for TNF-α, IL-6, and IFN-γ were obtained from eBioscience (San Diego, CA, USA). Antibodies against β-actin, IκBα, LaminB1, p65, and HRP-conjugatedgoat anti mouse IgG were purchased from CST. Inhibitors BAY11–7085 was purchased from Selleck (Houston, TX, USA).

2.3. Preparation of Bone Marrow-Derived dendritic cells (BMDCs)

The method of BMDCs preparation was modified from previously reported [17,18]. Briefly, mice were sacrificed by cervical dislocation, and the femur and tibia of the hind legs were dissected, then bone marrow cavities were flushed with 10 ml cold sterile phosphate buffered saline (PBS). After lysing red blood cells, the bone marrow cells were washed, resuspended, and differentiated into BMDCs in RMPI-1640 with 10% FBS, 20 ng/ml rmGM-CSF, 10 ng/ml rmIL-4, 100μg/ml streptomycin and 100 U/ml penicillin. Six days after initial BMDCs cell culture, the purity of $CD11c^+$ cells were > 90% (Fig. 1), as determined by flow cytometry.

2.4. Cytotoxicity and viability assay

Monolayers of BMDCs in 96-well microplate were cultured in RMPI-1640 supplemented with 10% FBS and incubated with SEC2 (0.1–100 μg/ml) for 48 h. The cytotoxicity was evaluated using MTS. Briefly, 20 μl of MTS were added to each well 4 h before the end of incubation. Thereafter, the OD absorbance was evaluated in a microplate reader at 490 nm.

Lactate dehydrogenase (LDH) release from damaged cells was determined 48 h after treatment with PBS (Blank control), SEC2 (0.1–100 μg/ml) or LPS (200 ng/ml). LDH activity in the culture supernatant was measured using an automatic microplate reader at 570 nm

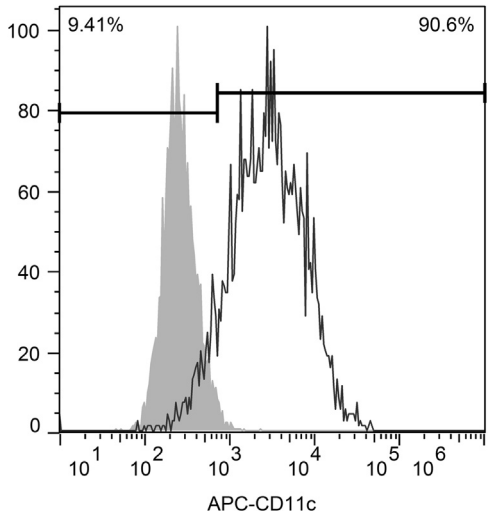

Fig. 1. The specific marker of BMDCs. The cells were treat as described in Section 2.2. Then, the cells were harvested and stain with antibodies to CD11c. The cells were analyzed with flow cytometry. The results were showed with gated percentage. Data are showed with one out of three independent experiments.

Table 1
Sequences of primers used for qPCR.

Gene	Primer sequence
β-actin	Forward : 5′-TACCCAGGCATTGCTGACAGG – 3′
	Reverse : 5′-ACTTGCGGTGCACGATGGA – 3′
TNF-α	Forward : 5′-CCCTCACACTCAGATCATCTTCT – 3′
	Reverse : 5′-GCTACGACGTGGGCTACAG – 3′
IFN-γ	Forward : 5′-AGACAATCAGGCCATCAGCA – 3′
	Reverse : 5′-TGGACCTGTGGGTTGTTGAC – 3′
IL-6	Forward : 5′-TAGTCCTTCCTACCCCAATTTCC – 3′
	Reverse : 5′-TTGGTCCTTAGCCACTCCTTC – 3′
TLR2	Forward : 5′-GCTGGAGGTGTTGGATGTTAG – 3′
	Reverse : 5′-AGGATAGGAGTTCGCAGGAG – 3′
TLR4	Forward : 5′-GGACTATGTGATGTGACCATTGAT – 3′
	Reverse : 5′-TTATAGATACACCTGCCAGAGACA – 3′
MyD88	Forward : 5′-CTACAGAGCAAGGAATGTGACT – 3′
	Reverse : 5′-CATATAGTGATGAACCGCAGGAT – 3′

Table 2
Sequences of primers used for siRNA.

siRNA	Primer sequence	
NC	Sense	5′- UUCUCCGAACGUGUCACGUTT – 3′
	Antisense	5′- ACGUGACACGUUCGGAGAATT – 3′
TLR2	Sense	5′- GGAACAGAGUGGCAACAGUTT – 3′
	Antisense	5′- ACUGUUGCCACUCUGUUCCTT – 3′
TLR4	Sense	5′- GCAUAGAGGUAGUUCCUAATT – 3′
	Antisense	5′- UUAGGAACUACCUCUAUGCTT – 3′

