Activation of lymphotoxin-beta receptor enhances the LPS-induced expression of IL-8 through NF-κB and IRF-1

Seok-Won Jang a, Su-Geun Lim a, Kyoungho Suk b, Won-Ha Lee a,∗

a School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu 702-701, Republic of Korea
b Department of Pharmacology, Brain Science & Engineering Institute, BK21 Plus KNU Biomedical Convergence Program, Kyungpook National University
School of Medicine, Daegu 700-422, Republic of Korea

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Lymphotoxin-beta receptor (LTβR), a receptor for LIGHT (tumor necrosis factor superfamily 14) and LTα1β2, is expressed on the epithelial, stromal, and myeloid cells. LTβR is known to affect the lymphoid organ development and immune homeostasis. However, its role in macrophage function has not been sufficiently elucidated. The effect of LTβR stimulation in the inflammatory activation of macrophages was investigated by treating the human macrophage-like cell line THP-1 with LTβR-specific monoclonal antibody. Interestingly, combined treatment with anti-LTβR antibody and LPS caused the synergistic induction of IL-8 expression at the transcriptional level. Analysis indicated that nuclear factor (NF)-κB activity was enhanced via the mitogen-activated protein kinase (MAPK) and glycogen synthase kinase (GSK)-3β/CAMP response element binding protein (CREB) pathways. In addition, LTβR stimulation induced the expression of interferon regulatory factor (IRF)-1, one of the major transcription factors of IL-8 gene. Down-regulation of IRF-1 expression reduced the enhancing effect caused by LTβR stimulation. This indicates that the LTβR stimulation enhances the LPS-induced expression of IL-8 via the combined action of NF-κB and IRF-1.

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1. Introduction

Lymphotoxin-beta receptor (LTβR) is a cellular receptor for LIGHT (tumor necrosis factor superfamily 14) and LTα1β2, which are expressed on the surface of the cellular counterparts. The LTβR expression can be detected on the stroma of secondary lymphoid organs and of thymus, and in cells of myeloid lineage [reviewed in [1]]. LTβR has diverse functionalities; its acts as the regulator of lymphoid organ development [2,3], mediator of cancer-associated inflammation [4,5], and homeostatic stimulator of dendritic cell expansion [6,7]. During inflammatory activation, ligand binding induces trimimerization of LTβR, which leads to the recruitment of TNF receptor-associated factor (TRAF) for the stimulation of the classical NF-κB pathway via TRAF2/5 [8,9] or the non-canonical NF-κB pathway via TRAF3 [10]. These inflammatory activities were observed in non-immune cells, such as the human embryonic kidney (HEK)293 cells and the mouse embryonic fibroblasts. Although macrophages express high levels of LTβR, its role in macrophage function has not been analyzed except by Wimmer et al. [11–13], who reported LTβR to be an inducer of cross-tolerance to the Toll-like receptor (TLR) ligands.

The cytokine IL-8 was initially identified for its strong chemotactic activity toward neutrophils and T-lymphocytes. However, it is currently believed to be multifunctional, as it has been determined to induce migration, invasion, proliferation, and angiogenesis. Aberrant expression of IL-8 is associated with the pathogenesis of various diseases. IL-8 is the major chemotractant for neutrophils in chronic inflammatory diseases [14,15]. In atherogenesis, IL-8 contributes to plaque formation as a potent angiogenic factor [16,17] and induces the migration and proliferation of T-lymphocytes, macrophages, endothelial cells, and smooth muscle cells [18–20]. During cancer development, IL-8 expression is elevated in the tumor microenvironment; this leads to the enhancement of cancer-associated activities, such as angiogenesis, proliferation, invasion and metastasis [21–26]. Because of this, IL-8 is considered to be a major therapeutic target for many diseases, including cystic fibrosis [27], ocular inflammation [28], melanoma [29], breast cancer [30], and colorectal cancer [31].

Although macrophages, along with endothelial cells, are a major source of IL-8, the regulation of IL-8 expression in macrophages has not been sufficiently elucidated. Interestingly, lipo polysaccharide (LPS)-induced expression of IL-8 was observed to be synergistically enhanced by the stimulation of LTβR in the human
macrophage-like cell line THP-1. An analysis of the molecular mechanisms responsible for these effects revealed the involvement of NF-κB, the major transcription factor involved in the regulation of inflammatory mediators, and IRF-1, a transcription factor involved in the host defense against pathogens, tumor prevention, and development of the immune system.

