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Deoxynivalenol induces ectodomain shedding of TNF receptor 1 and thereby inhibits the TNF-α-induced NF-κB signaling pathway

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ABSTRACT

Trichothecene mycotoxins are known to inhibit eukaryotic translation and to trigger the ribotoxic stress response, which regulates gene expression *via* the activation of the mitogen-activated protein (MAP) kinase superfamily. In this study, we found that deoxynivalenol induced the ectodomain shedding of tumor necrosis factor (TNF) receptor 1 (TNFRSF1A) and thereby inhibited the TNF-α-induced signaling pathway. In human lung carcinoma A549 cells, deoxynivalenol and 3-acetyldeoxynivalenol inhibited the expression of intercellular adhesion molecule-1 (ICAM-1) induced by TNF-α more strongly than that induced by interleukin 1α (IL-1α), whereas T-2 toxin and verrucarin A exerted nonselective inhibitory effects. Deoxynivalenol and 3-acetyldeoxynivalenol also inhibited the nuclear factor κB (NF-κB) signaling pathway induced by TNF-α, but not that induced by IL-1α. Consistent with these findings, deoxynivalenol and 3-acetyldeoxynivalenol induced the ectodomain shedding of TNF receptor 1 by TNF-α-converting enzyme (TACE), also known as a disintegrin and metalloproteinase 17 (ADAM17). In addition to the TACE inhibitor TAPI-2, the MAP kinase or extracellular signal-regulated kinase (ERK) kinase (MEK) inhibitor U0126 and the p38 MAP kinase inhibitor SB203580, but not the c-Jun N-terminal kinase (JNK) inhibitor SP600125, suppressed the ectodomain shedding of TNF receptor 1 induced by deoxynivalenol and reversed its selective inhibition of TNF-α-induced ICAM-1 expression. Our results demonstrate that deoxynivalenol induces the TACE-dependent ectodomain shedding of TNF receptor 1 *via* the activation of ERK and p38 MAP kinase, and thereby inhibits the TNF-α-induced NF-κB signaling pathway.

1. Introduction

Proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1), induce intracellular signaling pathways, one of which leads to the activation of the transcription factor nuclear factor κB (NF-κB) (Karin and Greten, 2005). When TNF-α and IL-1 engage with TNF receptor 1 (TNFRSF1A) and IL-1 receptor, respectively, they recruit distinct sets of adaptor proteins, this results in the activation of the inhibitor of κB (IκB) kinase as a common target (Hayden and Ghosh, 2008; Bhoj and Chen, 2009). Immediately after IκB kinase phosphorylates IκB, which prevents the nuclear translocation of the NF-κB subunits, phosphorylated IκB undergoes ubiquitination and proteolytic degradation by proteasomes (Perkins, 2006). The NF-κB subunits are released and translocated to the nucleus where they stimulate the transcription of diverse target genes that regulate inflammatory responses, including the gene encoding intercellular adhesion molecule-1 (ICAM-1) (Roebuck and Finnegan, 1999).

TNF-α-converting enzyme (TACE), also known as a disintegrin and metalloproteinase 17 (ADAM17), is a cell-surface metalloproteinase that mediates the ectodomain shedding of various ligands (e.g., TNF-α) and receptors (e.g., TNF receptor 1) (Scheller et al., 2011). TACE is ubiquitously expressed in many cell types and its activity is regulated by different mechanisms, including its posttranslational modification (Scheller et al., 2011). In response to various stimuli, extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein (MAP) kinase phosphorylate the cytoplasmic tail of TACE at threonine 735 and thereby TACE-dependent ectodomain shedding is induced (Díaz-Rodríguez et al., 2002; Soond et al., 2005; Liu et al., 2009; Xu and Derynck, 2010). Several translation inhibitors are known to induce the activation of the MAP kinase superfamily by interacting with ribosomes in an intracellular mechanism designated the ribotoxic stress response (Iordanov et al., 1997; Kataoka, 2012). Recently, we have shown that different translation inhibitors (acetoxycycloheximide and cytotrienin A) induce the ectodomain shedding of TNF receptor 1 *via* the activation of ERK and p38 MAP kinase (Ogura et al., 2008a, 2008b; Yamada et al., 2011a).

Trichothecene mycotoxins are a large group of sesquiterpenoids produced by *Fusarium* and other fungi. They are often found

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as contaminants in agricultural staples and are known to exert acute and chronic effects on animals and humans (Pestka et al., 2004; Pestka, 2010). Trichothecene mycotoxins interact with eukaryotic ribosomes and block the peptidyl transferase reaction. They also exhibit both immunosuppressive and immunostimulatory activities (Pestka et al., 2004; Pestka 2010). In addition to the induction of cell death in leukocytes, deoxynivalenol also upregulates the expression of proinflammatory cytokines (e.g., TNF-α) and chemokines in macrophages and monocytes *via* the ribotoxic stress response (Chung et al., 2003; Islam et al., 2006). However, it remains unclear whether deoxynivalenol modulates the intracellular signaling pathways induced by proinflammatory cytokines. In this study, we investigated the biological activities of deoxynivalenol and its structural analogs in the NF-κB signaling pathway and the gene expression in response to proinflammatory cytokines. Our results demonstrate for the first time that deoxynivalenol rapidly induces the ectodomain shedding of TNF receptor 1 and thereby inhibits the TNF-α-induced NF-κB signaling pathway.