2.5. Flow cytometry analysis

BMDCs were harvested at 24 h after simulated with medium only, 10 μg/ml SEC2 or LPS (200 ng/ml). Then, to detect the cell surface markers including CD80, CD86, and MHC II from different treatment groups, cells were respectively collected and stained with antibodies against CD11c, CD80, CD86, and MHC II for 30 min at 4 ℃ and then washed twice with PBS and analyzed in a FACScalibur flow cytometer (Becton-Dickinson). The experiment was repeated three times

2.6. Total RNA isolation and real-time quantitative PCR

BMDCs were harvested at 4/8/12/24 h after simulated with

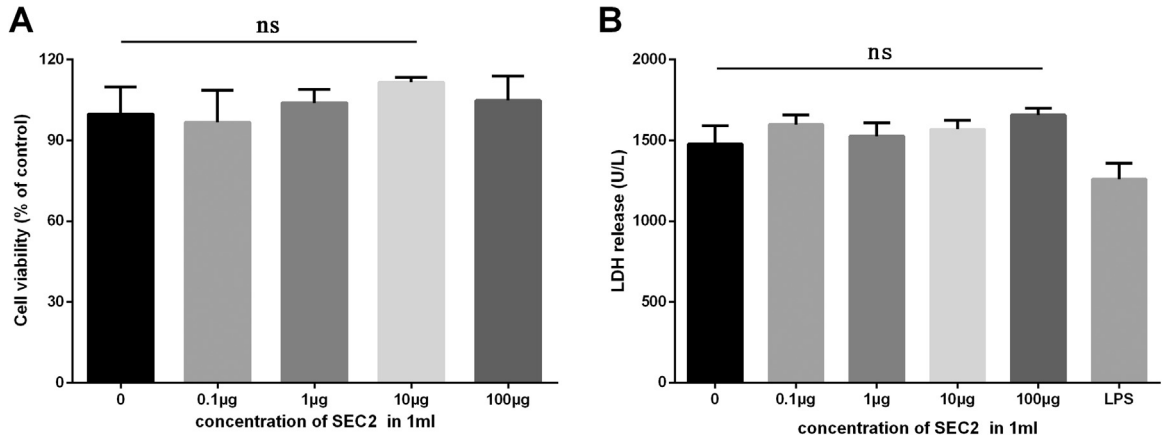

Fig. 2. The noncytotoxic dose of SEC2 on BMDCs was detected. (A) BMDCs were incubated with SEC2 at a concentration from 0.1 μg to 100 μg/ml with 10 times dilution for 48 h. The cell viability was determined by MTS and the results are expressed as the percentage of viable cells. (B) The release of LDH in media was tested as a marker to measure the death of BMDCs. BMDCs were treated with medium only, LPS (200 ng/ml) or SEC2 at a concentration from 0.1 μg to 100 μg/ml with 10 times dilution for 48 h, and the LDH activity in the supernatant was measured with a kit. The results were presented with mean ± S.D of five samples and *t*-test was carried out (* represent $P < 0.05$, ** represent $P < 0.01$).

