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Activation of LXR attenuates collagen-induced arthritis via suppressing BLyS production

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ABSTRACT

B-lymphocyte stimulator (BLyS) plays a critical role in the pathogenesis and progression of rheumatoid arthritis (RA). Liver X receptor (LXR), a nuclear receptor, has an important anti-inflammatory effect. However, it is unclear whether the BLyS expression is regulated by LXR. In this study, we found that treatment with LXR agonist in collagen-induced arthritis (CIA) mice significantly attenuated arthritis progression, and markedly decreased BLyS production in serum and splenocytes as well as the production of serum IFNγ and TGFβ. Activation of LXR in B lymphocytes dramatically suppressed the basal and IFNγ/TGFβ-induced BLyS expression. Moreover, LXR agonist prominently suppressed the binding of NF-κB to *BLyS* promoter region, and decreased the promoter's transcriptional activity. Additionally, activation of LXR obviously repressed IFNγ-induced STAT1 activation and TGFβ-induced SMAD3 activation. These results indicated that downregulation of BLyS may be a novel mechanism by which LXR ameliorates RA, and LXR/BLyS pathway may serve as a novel target for the treatment of RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, progressive, systemic inflammatory autoimmune disease that mainly targets the synovial membrane, cartilage, and bone. Although the exact cause is incompletely understood, it has been demonstrated that B lymphocytes interact with pro-inflammatory cytokines play a critical role in the pathological processes of RA [1,2]. B lymphocytes contribute to RA pathogenes is not only through autoantibody production, antigen presentation, but also through the cytokines release and T cell activation [3,4]. Therefore, it is necessary to investigate the abnormal expressed molecular in B lymphocytes, which was associated with RA processes.

B lymphocyte stimulator (BLyS) is produced as a membrane-bound or soluble form by B lymphocytes, monocytes and macrophages. It has an essential pathogenic role in B lymphocyte proliferation, differentiation and autoantibody production [5–7]. Previous study has shown that overproduction of BLyS can destroy B lymphocytes self tolerance status, which is closely involved in the pathogenesis and progression of RA [8]. In patients with RA, serum levels of BLyS are considerably higher than that in the healthy controls [9]. Furthermore, BLyS levels positively correlate with rheumatoid factor titers and the intensity of inflammatory response [10]. Elevated BLyS levels is paralleled with the production of anti-collagen type II (CII) autoantibody in collagen-induced arthritis (CIA) mice [11,12]. In addition, transgenic mice overproduction of BLyS leads to an enlarged spleen and increased number of mature B cells [13,14]. Thus, BLyS has become an ideal therapeutic target for RA, and suppression of BLyS expression may be considered as an alternative therapeutic approach to targeting B cells.

Liver X receptors (LXRα and LXRβ), ligand-activated transcription factors of the nuclear receptor superfamily, were initially found as key regulators of lipid and cholesterol metabolism [15,16]. Recent studies indicated activation of LXR possesses anti-inflammatory properties, mainly through inhibit the expression of genes which involving in inflammatory [17–19]. In fibroblast-like synoviocytes (FLS) from patients with RA, LXR exerts beneficial effects by inhibiting the expression of pro-inflammatory mediators, such as IL-1β, IL-6, MMP-9 and COX-2, and by suppressing the invasion of FLS [17,20]. Moreover, activation of LXR suppresses the degree of joint damage by inhibiting the expression of pro-inflammatory mediators in CIA mice [21,22]. Previous studies have reported that LXR was constitutively expressed and functional in human B lymphocytes, which inhibited IgE expression and prevented homocysteine-induced production of IgG antibodies via NF-κB pathway [23,24]. However, it is not well known about the mechanisms by which LXR inhibits RA. Especially, it is unclear whether the anti-RA effect of LXR is associated with regulation of BLyS expression. In this study, we found that activation of LXR by GW3965 significant attenuated arthritis progression in CIA mice, which was accompanied with suppresses of BLyS production in serum and splenocytes as well as decreases of

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serum interferon-γ (IFNγ) and transforming growth factor β (TGFβ). Importantly, we found that LXR inhibited basal BLyS expression in B lymphocytes via suppression of NF-κB activation, and LXR repressed IFNγ/TGFβ-induced BLyS expression possibly through negatively interfering with STAT1 and Smad3 signaling, respectively. These studies suggest that BLyS may play an important role in LXR-mediated suppression of RA, and inhibition the production of BLyS may be a novel mechanism by which LXR ameliorates RA.

2. Materials and methods

2.1. Induction and treatment of collagen-induced arthritis

Male DBA/1 J mice were purchased from the Laboratory Animal Center of China. All mice were used at 7–9 week-old and maintained under specific pathogen-free (SPF) conditions. Animal studies were complied with the World Medical Association Declaration of Helsinki and were approved by the Animal Care Committee of the Third Military Medical University.

DBA/1 J mice were immunized with chicken type II collagen (CII) (Chondrex, Redmond, USA) as described previously [25]. To examine the anti-arthritic effect of the specific LXR agonist GW3965 (Sigma, St. Louis, USA), mice with CIA were injected intraperitoneally with GW3965 (0.5 mg/kg) daily from day 28 after the first immunization. Control mice received vehicle alone. The severity of arthritis in all four paws of mice was evaluated as previously described [26].