2. Materials and methods

2.1. Reagents

The mouse IgG1 isotype control and mouse monoclonal antibodies (mAbs) to LTβR and IRF-1 were obtained from R&D Systems (Minneapolis, MN, USA). SB203580 was obtained from Calbiochem International Inc. (La Jolla, CA, USA). U0126, rabbit polyclonal antibodies to extracellular signal-regulated kinase (ERK)-1/2 (p42/44 mitogen-activated protein kinase (MAPK)), phospho-ERK-1/2 (Thr202/Tyr204), IκB-α, p38 MAPK, phospho-p38 MAPK (Thr180/Tyr182), and Jun amino-terminal kinase (JNK) MAPK, rabbit mAbs to cAMP response element-binding protein (CREB) and phospho-NF-κB p65 (ser536) (9H11), and mouse mAb to phospho-JNK MAPK (Thr183/Tyr185) (G9), phospho-IκB-α (Ser32/36) (SA5), and phospho-CREB (ser-133) (1B6) were acquired from Cell Signaling (Danvers, MA, USA). Mouse mAb to NF-κB p65 and goat polyclonal antibody to β-actin were obtained from Santa Cruz (Santa Cruz, CA, USA). Bacterial LPS and SP600125 were purchased from Sigma–Aldrich (St. Louis, MO, USA); TWS119 was acquired from Selleck Chemicals (Houston, TX, USA).

2.2. Cell culture and activation

The human macrophage-like cell line THP-1 was cultured in RPMI 1640 (WelGENE Inc, Korea), supplemented with 10% FBS, 0.05 mM β-mercaptoethanol, and 1 x streptomycin–penicillin at 37 °C in 5% CO2. THP-1 cells (1.0 x 10^6 cells/well in 100 μl of RPMI 1640 medium in 96 well plate) were pretreated for 30 min with 0.5–5 μg/ml of anti-LTβR mAb (clone 71319, R&D Systems) and then stimulated with 100 ng/ml LPS for 2–24 h. For cross-tolerance experiment, the pretreatment period was extended to 30 h. If required, cells were treated with various inhibitors for 30 min before the mAb pretreatment: 10 μM U0126 for ERK MAPK, 5 μM SB203580 for p38 MAPK, 10 μM SP600125 for JNK MAPK, and 10 μM TWS119 for GSK3β. All the inhibitors were dissolved DMSO. Triplicate samples were prepared for ELISA measurements.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The quantity of cytokines present in the supernatant was measured using a sandwich ELISA (R&D Systems). Briefly, flat-bottom 96 well plates were coated with capture antibodies (diluted to 1/250 in coating buffer) and incubated overnight at 4 °C. Wells were washed three times with 0.05% Tween-20 diluted in PBS (wash buffer) and blocked with 200 μl of 1 x Assay diluent for 1 h at RT. After washing, 100 μl of samples/well were added and incubated for 2 h at RT. The wells were sequentially incubated 1 h at RT with 100 μl of detection antibodies (diluted to 1/250 in 1 x Assay diluent), streptavidin-HRP (diluted to 1/250 in 1 x Assay diluent) for 30 min at RT, 100 μl of substrate solution (e Bioscience) for 30 min at RT, and 50 μl 2N H2SO4 to stop the reaction. The colorimetric change was detected by microplate reader set to 450 nm (corrected by absorption at 540 nm). The measurement was performed in triplicate and the detection limit was <10 pg/ml.

2.4. Western blot analysis

Cell lysates were obtained for Western blot at various time points after activation and analysis was performed as described previously [32,33]. Briefly, cell lysates were collected using NP-40 lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris, pH 8.0) containing 1 x protease inhibitor cocktail (Calbiochem). Proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Millipore, USA), the membrane was incubated in blocking solution (TBS-T: 5% nonfat dried milk in TBS containing 0.1% Tween-20), washed with TBS-T, incubated at 4 °C with primary antibodies in blocking solution for 1–13 h, washed with TBS-T, incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at 4 °C for 1 h and then washed. Bands were visualized by using enhanced chemiluminescence detection reagents (Pierce, Rockford, USA) and by exposure to X-ray films. For the densitometric analysis of Western blot data, band intensities were measured by densitometer and normalized with band intensities of corresponding actin loading control.