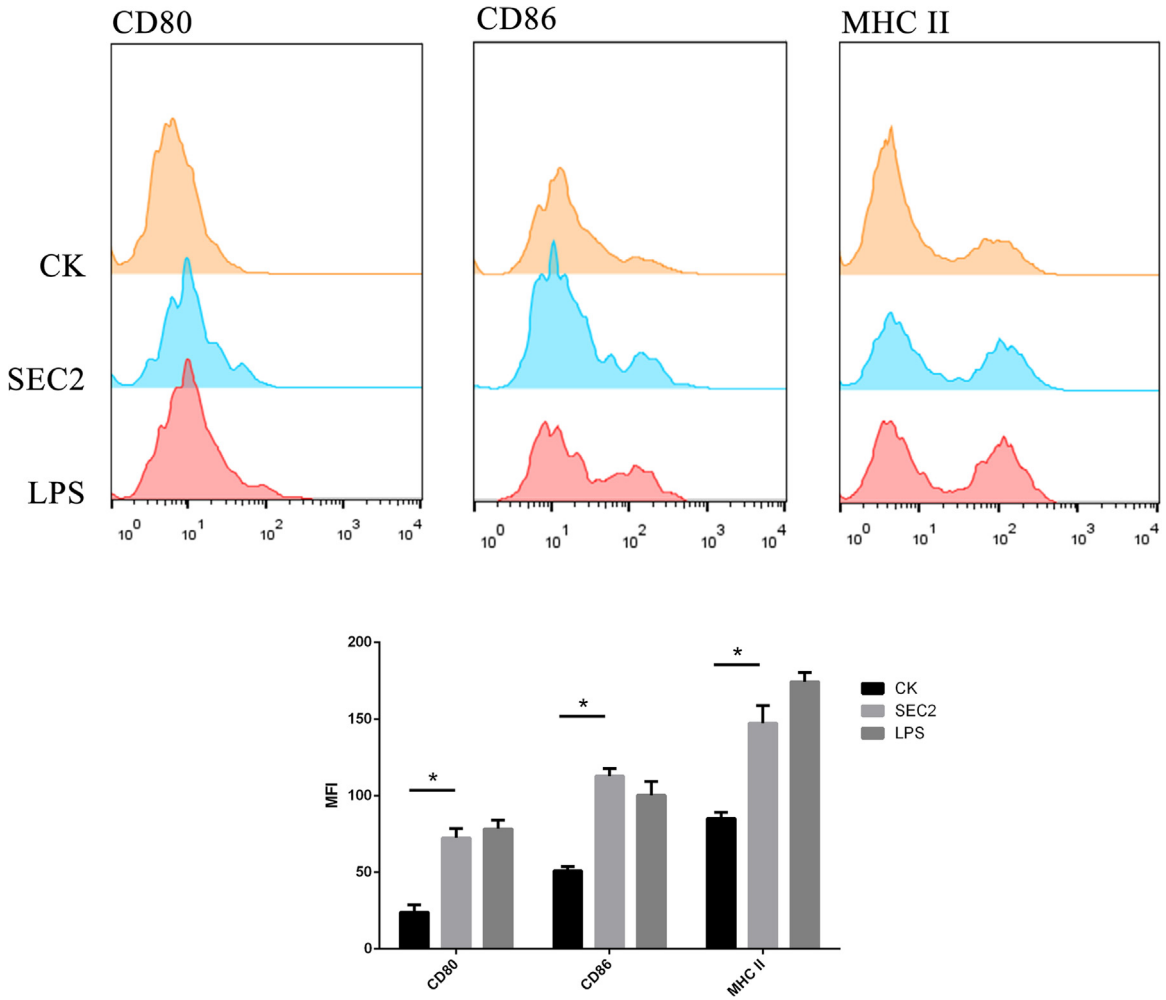

Fig. 3. The induction of co-stimulatory molecules improved by SEC2 on BMDCs. BMDCs were treated with medium only, LPS (200 ng/ml) or SEC2 (10 μg/ml) for 24 h and the cells were collected and stained with antibodies to CD80, CD86 and MHC II. Then, the cells were analyzed with flow cytometry. The results were showed with gated percentage. Data are showed with one out of three independent experiments.

