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Enhanced transglutaminase 2 expression in response to stress-related catecholamines in macrophages

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ABSTRACT

Transglutaminase 2 (TG2) is a multifunctional protein that contributes to inflammatory disease when aberrantly expressed. Although macrophages express TG2, the factor stimulating TG2 expression remains poorly characterized in these cells. In the present study, we examined the effects of the stress-related catecholamines adrenaline and noradrenaline on macrophage expression of TG2 in RAW264.7 murine macrophages and murine bone marrow-derived macrophages. Treatment with adrenaline markedly increased TG2 mRNA expression and increased TG2 protein levels. While the β_2-adrenoceptor-selective antagonist ICI 118,551 completely blocked adrenaline-induced TG2 mRNA expression, the β_2-adrenoceptor specific agonist salmeterol increased TG2 expression. Noradrenaline also increased TG2 mRNA expression at higher doses than the effective doses of adrenaline. The effect of adrenaline on TG2 mRNA expression was mimicked by treatment with the membrane-permeable cAMP analog 8-Br-cAMP. Thus, increased intracellular cAMP following stimulation of β_2-adrenoceptors appeared to be responsible for adrenaline-induced TG2 expression. Because stress events activate the sympathetic nervous system and result in secretion of the catecholamines, adrenoceptor-mediated increase in macrophage TG2 expression might be associated with stress-related inflammatory disorders.

Introduction

Transglutaminase 2 (TG2) is a multifunctional protein and a calcium-dependent protein-crosslinking enzyme (Kumar and Mehta 2013). TG2 is found in the cytoplasm, nucleus, mitochondria, cell surface, and extracellular matrix (ECM) (Griffin et al. 2002; Piacentini et al. 2005). Irreversible crosslinking of ECM proteins by TG2 appears to promote net formation of ECM molecules (Verderio et al. 2004). Moreover, various roles of TG2 have been shown in cell proliferation, differentiation, signal transduction, apoptosis, inflammation, and wound healing (Fesus and Piacentini 2002; Telci and Griffin 2006).

TG2 also plays a pathogenic role in inflammatory disorders, such as celiac disease (Dieterich et al. 1997) and tissue fibrosis (Griffin et al. 2002). Recently, it was reported that TG2 is a critical inducer of T helper type 17 (T_h17) differentiation and subsequent inflammatory amplification after noninfectious pulmonary injury in a murine model of pulmonary fibrosis (Oh et al. 2011). Moreover, TG2 exacerbates mouse experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, by increasing numbers of interleukin (IL)-17- and interferon (IFN)-γ-producing cells in the spinal cord (Oh et al. 2012). Accordingly, multiple roles of TG2 in immune-mediated inflammation have been demonstrated, and therapeutic targeting of this molecule appears to be effective against inflammatory disorders.

Stress events activate the sympathetic nervous system and result in secretion of adrenaline (Ad) and noradrenaline (NA), which are responsible for the "fight-or-flight" response. However, excessive stress can be deleterious to biological functions, and stress events are associated with the pathogenesis and progression of various diseases (Chrousos 2005). Several stressors are immunosuppressive and thereby affect susceptibility to infectious diseases

Abbreviations: Ab, antibody; Ad, adrenaline; AR, adrenoceptor; BMM, bone marrow-derived macrophages; ECM, extracellular matrix; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HRP, horseradish peroxidase; IL, interleukin; IFN, interferon; LPS, lipopolysaccharide; NA, noradrenaline; PCR, polymerase chain reaction; RT, reverse transcription; TG2, transglutaminase 2; T_h17, T helper type 17; TNF, tumor necrosis factor.

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and severity of immune disorders (Glaser and Kiecolt-Glaser 2005; Franco et al. 2007). Ad and NA inhibit the expression of the proinflammatory cytokines tumor necrosis factor (TNF), IL-12, and IL-23 by innate-immune cells, such as macrophages and dendritic cells (Goyarts et al. 2008; Zinyama et al. 2010; Yanagawa et al. 2010). In addition, these catecholamines can increase the secretion of the anti-inflammatory cytokine IL-10. Thus, Ad- and NA-mediated alternations in cytokine balance may be associated with, at least in part, stress-related immune dysfunction.

In contrast, stress may promote inflammation and influence the development and severity of inflammatory disorders, such as inflammatory bowel disease, rheumatoid arthritis, and psoriasis (Rampton 2011), which may be associated with secretion and effects of catecholamines on immune cells, such as macrophages (Bonaz and Bernstein 2013). However, the mechanisms underlying stress-induced exacerbation of inflammatory disease are not precisely understood.