2.5. Reverse transcription polymerase chain reaction (RT-PCR)

Conventional and real time RT-PCR was performed as described previously [34,35]. Briefly, 5 μg of total RNAs isolated from cells were treated with RNase-free DNase (BD-Pharmingen, San Jose, CA, USA). The RNA was used as a template for the generation of first-strand complementary DNAs (cDNAs) using a RevertAid™ first strand cDNA synthesis kit (Fermentas, Hanover, MD, USA) with 500 ng oligo (dT)12–18 primers. PCR primers were designed using the ABI PRISM Primer Express 2.0 (Applied Biosystems), and prepared by Geno Tech Corp (Daejeon, Korea). Primer sequences were 5’−CAA ATC CGG GTC ATC TGG-3’ (forward) and 5’−CTG CCT TTG CCC CTG TGT-3’ (reverse) for IRF-1 and 5’−TGC AGT GGC AAA GTG GAG ATT-3’ (forward) and 5’−TTG AAT TGG CCG TGA GTG GA-3’ (reverse) for GAPDH. The real time PCR reaction was performed in the ABI PRISM 7300 sequence detector (Applied Biosystems) using the SYBR green PCR mix (Applied Biosystems) with cDNA corresponding to 125 ng of original RNA and 400 nM primers in a volume of 20 μl. The threshold cycle (Ct) values obtained for each reaction were normalized using the GAPDH Ct value. Primers corresponding to IL-8 and GAPDH for conventional RT-PCR were designed using the national center for biotechnology information (NCBI) primer-basic local alignment search tool (BLAST), and synthesized by Geno Tech Corp (Daejeon, Korea). Primer sequences were 5’−CAA GGA AAA CTG GGT GCA GA-3’ (forward) and 5’−TGG GAT ATT CTG TGG GCC CT-3’ (reverse) for IL-8 and 5’−ATC GCC ACC CAG AAG AC-3’ (forward) and 5’−TGA GCT TGA CAA AGT GGT CG-3’ (reverse) for GAPDH. After heating at 94 °C for 5 min, PCR was performed under following conditions: 35 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min. After second step, PCR was performed 1 cycle of 72 °C for 7 min. PCR products were resolved on a 1.2% agarose gel stained with ethidium bromide. For the densitometric analysis of RT-PCR data, band intensities were measured by densitometer and normalized with intensities of corresponding GAPDH bands.

2.6. RNA interference

The IRF-1 expression was suppressed using a mixture containing three specific small interfering RNA (siRNA) coding for IRF-1, purchased from Santa Cruz Biotechnology. The siRNA were transfected into cell lines as described in a previous study [36] using Dharma FECT (Dharmacon Inc., Lafayette, CO, USA), as per the manufacturer protocols.
2.7. ELISA-based measurement of NF-κB binding activity

NF-κB binding activity was measured as per a previously described method [37]. Briefly, biotin-labeled double-stranded oligonucleotides containing a consensus NF-κB binding site (5′-CAC AGT TGA GGG CAC TTT CCC AGG C-3′) (0.02 nm/well) was immobilized in streptavidin-coated 96-well culture plates. Whole cell or nuclear lysates were then added to the NF-κB oligo plates (100 μl/well in phosphate buffered saline), and incubated at room temperature for 1 h. The whole cell and nuclear lysates were observed to produce the same results. The plates were then sequentially incubated with antibodies specific to NF-κB p65 subunit, horseradish peroxidase (HRP)-labeled secondary antibody and tetramethylbenzidine (chromogen). The absorbance (450–500 nm) of the solution was then measured, and the values normalized by subtracting the background values. For blocking, cell lysates were pre-incubated with 1.0 nm/sample of double-stranded oligonucleotides containing the wild type NF-κB binding sequence or a mutant sequence (5′-CAC AGT TGA GGC CAC TTT CCC AGG C-3′) before addition to the NF-κB oligo plates.

2.8. Statistical analysis

All data were presented as mean ± standard error of mean (SEM), with the number of independent experiments indicated in the figure legends. All analyses were performed using the SPSS software (IBM, Armonk, NY, USA) with one-way ANOVA or the paired or unpaired Student’s t-test, as appropriate. Differences were considered significant at p < 0.05.