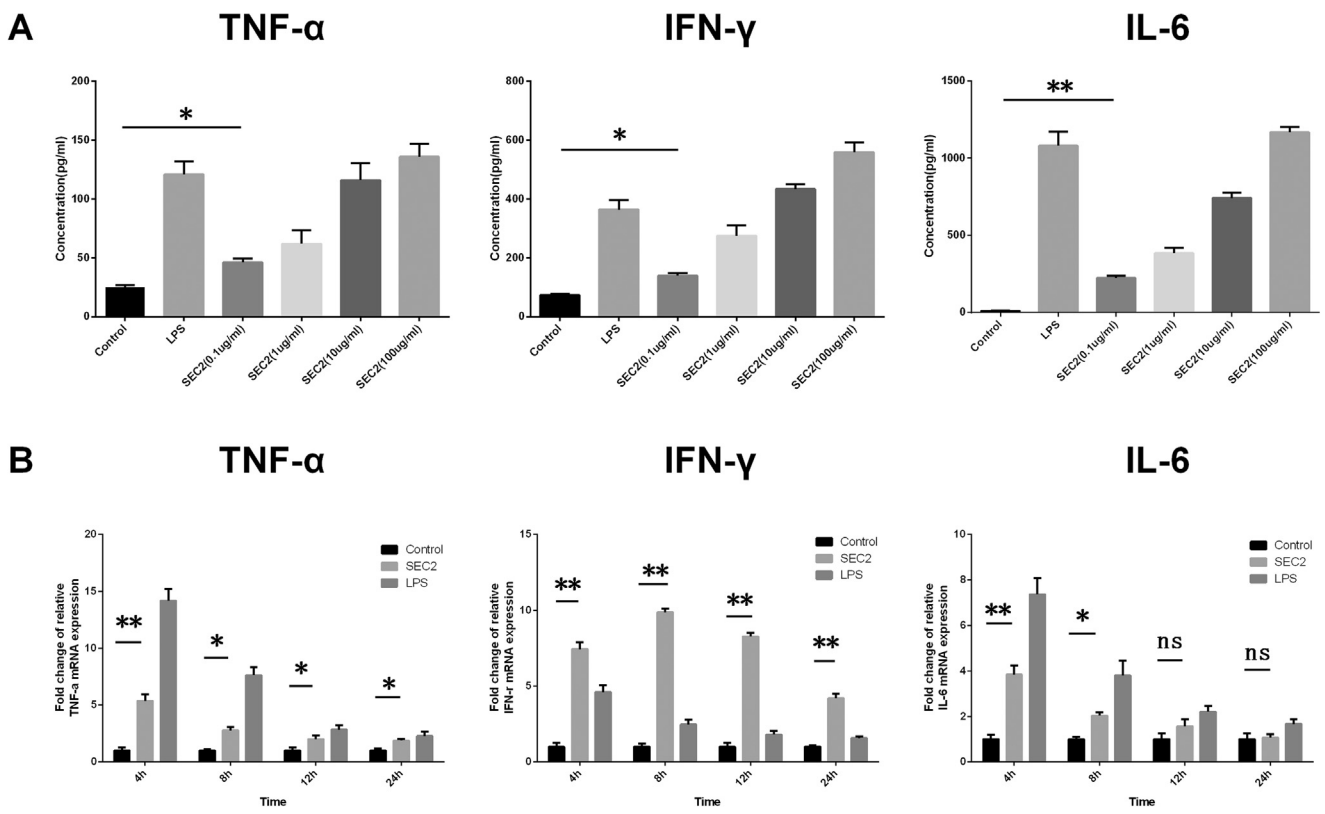

Fig. 4. The effect of SEC2 on cytokines released in BMDCs. (A) BMDCs were treated with SEC2 at a concentration from 0.1 μg to 100 μg/ml for 24 h. Then, the secretions of TNF-α , IFN-γ and IL-6 were quantified by ELISA. (B) BMDCs were treated with medium only, LPS (200 ng/ml) or SEC2 (10 μg/ml) and harvested 4/8/12/24 h later and total RNA of cells were extracted to measure the expression of TNF-α, IFN-γ and IL-6 genes. Data are expressed as mean ± S.D from three independent experiments and *t*-test was carried out (* represent $P < 0.05$, ** represent $P < 0.01$).

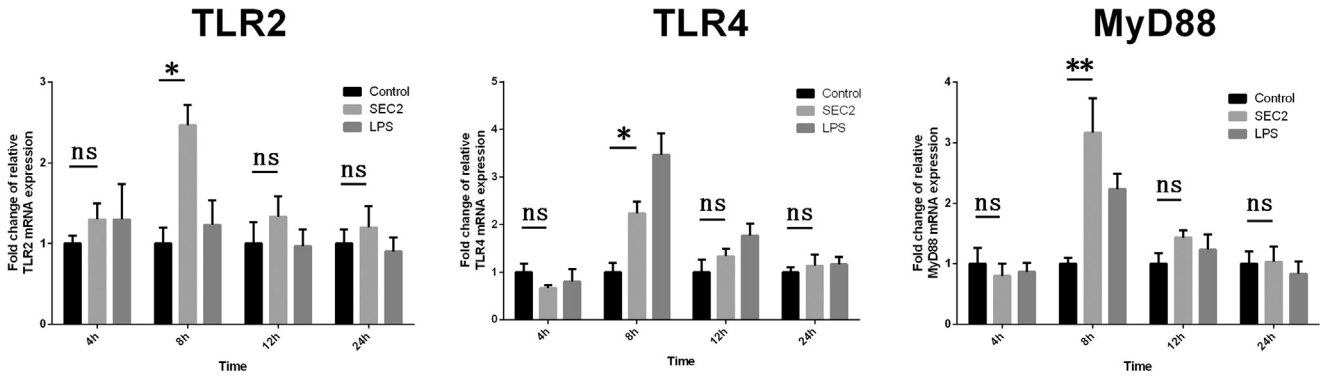

Fig. 5. The effect of SEC2 on TLRs expression in BMDCs. BMDCs were treated with medium only, SEC2 (10 μg/ml) or LPS (200 ng/ml) and harvested 4/8/12/24 h later and total RNA of cells were extracted to measure the expression of TLR2, TLR4 and MyD88 genes. Data are expressed as mean ± S.D from three independent experiments and *t*-test was carried out (* represent $P < 0.05$, ** represent $P < 0.01$).

