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TL1A induces the expression of TGF-β-inducible gene h3 (βig-h3) through PKC, PI3K, and ERK in THP-1 cells

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ABSTRACT

βig-h3, an extracellular matrix protein involved in various biological processes including cellular growth, differentiation, adhesion, migration, and angiogenesis, has been shown to be elevated in various inflammatory processes. Death receptor 3 (DR3), a member of the TNF-receptor superfamily that is expressed on T cells and macrophages, is involved in the regulation of inflammatory processes through interaction with its cognate ligand, TNF-like ligand 1A (TL1A). In order to find out whether the TL1A-induced inflammatory activation of macrophages is associated with the up-regulation of βig-h3 expression, the human acute monocytic leukemia cell line (THP-1) was stimulated with either recombinant human TL1A- or DR3-specific monoclonal antibodies. Stimulation of DR3 up-regulated the intracellular levels as well as the secretion of βig-h3. Utilization of various inhibitors and Western blot analysis revealed that activation of protein kinase C (PKC), extracellular signal-regulated kinase (ERK), phosphoinositide kinase-3 (PI3K), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is required for TL1A-induced βig-h3 expression. PKC appears to be the upstream regulator of PI3K since the presence of PKC inhibitor blocked the phosphorylation of AKT without affecting ERK phosphorylation. On the other hand, suppression of either PI3K or ERK activity resulted in the suppression of IκB phosphorylation. These findings indicate that TL1A can regulate the inflammatory processes through modulation of the βig-h3 expression through two separate pathways, one through PKC and PI3K and the other through ERK, which culminates at NF-κB activation.

1. Introduction

As a member of TNF-receptor superfamily, death receptor 3 (DR3, TNFRSF25, Apo-3, TRAMP, LARD, or WSL-1) contains a conserved death domain in its cytoplasmic region similar to tumor necrosis factor receptor-1 (TNFR-1) and CD95 (also called FAS or APO-1) [1]. DR3 was found to be expressed primarily in T lymphocytes and myeloid lineage cells [2–4]. The ligand for DR3 was identified to be TNF-like ligand 1A (TL1A, TNFSF15) [5]. TL1A regulates, through its interaction with DR3, the function of T and NK cells and the negative selection process during thymocyte development [2,4]. TL1A treatment or cross-linking DR3 with monoclonal antibodies (mAb) induced proliferation and/or cytokine/chemokine production in T cells and macrophages [3,6]. Accordingly, DR3 has been implicated in the pathogenesis of inflammatory diseases such as irritable bowel disease [7], atherosclerosis [3], and rheumatoid arthritis (RA) [2].

βig-h3 (TGFBI, MP78/70, RGD-CAP, keratoepithelin) was first identified in the human adenocarcinoma cell line A549 that had been treated with TGF-β [8–11]. βig-h3 has been shown to bind to extracellular matrix (ECM) proteins such as collagen type I, II, IV, and VI [9,12], and fibronectin [13] through integrins and modulate the adhesion, migration, and chemotactic potential of cells [14,15]. The over-expression of βig-h3 was first detected in inflammatory processes associated with atherosclerosis, wound healing, diabetic angiopathy, cyclosporine nephropathy, and RA [16–20]. These observations suggest that βig-h3 is involved with the modulation of inflammatory responses and regulation of its expression could be a potential target for the treatment of chronic inflammatory diseases. In this study, βig-h3 expression patterns were investigated in the human macrophage-like cell line, THP-1 after stimulation of DR3 using recombinant human TL1A (rhTL1A) or DR3-specific mAb. The signaling pathway involved in DR3-induced βig-h3 expression was also investigated.

2. Materials and methods

2.1. Antibodies, reagents, and cell culture

Human TL1A and mouse IgG isotype control were purchased from R&D System (Minneapolis, MN, USA). The anti-human

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βig-h3 mAb (clone 18B3) were generated as previously described [21]. Rottlerin, GÖ6976, Ro31-8425, SB203580, c-Jun N-terminal kinase (JNK) inhibitor I (JNK-I1), negative control for JNK inhibitor I and LY294002 were obtained from Calbiochem International Inc. (La Jolla, CA, USA). Mouse mAb against DR3 (clone 4E3) was purchased from ABGENT (San Diego, USA) or from Abnova (clone M07) (Taipei City, Taiwan). Both antibodies induced similar responses in the THP-1 cells. Rabbit polyclonal antibodies to ERK (p42/44 MAPK), phospho-ERK (Thr202/Tyr204), IκB-α, AKT, phospho-AKT (Ser473), mouse mAb to phospho-IκB-α (Ser32/36) (5A5), PD98059, and U0126 were purchased from Cell Signaling (Danvers, MA, USA). Rabbit polyclonal antibodies to p50 and actin were purchased from Santa Cruz (CA, USA). Ethyl pyruvate, sulfasalazine, bacterial lipopolysaccharide (LPS), saponin, and brefeldin-A were purchased from Sigma (St. Louis, MO, USA). The human monocytic cell line, THP-1, cells were cultured according to the supplier's instruction (American Type Culture Collection, Manassas, VA, USA). The THP-1 cells were grown in RPMI 1640 medium (WelGENE Inc., Daegu, Korea) which was supplemented with 10% FBS, 0.05 mM β-mercaptoethanol, glucose, and streptomycin–penicillin at 37 °C in 5% CO_2 incubators.