2.2. Preparation of splenocyte suspensions from CIA mice

On day 49 after the first immunization, spleens were aseptically removed from mice in each group and washed with cold PBS. Then the splenocyte suspensions were prepared by mechanical disruption of the mice spleens through a 100-μm-cell strainer. The suspensions were centrifuged on Ficoll–Paque at 1200 g for 25 min and the cell layers were collected and incubated for 2 h at 37 °C to allow adherence of macrophages. Nonadherent cells were collected and cultured in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum (FBS) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37 °C in 5% CO_2.

2.3. Measurement of cytokine levels in serum and splenocytes

For the analysis of serum cytokine levels, BLyS was quantified using ELISA-specific quantification kits (R&D Systems, MN, USA) according to the manufacturer's protocol. Other cytokine concentrations in serum were detected by a 25-plex mouse cytokine assay (Minneapolis, MN, USA) according to the manufacturer's instructions using a luminex platform. For the analysis of lymphocytes from spleen cytokine levels, single-cell suspensions (1×10^6 cells/ml) were cultured in triplicate to 48-well plates. IFNγ and TGFβ levels in the culture media supernatants were measured by ELISA, as previously described [27].

2.4. Histological analysis

The hind paws were fixed in 10% neutral-buffered formalin. Subsequently, these paws were decalcified in 15% EDTA. Sections (5 μm) were stained with hematoxylin and eosin (H&E) according to standard methods. The joint pathology was examined and scored as previously described [21].

2.5. Reagents and cell culture

TRIzol reagent, M-MLV reverse transcriptase and all products for cell culture were purchased from Invitrogen (Carlsbad, CA, USA). TurboFect transfection reagent was obtained from Fermentas (Glen Burnie, MD, USA). Oligo (dT) primer, luciferase assay system, β-galactosidase assay system and the firefly luciferase reporter vector pGL3-basic were from Promega (Madison, Wisconsin, USA). LightShift chemiluminescent EMSA kit was from Pierce (Pierce, Rockford, USA). ChIP kit was from Upstate (Lake Placid, NY, USA). Human IFNγ and TGFβ were from PeproTech (Princeton, USA). Human B lymphoma cell lines (Namalwa and Daudi) were from American Type Culture Collection, and cultured in DMEM supplemented with 10% (v/v) fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37 °C in 5% CO2. Cells were treated with or without GW3965 at indicated concentration (0.5 μM or 5 μM) according to previous studies [23,24], taking 0.1% DMSO as the vehicle control.

2.6. Quantitative real-time RT-PCR

Total RNAs were extracted with TRIzol reagent. Reverse transcription (RT) and real time quantitative PCR (qRCR) were performed using the SYBR Green Mix on iQ5 system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The following primers were used: for human BLyS, 5′-TTCCATGGCTTCTCAGCTTT-3′ (forward primer) and 5′-GTCCCATGGCGTAGGTCTTA-3′ (reverse primer); for human β-actin, 5′-GTGAAGGTGACAGCAGTCGGTT-3′ (forward primer) and 5′-GAAGTGGGGTGGCTTTTAGGA-3′ (reverse primer); for human ABCA1, 5′-GAACATTACAGCAGATCAAGAAATCC-3′ (forward primer) and 5′-ACTTTGGGAGAGAGAGGTTGTGA-3′ (reverse primer); for mouse BLyS, 5′-TACACATTTGTTCCATGGCTTC-3′ (forward primer) and 5′-GCAAGCTGAATCTCATCTCCTT-3′ (reverse primer); For mouse β-actin, 5′-TGTTACCAACTGGGACGACA-3′ (forward primer) and 5′-GGGGTGTTGAAGGTCTCAAA-3′ (reverse primer). Relative expression was calculated with normalization to β-actin values by using the $2^{-\triangle\triangle Ct}$ method.

2.7. Western blot analysis

Protein extraction and Western blot analysis were performed as previously described [28]. Antibodies against BLyS (sc-80337), β-actin (sc-47778) and NF-κB p65 (sc-109X) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit anti-phospho-STAT1 (Ser727), anti-STAT1, anti-phospho-Smad3 (Ser423/425) and anti-STAT1 antibodies were purchased from Cell Signaling Technology (Cell Signaling, MA, USA). Horseradish peroxidase (HRP)-conjugated goat anti-mouse or anti-rabbit IgG and the ECL chemiluminescence kit were from Pierce (Rockford, IL, USA).

2.8. Transfection assays

Namalwa cells were in logarithmic growth phase at the time of transfection. The transfection procedure mediated by TurboFect was performed according to the manufacturer's instruction. The luciferase reporter (pBLyS/1082, or pBLyS/571) have been constructed in our previous studies [29], and pGL3-basic plasmid was added to ensure identical amounts of DNA in each well. Five hours after transfection, the cells were treated with GW3965 (5 μM) or vehicle alone for 24 h. Transfection efficiency was determined by cotransfection of pCMV-β-galactosidase plasmid (pCMV-β-gal, Stratagene, USA). The luciferase and β-gal activities in cell extracts were measured as described [30], and the luciferase activity was normalized against β-gal activity. Transfection experiments were carried out 3 times in triplicate. Data were represented as the ratios of luciferase activities/β-galactosidase activities. Small interfering RNAs (siRNAs) specific for LXRα (sc-38828) or LXRβ (sc-45316) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and transfection of siRNAs was performed according to manufacturer's instructions. Then the cells were harvested and the assays were performed by Western blot.