medium only, 10 μg/ml SEC2 or LPS (200 ng/ml). The total RNA from each treated sample was isolated by RNAiso kit according to the manufacturer's protocol. And 0.5 μg of total RNA from each sample was reverse transcribed to cDNA. Quantitative real-time PCR was performed in triplicates using the SYBR Premix Ex Taq™ Kit with ABI Prism 7000. Amplification was performed in a total volume of 25 μl containing 1 μl of cDNA template, 1 μl of forward primer, 1 μl of reverse primer, 9 μl of nuclease-free water, 0.5 μl of ROX, and 12.5 μl of SYBR premix Taq™ (2 ×). The thermal cycling parameters were as follows: initial denaturation at 94 °C (30 s), followed by 40 cycles at 94 °C (25 s), 60 °C (45 s) and 72 °C (45 s). The house keeping gene (β-actin) was used to correct minor variations. Primers for target genes were listed in Table 1. Data analysis was performed by comparative Ct method of relative quantification using β-actin as endogenous control. The equation is following: $\triangle Ct$ = Ct (sample) – Ct (endogenous control); $\triangle\triangle Ct$ = $\triangle Ct$ (sample) $-\triangle Ct$ (untreated); and fold change = $2^{-\triangle\triangle Ct}$. The experiment was repeated three times

2.7. Cytokine assay

BMDCs were harvested at 24 h after simulated with medium only, SEC2 (0.1–100 μg/ml) or LPS (200 ng/ml). Levels of TNF-α, IL-6, and IFN-γ in the culture supernatants were quantified using a sandwich ELISA kit (eBioscience) according to the manufacturer's instructions. The experiment was repeated three times.

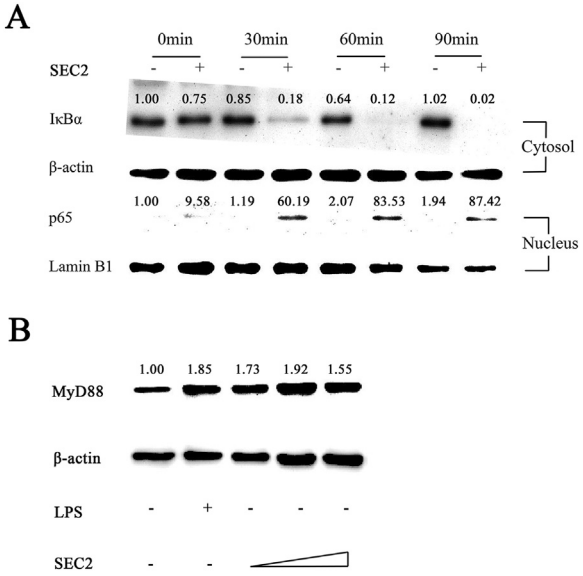

Fig. 6. The effect of SEC2 on MyD88, IκB and p65 in BMDCs. (A) BMDCs were treated with SEC2 (10 μg/ml) for 30/60/90 min. Then, the cells were harvested and the proteins were separate into nuclear and cytosolic. Western blotting was carried out to measure the change of p65 (nuclear) and IκB (cytosolic). (B) BMDCs were treated with medium only, LPS (200 ng/ml) or SEC2 (0.1, 1 or 10 μg/ml) for 12 h. Then, the cells were harvested and MyD88 was detected by western blotting. Data are showed with one out of three independent experiments.

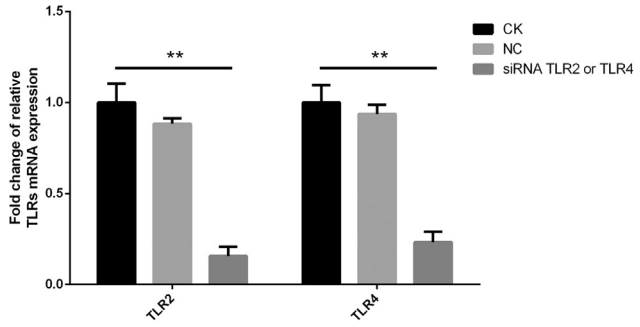

Fig. 7. BMDCs transfected with siRNA target murine TLR2 and/or TLR4 were incubated for 48 h. The expression of TLR2 and TLR4 mRNA were examined by qRT-PCR. Data are expressed as mean ± S.D from three independent experiments and *t*-test was carried out (* represent $P < 0.05$, ** represent $P < 0.01$).

2.8. Western blotting analysis

BMDCs were treated with medium only or SEC2 (10 μg/ml) for 30/60/90 min for the detection of IκB and NFκB. Besides, BMDCs were treated with medium only, LPS (200 ng/ml) or SEC2 (0.1, 1 or 10 μg/ml) for 12 h for the detection of MyD88. Total cell lysates were lysed with RIPA. Nuclear and cytosolic extracts were fractionated using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Biotechnology) according to the manufacturer's instructions. Equal amounts of proteins from each sample were subjected to SDS-PAGE followed by being transferred to nitrocellulose membranes. Membranes were blocked in 5% Non-fat milk and incubated with a primary antibody overnight at 4 °C. After washing with TBST, membranes were incubated with secondary antibody linked to HRP. The blots were then developed with an ECL detection system as per the manufacturer's instructions.