3. Results

3.1. LTβR stimulation enhances the LPS-induced expression of IL-8

THP-1 is a human macrophage-like cell line that expresses LTβR, as determined by flow cytometry (data not shown). Monoclonal antibody (mAb) against LTβR was used for the specific stimulation of LTβR since utilization of natural ligands such as LIGHT or lymphotixin may cross activate other receptors of TNF receptor superfamily. As seen in Fig. 1A, anti-LTβR mAb alone failed to induce cytokine production. The application of mAb for a 30 min pretreatment caused a strong increase in LPS-induced production of IL-8 (Fig. 1A and B). This result indicates that the mAb has agonistic activity. On the other hand, the isotype-matching mouse IgG did not enhance the LPS response, indicating that this enhancement did not result from non-specific interactions of the antibody with the FcR present on the macrophage surface. Other cytokines such as TNF-α, and MCP-1 was similarly increased by the mAb but the enhancing effect was less than that of IL-8. Therefore, further studies were focused on IL-8. In order to determine if the enhancement of IL-8 production occurred at the transcriptional level, RT-PCR analysis of the cytokine mRNA was performed. As seen in Fig. 1C, LPS-induced IL-8 mRNA levels were increased by antibody pre-treatment indicating that the pretreatment with anti-LTβR mAb resulted in the enhancement of LPS-induced signaling pathway, which in turn lead to transcription activation of the IL-8 gene.

3.2. LTβR induces NF-κB activation via the MAPK and glycogen synthase kinase (GSK)3β/CREB pathways.

As the MAPK activation is induced by LPS, MAPK activation was investigated in THP-1 cells pretreated with anti-LTβR mAb and stimulated with LPS. As seen in Fig. 2A, ERK and JNK MAPK phosphorylation was enhanced by the antibody pretreatment, while that of p38 MAPK was not affected. In agreement with this observation, the inhibitors of ERK or JNK MAPK efficiently blocked IL-8 production in cells treated with the antibody and LPS (Fig. 2B).

MAPK activation is known to activate NF-κB [38–40], the major transcription factor involved in inflammatory activation in the macrophages. In order to test the functional activity of NF-κB, the NF-κB binding sequence elements were immobilized in a culture plate, and the binding activity was tested in cells stimulated with the antibody and LPS. As seen in Fig. 3A, LPS-induced NF-κB binding was enhanced by antibody pretreatment (with statistical significance), indicating that LTβR-mediated activation of ERK and JNK MAPKs leads to the enhancement of NF-κB activity.

CREB is a ubiquitous transcription factor involved in proliferation, survival, development, and tumorigenesis. During inflammatory response, phospho-CREB antagonizes NF-κB activity (reviewed in [41]). In order to determine if LTβR activation affects CREB activity, CREB phosphorylation was examined. As seen in Fig. 3B, pretreatment with anti-LTβR mAb reduced LPS-induced phosphorylation of CREB.

Recent studies have revealed GSK3 to be a pivotal regulator of inflammation [42] and an upstream regulator of CREB [41]. GSK3β inactivates CREB via the direct phosphorylation of ser-129 [43], as well as the indirect de-phosphorylation of ser-133 [44]. As the CREB phosphorylation levels were altered as a result of LTβR and TLR4 stimulation, GSK3β was believed to regulate this process. In order to examine this possibility, THP-1 cells were stimulated with anti-LTβR and LPS in the presence of a GSK3β inhibitor. As seen in Fig. 3C, GSK3β inhibition resulted in the increased phosphorylation of CREB (ser-133). This indicated that LTβR affects NF-κB activity via the GSK3β/CREB pathway (in addition to the MAPK pathway). In agreement with this observation, the IL-8 enhancing effect of LTβR was greatly suppressed in the presence of the GSK3β inhibitor (Fig. 3D).

3.3. LTβR stimulation enhances the expression of IRF-1, a transcription factor for IL-8

The enhancement of LPS-induced activation of NF-κB by anti-LTβR mAb pretreatment appeared to be responsible for the slight increase in TNF-α and MCP-1. However, the extent of increase in IL-8 production was much greater that of TNF-α or MCP-1. This indicated that LTβR stimulation activated some additional IL-8 specific factors. Interestingly, the expression IRF-1, a transcription factor involved in the expression of IL-8, but not that of TNF-α or MCP-1, was induced in the THP-1 cells by anti-LTβR mAb or LPS treatment, as determined by real time RT-PCR (Fig. 4A). Accordingly, Western blot analysis revealed that the cells serially treated with anti-LTβR and LPS displayed higher overall levels of IRF-1 when compared to the cells treated with LPS alone (Fig. 4B).