2. Materials and methods

2.1. Cell culture

Human lung carcinoma A549 cells (JCRB0076) and human hepatocellular carcinoma HepG2 cells (RCB1648) were provided by the Health Science Research Resources Bank (Tokyo, Japan) and RIKEN BRC through the National Bio-Resource Project of MEXT, Japan (Tsukuba, Japan), respectively. A549 cells and HepG2 cells were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) heat-inactivated fetal calf serum (JRH Biosciences, Lenexa, KS, USA) and a mixed penicillin–streptomycin solution (Nacalai Tesque Inc., Kyoto, Japan).

2.2. Reagents

Deoxynivalenol, 3-acetyldeoxynivalenol, verrucarin A, and 1,9-pyrazoloanthrone (SP600125) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). T-2 toxin (Enzo Life Sciences International, Inc., Plymouth Meeting, PA, USA), *N*-(*R*)-(2-(hydroxyaminocarbonyl)methyl)-4-methylpentanoyl-L-*t*-butyl-glycyl-L-alanine 2-aminoethyl amide (TAPI-2; Peptide Institute, Inc., Osaka, Japan), 1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene (U0126; Wako Pure Chemical Industries, Ltd., Osaka, Japan), and 4-(4-fluorophenyl)-2-(4-methysulfinylphenyl)-5-(4-pyridyl)1H-imidazole (SB203580; Cayman Chemical Co., Ann Arbor, MI, USA) were obtained commercially. Recombinant human TNF-α and human IL-1α were kindly provided by Dainippon Pharmaceutical (Osaka, Japan).

2.3. Antibodies

Antibodies to β-actin (AC-15; Sigma-Aldrich), cyclooxygenase-2 (Cox-2) (Clone 33; BD Biosciences, Franklin Lakes, NJ, USA), ERK1/ERK2 (#9102; Cell Signaling Technology, Inc., Danvers, MA, USA), cellular FLICE-inhibitor protein (c-FLIP) (Dave-2; Alexis Co., Lausen, Switzerland), ICAM-1 (clone 15.2; Leinco Technologies, Inc., St. Louis, MO, USA), ICAM-1 (clone 28; BD Biosciences), NF-κB p50 (H-119; Santa Cruz Biotechnology, Santa Cruz, CA, USA), NF-κB p65 (C-20; Santa Cruz Biotechnology), p38 MAP kinase (#9212; Cell Signaling Technology), poly(ADP–ribose) polymerase (PARP) (C-2–10; Sigma-Aldrich), phospho-ERK1/ERK2 (Thr202/Tyr204) (#9101; Cell Signaling Technology), phospho-p38 MAP kinase (Thr180/Tyr182) (#9211; Cell Signaling Technology), and TNF receptor 1 (H-5; Santa Cruz Biotechnology) were obtained commercially.

2.4. Assay for cell-surface expression of ICAM-1

A549 cells were washed twice with phosphate-buffered saline (PBS) and incubated with 1% paraformaldehyde–PBS for 15 min. The fixed cells were washed twice with PBS and incubated overnight in the presence of 1% bovine serum albumin (Sigma-Aldrich)–PBS for blocking. Then, the cells were incubated with mouse anti-human ICAM-1 IgG antibody (clone 15.2) for 60 min and thereafter washed three times with 0.02% Tween 20–PBS. The cells were further incubated with horseradish peroxidase (HRP)-linked anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) for 60 min and then washed three times with 0.02% Tween-20–PBS. The cells were incubated with the substrate solution (0.2 M sodium citrate (pH 5.3), 0.1% *o*-phenylenediamine dihydrochloride, 0.02% H_2O_2) at 37 °C for 20 min. The absorbance at 415 nm was measured with a Model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA).

2.5. Assay for macromolecular synthesis

A549 cells were pulse-labeled with [4,5-^{3}H]L-leucine (41.66 TBq/mmol; Moravek Biochemicals, Inc., Brea, CA, USA). The cells were washed three times with PBS and lysed with 0.25 M NaOH for 15 min. The proteins were precipitated by incubation on ice for 1 h in the presence of 5% trichloroacetic acid. Cell lysates were separated into supernatants and precipitates by centrifugation (10,000 × *g*, 5 min). The precipitates were washed once with 5% trichloroacetic acid. Radioactivity of the supernatants and the precipitates was measured with a 1900CA TRI-CARB® liquid scintillation analyzer (Packard Instrument Co., Meriden, CT, USA).

2.6. Preparation of cell lysates and western blotting

A549 cells were washed once with PBS and lysed with Triton X-100 lysis buffer (50 mM Tris–HCl (pH 7.4), 1% Triton X-100, the protease inhibitor mixture Complete™ (Roche Diagnostics, Mannheim, Germany), 2 mM DTT, 2 mM orthovanadate). Cell lysates were centrifuged (10,000 × *g*, 5 min) and separated into supernatants as cytoplasmic fractions and pellets. The pellets were washed twice with Triton X-100 lysis buffer and then solubilized as nuclear fractions. The culture medium was centrifuged (10,000 × *g*, 5 min) to remove cell debris and insoluble materials. The proteins were then precipitated with chloroform/methanol. The protein samples (30 μg/lane) were separated by SDS–PAGE and transferred onto Hybond-ECL nitrocellulose membranes (GE Healthcare, Piscataway, NJ, USA). The membranes were incubated overnight with 4% skim milk in 0.5% Tween 20–PBS for blocking, and then incubated with the primary antibodies and HRP-linked secondary antibodies (Jackson ImmunoResearch). The protein bands were detected with ECL Western blotting detection reagents (GE Healthcare) and analyzed with the ImageQuant LAS 4000 mini (GE Healthcare).

2.7. Statistical analysis

Statistical significance was assessed with one-way ANOVA followed by the Tukey test for multiple comparisons. Differences with P values of <0.05 were considered to be statistically significant.