2.9. Blocking experiment

BMDCs were treated with medium (contains 5 μl DMSO as negative control), SEC2 (10 μg/ml) or SEC2 with Bay11–7085 (diluted with DMSO in 5 μl with a final concentration of 5 μm/ml). The Bay 11–7085 was prior to SEC2 and incubated for 30 min. Then, BMDCs were incubated for another 48 h. After that, the supernatants were harvested and TNF-α, IFN-γ and IL-6 were measured by ELISA.

2.10. Small-interfering RNA transfection

Small-interfering RNAs (siRNAs) which target murine TLR2 [19] or TLR4 [20] were synthesized by GenePharma Co., Ltd (Shanghai, China) and the primers were listed in Table 2. BMDCs cultured in 12-well plate with a density of 5×10^5 cell/ml were transfected with either 40 p.M. siRNA or negative control (NC) siRNA by using RNAi-Mate (GenePharma Co., Ltd) according to the manufacturer's instructions. qRT-PCR was performed to evaluate the efficiency of transfection after 48 h incubation. Then, SEC2 (10 μg/ml) was added, the maturation was detected by flow cytometry, the expression of protein was evaluated by western blot and the release of pro-inflammatory cytokine.

2.11. Statistical analysis

Results are presented as mean ± SD of three independent experiments. Statistical analyses were performed using two-tailed Student's *t*-test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Cytotoxic effects of SEC2 on BMDCs

To evaluate the cytotoxicity of SEC2 on BMDCs, BMDCs were incubated with SEC2 in ten-time serial diluted concentrations from 0.1 100 μg/ml. As showed in Fig. 2(A), SEC2 did not make any change in the growth and viability of BMDCs even in high concentration. Besides, to confirmed the cell damage of SEC2 on BMDCs, we measuring the release of the cytosolic marker LDH from BMDCs. After 48 h incubation, no significant difference of LDH release was founded compared with control group, the result was showed in Fig. 2(B).

3.2. Effect of SEC2 on the induction of co-stimulatory molecules expression on BMDCs

As important molecular makers of BMDCs maturation, the expression of CD80, CD86, and MHC II on BMDCs were analyzed by flow cytometry. As showed in Fig. 3, untreated BMDCs expressed a basal level of CD80, CD86, and MHC II. As we expected, the expressions of CD80, CD86, and MHC II were elevated in positive control group which was treated with LPS. Likewise, BMDCs treated with SEC2 significantly increased the expression of all these three molecules compared with negative control group. This result implied that SEC2 could directly act on BMDCs and induce BMDCs maturation.

3.3. Effect of SEC2 on cytokines levels in BMDCs

It is well known that DC could produce various cytokines which are important in inducing a number of cellular and molecular events and eventually leading to eliciting immune responses [21]. To determine if SEC2 has effects on the cytokine expression of DC, IFN-γ, TNF-α, and IL-6 expressed in BMDCs were evaluated in both protein and mRNA levels. From ELISA assay results (Fig. 4(A)), as predicted, BMDCs stimulated with LPS showed significantly increased levels of IFN-γ, TNF-α and IL-6 compared with negative control group. Similarly, BMDCs treated with SEC2 also exhibited dramatic increasement in expressions of IFN-γ, TNF-α and IL-6 compared with negative control group. Dose-dependent