2.2. Reverse transcriptase polymerase chain reaction (RT-PCR)

To quantify the βig-h3 mRNA expressions in the THP-1 cells, RT-PCR was performed. THP-1 cells (2×10^6 cells/well in 24 well plates containing 2 ml serum-free RPMI medium) were stimulated with 30 μg/ml immobilized anti-DR3 mAb for 12, 18, and 24 h. Total cellular RNA was extracted from whole cells and 3 μg of RNA was treated with RNase free DNase (Takara, Otsu Shiga, Japan) for the synthesis of first-strand cDNA using a RevertAid First-Strand cDNA Synthesis Kit (Fermentas, Hanover, USA) according to the manufacturer's protocol. Primer sequences for the 660 bp-long βig-h3 product were 5′CCA TCA CCA ACA ACA TCC AG3′ (forward primer) and 5′GAG TTT CCA GGG TCT GTC CA3′ (reverse primer). For GAPDH (391 bp products), 5′ATC ACT GCC ACC CAG AAG AC3′ (forward primer) and 5′TGA GCT TGA CAA AGT GGT CG3′ (reverse primer) were used. A PCR was carried out under following conditions: 1 cycle of 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 56.6 °C for 40 s and 72 °C for 40 s followed by one cycle of 72 °C for 7 min. PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide.

2.3. Western blotting

For the detection of βig-h3, ERK, phospho-ERK, AKT, phospho-AKT, IκB, and phospho-IκB, the THP-1 cells were incubated in a serum-free RPMI medium and stimulated with anti-DR3 mAb that had been immobilized at 2–20 μg/ml concentrations. Supernatants were collected for the analysis of secreted form of βig-h3 and cell lysates were collected to assess cell-associated βig-h3. For the cell lysis, NP-40 (IGEPAL CA-630) lysis buffer (150 mM NaCl, 1% IGEPAL CA-630, 50 mM Tris, pH 8.0) with a protease inhibitor cocktail (Calbiochem) was used. The lysates were clarified by centrifugation (12,000 rpm for 15 min at 4 °C). 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.

2.4. Intracellular staining and flow cytometry

The THP-1 cells were stimulated with anti-DR3 mAb or mouse IgG that had been immobilized at 20 μg/ml concentration for 24 h. Six hours after stimulation, cells were incubated with 3 μg/ml of brefeldin-A. The cells were washed once with ice-cold staining buffer (1 mM Sodium azide, 0.5% BSA in PBS) and resuspended in 100 μl PBS. The cells were then fixed by adding 100 μl of 4% paraformaldehyde, incubated at room temperature in dark for 20 min, and washed twice with ice-cold staining buffer. The fixed cells were then permeabilized with staining solution containing 0.5% saponin at room temperature (RT) for 10 min. The cells were stained with anti-βig-h3 mAb for 30 min in the dark, washed twice, and incubated with 0.5 μg of fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG (Caltag-Medsystems, Buckingham, UK). For background level fluorescence, cells were stained with an isotype matching control antibody. Flow cytometry was performed on the FACS-calibur system (Becton Dickinson, Mountain View, CA). Fluorescence profiles of 1×10^4 cells were collected and analyzed.

2.5. Immunofluorescence assay

For the detection of subcellular location of NF-κB p50 subunit, THP-1 cells (1.5×10^5 cells/well in 96-well plates) were treated with anti-DR3 antibody, lipopolysaccharide (LPS), or mouse IgG (isotype control). After treatment, cells were washed with PBS, resuspended in 10 μl of 4% paraformaldehyde in PBS, and placed onto slide glass for 30 min that was followed by 5 min washing in PBS. The fixed cells were then stained with 1.43 μM 4′,6-diamidino-2-phenylindole (DAPI) (Molecular probe, Eugene, USA) for 10 min at 37 °C and then washed with PBS for 5 min. Cells were then permeabilized with 1% Triton X-100 in PBS for 10 min at RT, washed with 0.02% Tween-20 in PBS for 5 min, and 0.02% Tween-20/1% BSA in PBS for 5 min. Cells were then treated with 20 μg/ml anti-p50 polyclonal antibody for 45 min at 37 °C in a humid chamber, and washed with 0.02% Tween-20/1% BSA in PBS for 5 min. Alexa Fluor 594-labeled goat anti-rabbit antibody (20 μg/ml) (Molecular probe, Eugene, USA) was then added for 45 min at 37 °C in a humid chamber, and then cells were sequentially washed with 0.02% Tween-20 in PBS for 5 min and PBS for 5 min. Finally, the cells were dried at RT and mounted with a mounting medium (Dako, Glostrup, Denmark). For the calculation of the percentage of cells with nuclear NF-κB, pictures of five random high-power fields were taken (with a total of about 100 cells in each sample) and cells were counted with bare eyes.

2.6. ELISA-based measurement of NF-κB binding activity

A previously described method [22] was followed for the measurement of NF-κB binding activity. Briefly, biotin labeled double-stranded oligonucleotides containing a consensus NF-κB binding site (5′cacagttgaggggactttcccaggc3′) was immobilized (0.02 nm/well) onto streptavidin-coated 96-well culture plates to obtain NF-κB oligo plates. Cells were stimulated with LPS or immobilized anti-DR3 mAb/mouse IgG for 15–90 min and total cell lysates were obtained using NP-40 lysis buffer. Cell lysates (10 μg/well) were then added into the NF-κB oligo plates and incubated at room temperature for 1 h with mild agitation. The plates were then incubated with mAb specific to NF-κB p65 subunit (clone F-6, Santa Cruz). The amounts of bound mAbs were then detected with sequential incubation with HPR-labeled goat anti-mouse IgG and tetramethylbenzidine (chromogen). Absorbance (450–540 nm) was then measured and the values were normalized by subtracting the background values which were obtained in wells treated with all the reagents except cell lysate. For the blocking, cell lysate were