In order to confirm the role of IRF-1 in the enhancement of LPS-induced expression of IL-8, the expression of IRF-1 was down-regulated by RNA interference. A mixture of three LTβR-specific siRNA was used to transfect the THP-1 cells. IRF-1 expression of was successfully down-regulated by the IRF-1 specific siRNAs, but not by the control siRNA (Fig. 4C). These transfecants were pretreated with anti-LTβR mAb and stimulated with LPS. As seen in Fig. 4D, the enhancing effect of anti-LTβR mAb was decreased in cells transfected with IRF-1 specific siRNA compared to the cells transfected with the control siRNA.

3.4. LTβR-induced cross tolerance applies to TNF-α, but not to IL-8

A previous analysis of LTβR expression in macrophages revealed that the stimulation of LTβR for an extended period of time induces tolerance against later stimulation by TLR ligands. Wimmer et al.
reported that the pretreatment of macrophages with anti-LTBR mAb for 30 h (instead of 30 min, as in our analysis) induced cross-tolerance to TLR4 and TLR9 ligands which was manifested by the decrease in the expression of TNF-α or IL-6. In order to examine if this type of LTBR-induced cross tolerance applied to IL-8, THP-1 cells were treated with anti-LTBR mAb for 30 h and stimulated with LPS. As seen in Fig. 5, the LPS-induced production of TNF-α was significantly blocked, while the production of IL-8 was unaffected by LTBR pretreatment.

4. Discussion

Our data demonstrate that LTBR has an enhancing effect on LPS-induced IL-8 expression in the human macrophage-like cell line THP-1. The effect of LTBR on IL-8 expression was also observed in cell types other than macrophages. In bronchial epithelial cells, the stimulation of LTBR with LIGHT had a major effect on the IL-6 and IL-8 expression via the MAPK/NF-κB pathway [45]. Overexpression of LTBR in HEK293 cells led to an NF-κB- and AP-1-dependent increase in IL-8 promoter activity [46]. Our data demonstrated that the stimulation of LTBR enhances LPS-induced activation of NF-κB and IRF-1 in macrophages. This is the first report that linked LTBR activity to IRF-1 activation. The macrophage specificity of this event remains unclear, and must be investigated further. The synergistic action of NF-κB and IRF-1 is responsible for the enhanced expression of IL-8 in cells treated with anti-LTBR and LPS. As only NF-κB (and not IRF-1) is involved in TNF-α or MCP-1 expression, the effect of LTBR stimulation on these cytokines would be less, compared to that on IL-8 expression (Fig. 1A). As both IRF-1 and NF-κB are involved in IL-8 expression, the blocking effect of IRF-1-specific siRNA was determined to be incomplete in IL-8 expression (Fig. 4).
Pretreatment with anti-LTβR antibody for a longer period of time caused a reduction in the LPS-induced production of TNF-α, while that of IL-8 was unaffected. This indicated that LTβR-mediated cross-tolerance may apply to some, but not all, of the proinflammatory mediators. During the extended pretreatment period, LTβR-mediated signaling activated the expression of the tripartite motif protein (TRIM)30α, a negative regulator of NF-κB [47], in a TNF receptor-associated factor (TRAF)3-dependent manner.

**Fig. 3.** Stimulation of LTβR enhances NF-κB activation through the suppression of CREB activity. (A) THP-1 cells were pretreated with 5 μg/ml anti-LTβR mAb for 30 min and then stimulated with 100 ng/ml LPS for 30 min. The functional activation of NF-κB was then measured using an ELISA-based NF-κB binding assay as described in Section 2. **p<0.001. (B) Cells were treated as in (A) for the indicated times. The protein levels of phospho-CREB, CREB and actin were analyzed using Western blot. Band intensities were then measured using densitometer and normalized with band intensities of corresponding actin control. Values indicate measurements relative to the zero time no treatment control (***p<0.001 when compared to the zero time control). (C) Cells were pretreated with 10 μM TWS119 (GSK3β inhibitor) for 30 min, and with 5 μg/ml anti-LTβR mAb for 30 min. The cells were then stimulated with 100 ng/ml LPS for the indicated times for the analysis of phospho-CREB and actin using Western blot. Band intensities were then measured using densitometer and normalized with band intensities of corresponding actin control. Values indicate measurements relative to the zero time no treatment control (**p<0.05 and ***p<0.01 when compared to the zero time control), (D) Cells were treated as in (C) for 24 h. Culture supernatants were then collected for TNF-α and IL-8 concentration measurement using ELISA (***p<0.001 when compared to the corresponding LPS-treated positive control). VC, 0.04% DMSO.