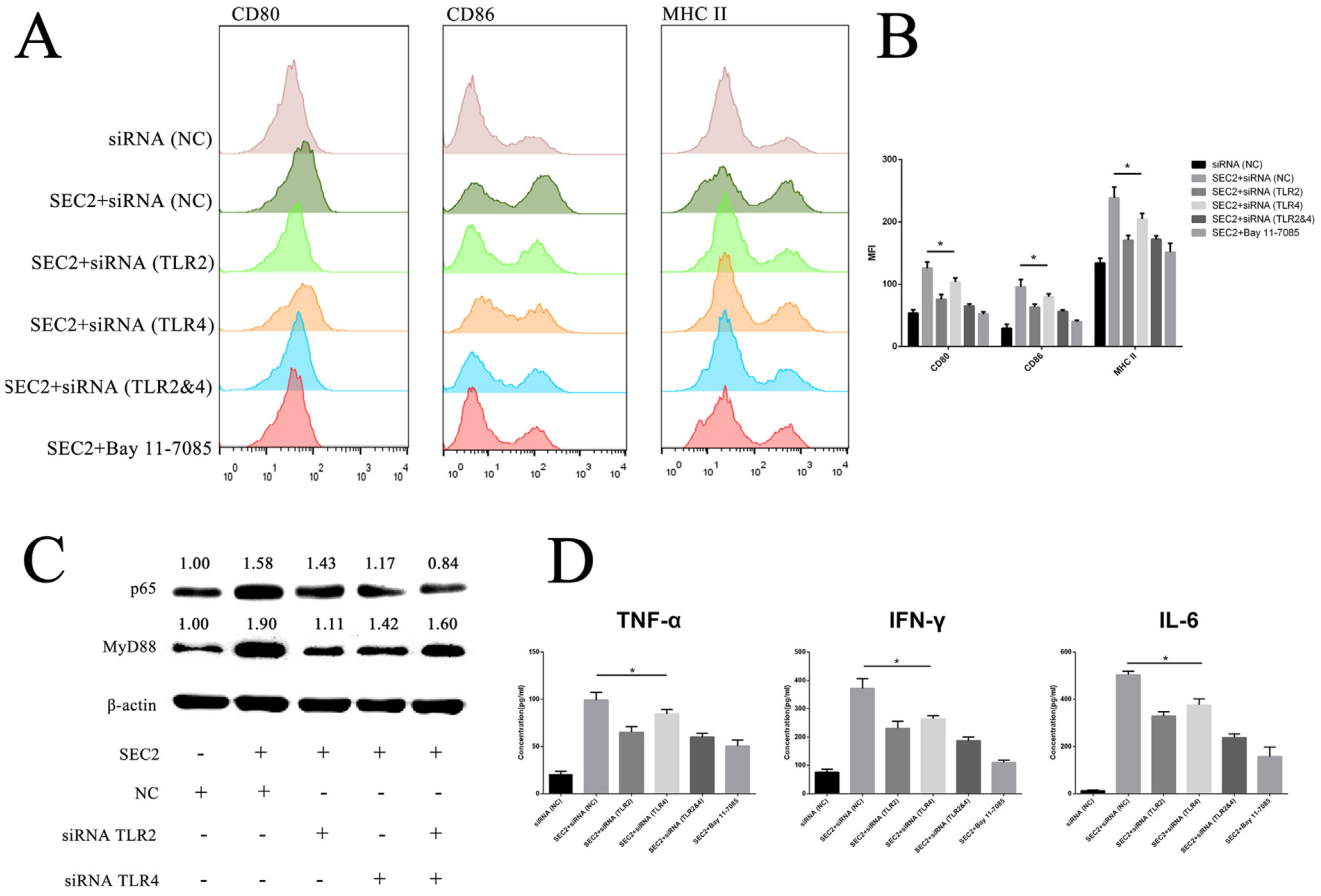

Fig. 8. BMDCs were transfected with siRNA (40 pM for each well) for 48 h or pre-treated with Bay 11–7085 (5 μm/ml. as final concentration) for 30 min, then the BMDCs were treated with SEC2 (10 μg/ml) for another 48 h. The expression of CD80, CD86 and MHC II were examined by flow cytometry (A) with the median fluorescent intensity (B). The expression of MyD88 and p65 were detected by western blot (C). And the release of TNF-α, IFN-γ and IL-6 were measured by ELISA (D). Data are expressed as mean ± S.D from three independent experiments and *t*-test was carried out (* represent $P < 0.05$, ** represent $P < 0.01$).

manners of the effects of SEC2 were also observed and even when the final concentration of SEC2 is only 0.1 μg/ml, the statistical difference is significant ($p < 0.05$ for IFN-γ, TNF-α, $p < 0.01$ for IL-6). The same tendencies were also found on the level of mRNA as shown in Fig. 4(B). The gene fold change of all these three cytokines were upregulated after treated with SEC2 for 4 h compared with negative control group. The significant difference last for 24 h in the aspect of TNF-α and IFN-γ, last for 8 h in the aspect of IL-6.

3.4. Effect of SEC2 on mRNA expression of TLR2 and TLR4

Toll-like receptors (TLRs) can recognize a wide variety of microbial compounds and elicit immune activation. In our preliminary experiments, we found that SEC2 could especially upregulate the expression of TLR2 and TLR4 among all of the nine TLRs from TLR1 to TLR9 on the surface of murine BMDCs (data not shown). In this study, we determined the mRNA expression levels of TLR2, TLR4 and MyD88 in murine BMDCs stimulated with SEC2 in different time (4, 8, 12 and 24 h). Treat with LPS showed significant up regulation of TLR2, TLR4 and MyD88 as positive control especially at 8 h. Also at 8 h, SEC2 increased the gene expression of all the three factors ($p < 0.05$ for TLR2 and TLR4, $p < 0.01$ for MyD88). All these results were showed in Fig. 5.

3.5. Effect of SEC2 on NFκB signaling pathway activation

It is well known that TLR-NFκB signaling is an important pathway for dendritic cells activation [22]. To investigate whether SEC2 also activates this cascade in the induction of BMDCs maturation, western blotting was carried out to examine the change of MyD88, NFκB p65 and IκBα. As shown in Fig. 6(A), SEC2 triggered a gradual increasement of NFκB p65 protein in the nucleus, while corresponding decreasement of IκBα protein in the cytosol within 90 min of stimulation. The change of MyD88 was also observed after incubated for 12 h. Fig. 6(B) shows that SEC2 increased the expression of MyD88 in a dose-dependent manner.

3.6. SEC2 induce the maturation of BMDCs is mediated by NFκB signaling

To determine whether the maturation of BMDCs induced by SEC2 is related to TLR-NFκB signaling pathway, BMDCs was transfected with siRNA target murine TLR2 or TLR4 for 48 h, or pretreated with Bay 11–0785(inhibitor for NFκB p65) for 30 min and then incubated with SEC2 for 48 h. The expression of TLR2 and TLR4 were significant inhibited by its specific siRNA respectively, the result of this part were shown in Fig. 7. As shown in Fig. 8, transfected with siRNA or pretreated with Bay 11–0785 significantly decreased SEC2 induced upregulation of CD80, CD86 and MHC II. The production of IFN-γ, TNF-α, IL-6 was also reduced ($p < 0.01$ for all three cytokines). What's more, the expression of MyD88 and p65 were also decreased after transfected with siRNA. All these data indicated that TLR-NFκB is required for SEC2 induced maturation of BMDCs.

4. Discussion

As the most powerful APC, DC connect innate and adaptive immune

responses with capturing, degradation and antigens presentation to activate immune responses [23–25]. During this process, TLRs play an important role with upregulation of co-stimulatory molecules and secretion of cytokines and chemokines [26]. Staphylococcal enterotoxins (SEs) produced by Staphylococcus aureus belong to the family of superantigens. SEs can activate a large proportion of T cells and release large amounts of cytokines [10,27]. As one of the members of superantigens, SEC2 and its mutants showed potent activities in stimulating T cells [12,16,28]. Besides, it is reported that SEA and SEB, two superantigens with high homologous to SEC2, could upregulate the expression of the key signaling molecules in TLRs such as MyD88 [29]. All these enlighten the possible relation between SEC2 and TLRs in DCs. To verify our hypothesis, in this research, we study the role of SEC2 in stimulating murine BMDCs maturation through TLRs and associated factors for the first time.

Firstly, we examined the proliferation and viability of BMDCs when treated with SEC2 in different concentration from 0.1 to 100 μg/ml. SEC2 could not induce any proliferation in BMDCs even in high concentrations, which is extremely different with in T cells. SEC2 also could not induce LDH releasing in BMDCs. In living cells, LDH is stably existing in cytoplasm. Only when the cell was damaged, LDH release from cytoplasm to extracellular matrix [30,31]. So, it is safe for BMDCs when treated with SEC2 under a concentration not exceeding 100 μg.

Marker molecules CD80, CD86, and MHCII on DC cell surface are important and associated with the antigen presentation and T lymphocyte activation [32]. Our results showed that SEC2 markedly increased the expression of CD80, CD86, and MHCII molecules on murine BMDCs. We also found that SEC2 significantly upregulated the expression of pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-6 which play an important role in the early stages of infection. This result was similar with others studies that both SEA and SEB could induce pro-inflammatory cytokines production in murine spleen cells primarily in MyD88-dependent manner. [14,33].

It is well known that MyD88 is an adaptor protein and the key factor of TLRs signaling pathway. And the conclusions from other reports also enlightened that the possible pathway involved in SEC2 inducing BMDCs maturation could be TLRs signaling [29,34,35]. TLRs recognize various of pathogen and regulate various inflammatory cytokines expression through NFκB family. The p65 subunit is a major component of NFκB complexe and is responsible for trans-activation. As the inhibitor of NFκB, IκB bind with NFκB usually, and IκBα is the most main protein of IκB family. However, IκB phosphorylate and detach from NFκB if foreign antigen was recognized by TLRs. Then, NFκB translocate from the cytoplasm to the nucleus and regulate the transcription of a large number of genes [31]. In this study, SEC2 improved the genes expression of TLR2, TLR4 and MyD88 in murine BMDCs after 8 h incubation. Furthermore, in this process, both the phosphorylation of IκBαand the translocation of p65 from the cytoplasm to the nucleus were also observed.

Lastly, inhibition of TLR-NFκB activation by murine TLR2 or TLR4 specific siRNAs and NFκB specific inhibitor BAY11–7085 were examined. The expression of CD80, CD86 and MHC II which were upregulation by SEC2 were significantly decreased. The release of TNF-α, IFN-γ and IL-6 were also reduced after transfected with siRNA or treated with BAY11–7085. Besides, the results of western blot showed that the up-regulations of MyD88 and p65 induced by SEC2 were decreased after the silence of TLR and/or TLR4.

To sum up, all our results indicated that SEC2 stimulate the maturation of BMDCs through TLR-NFκB signaling pathway.

Acknowledgments

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Conflict of interest statement

The authors declare no conflict of interest.

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