TRIM30α subsequently suppressed the expression of TNF-α and IL-6 in cells stimulated with TLR ligands [11,13]. As the extended stimulation of LTβR did not affect IL-8 expression, it appeared that the IRF-1 activity induced by anti-LTβR treatment persisted during the 30 h treatment period and this was strong enough to overcome the NF-κB inactivation. This further indicated that IRF-1 activity could sustain and induce IL-8 expression, even when the NF-κB activity was suppressed.

**Fig. 4.** Stimulation of LTβR induces the expression of IRF-1, which in turn is responsible for the activation of IL-8 gene expression. (A) THP-1 cells were treated with 5 μg/ml anti-LTβR mAb or LPS for 2 h, and the IRF-1 gene expression measured using real-time RT-PCR (**p<0.001 when compared to the control), (B) Cells were pretreated with 5 μg/ml anti-LTβR for 30 min and stimulated with 100 ng/ml LPS for the indicated times. The IRF-1 protein levels were analyzed using Western blot. Band intensities were then measured using densitometer and normalized with band intensities of corresponding actin control. Values indicate measurements relative to the zero time no treatment control (**p<0.05 and ***p<0.01 when compared to the zero time no treatment control), (C) Cells were transfected with control siRNA (siCON) or IRF-1-specific siRNA (siIRF1). The IRF-1 and actin expression were analyzed using Western blot at 48 h after transfection. (D) Control or siIRF1 transfectants were treated as in (B) for 24 h. Culture supernatants were then collected to measure the IL-8 concentration (ELISA). *p<0.05, NS, no significance.
Current data indicate that LTβR enhanced the LPS-induced NF-κB activity through two separate mechanisms; the MAPK and GSK3β/CREB pathways. MAPKs play a pivotal role in LPS-induced activation of pro-inflammatory cytokines and MAPK inhibition blocks the LPS-induced activation of NF-κB [38–40] and TNF-α expression [48–50]. The NF-κB activity is manifested through its interaction with the transcription coactivator CREB-binding protein (CBP) [51,52]. This protein, along with its close relative p300, serves as a protein scaffold and connects diverse transcription factors to the transcription apparatus [45,53]. CBP/p300 also have histone acetyltransferase (HAT) activity, which endows p300/CBP with the capacity to influence chromatin activity by modulating the histones in the nucleosome [45]. The KIX region in the CBP interacts with the NF-κB p65 subunit and the serine-133 phosphorylated form of CREB [51,52]. This results in a competition between NF-κB and CREB to interact with CBP; the dominance of NF-κB over CBP for CBP binding results in the suppression of CREB-mediated gene expression and vice versa [52,54,55]. Pretreatment with anti-LTβR mAb, and the subsequent stimulation with LPS led to the reduction in phospho-CREB levels (Fig. 3B). This indicates that the LTβR-mediated decrease in phospho-CREB and the resulting increase in the interaction between CBP and NF-κB (in addition to the MAPK/NF-κB pathway) could be the underlying mechanism responsible for the activation of NF-κB in our experimental setting.

GSK3 plays a central role in both innate and adaptive immune responses [42] and is an upstream regulatory molecules of CREB [41]. Current data show that the inhibition of GSK3β resulted in an increase in CREB phosphorylation, while almost completely eliminating IL-8 production, suggesting that the treatment with anti-LTβR mAb and LPS activates GSK3β, which then decreases phospho-CREB levels. Decrease in phospho-CREB will lead to the increased interaction between NF-κB and CBP. To our knowledge, this is the first report implicating GSK3β in the LTβR-mediated signaling pathway. Currently, the upstream signaling adaptor that links the LTβR to GSK3β activation is not known and this could be the subject for future research.

Current data indicate that the LTβR stimulation enhances the LPS-induced expression of IL-8 via the combined action of NF-κB and IRF-1. As IL-8 is involved in the pathogenesis of various diseases including chronic inflammatory diseases and cancer, proper regulation of IL-8 expression using LTβR or these transcription factors will contribute to the development of therapeutic applications. Furthermore, analysis of the expression pattern of LTβR in the disease specimens will enhance our understanding on the role of LTβR in the pathogenesis of these diseases.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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