Macrophages are representative inflammatory cells that play crucial roles in the regulation of inflammatory responses, wound healing, and inflammatory disease. Although macrophages express TG2 (Hodrea et al. 2010), the stimulators of TG2 expression remain poorly characterized. In the present study, we investigated the effects of the stress-related catecholamines NA and Ad on TG2 expression in RAW264.7 murine macrophages and murine bone marrow-derived macrophages (BMM). This study demonstrates that NA and Ad markedly upregulate TG2 mRNA and protein expression by inducing β_2-adrenoceptor (AR)-mediated cAMP signaling in macrophages.

Materials and methods

Reagents and antibodies (Abs)

RPMI-1640 medium was purchased from Sigma–Aldrich (Saint Louis, MO) and was supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin. Synthetic Ad, NA, prazosin (α_1-AR antagonist), yohimbine (α_2-AR antagonist), propranolol (β-AR antagonist), ICI 118,551 (β_2-AR antagonist), salmeterol (β_2-AR agonist), and 8-Br-cAMP were obtained from Sigma–Aldrich. Anti-TG2 monoclonal Ab (D11A6), anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) monoclonal Ab (14C10), and anti-rabbit IgG horseradish peroxidase (HRP)-linked Ab were purchased from Cell Signaling Technology (Beverly, MA). Lipopolysaccharide (LPS, ultra pure grade, *Escherichia coli* O111:B4) was obtained from InvivoGen (San Diego, CA).

Culture of cell lines

RAW264.7 cells were obtained from the American Type Culture Collection (ATCC). THP-1 cells were obtained from the European Collection of Cell Cultures (ECACC). RAW264.7 and THP-1 cells were cultured in 5% FCS RPMI-1640. Cells were cultured at a density of 2×10^6 (RAW264.7) or 1×10^6 (THP-1) cells/ml/well in a 24-well plate and were treated with Ad, NA, salmeterol, or 8-Br-cAMP for indicated time periods. In some experiments, cells were pretreated with prazosin, yohimbine, propranolol, or ICI 118,551 for 60 min and were then stimulated with Ad in the presence of each antagonist or inhibitor. After cell treatments, reactions were terminated by rapidly cooling the cells on ice.

Culturing BMM

Murine BMM were generated by an established method as previously described (Zamboni and Rabinovitch 2003), using L929-cell culture supernatant. L929 cells were obtained from ECACC and the culture supernatant was prepared by culturing the cells for 4 days in 10% FCS RPMI1640. Bone marrow cells were prepared from femur and tibial bone marrow of female C57BL/6 mice (Japan SLC Inc., Hamamatsu, Japan). The cells were cultured in 10% FCS RPMI-1640 medium containing 20% L929 culture supernatant, at a density of 2×10^7 cells/10 ml/10 cm dish (non-treated, Corning Incorporated, Corning, NY). On day 4, 10 ml of the fresh medium was added. On day 7, the cells were detached by treatment with 3 mM ethylenediaminetetraacetic acid for 5 min at 37 °C. Cells were then collected and used as BMM (>85% F4/80$^+$).

BMM were cultured in 5% FCS RPMI-1640 medium at a density of 1×10^6 cells/ml/well in a 24-well plate and were stimulated with Ad or salmeterol for indicated time periods. In some experiments, BMM were pretreated with ICI 118,551 for 60 min and were then stimulated with Ad for the indicated time periods in the presence of ICI 118,551. After cell treatments, reactions were terminated by rapidly cooling the cells on ice.

All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of the Health Sciences University of Hokkaido.

Quantification of mRNA expression by reverse transcription (RT)-polymerase chain reaction (PCR)

Total RNA from the cells was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA). Levels of mRNA for TG2 in the RNA samples were determined by quantitative RT-PCR using the SYBR® PrimeScript® RT-PCR Kit II (Takara Bio, Shiga, Japan) as previously described (Yanagawa et al. 2011). PCR was carried out using the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Primer sequences for TG2 and GAPDH genes were obtained from the PrimerBank, a public database for PCR primers (http://pga.mgh.harvard.edu/primerbank/). The primer sequences and amplicon sizes were as follows: mouse TG2 (PrimerBank ID 6678329a1) forward, 5′-GACAATGTGGAGGAGGGATCT-3′ and reverse, 5′-CTCTAGGCTGAGACGGTACAG-3′ (120 bp); human TG2 (PrimerBank ID 39777596c1) forward, 5′-GAGGAGCTGGTCTTAG AGAGG-3′ and reverse, 5′-CGGTCACGACACTGAAGGTG-3′ (184 bp); murine IL-1β (PrimerBank ID 6680415a1) forward, 5′-GCAAC TGTTCCTGAACTCAACT-3′ and reverse, 5′-ATCTTTTGGGGTCCGTC AACT-3′ (89 bp); murine GAPDH (PrimerBank ID 6679937a1) forward, 5′-AGGTCGGTGTGAACGGATTTG-3′ and reverse, 5′-TGTAG ACCATGTAGTTGAGGTCA-3′ (123 bp); human GAPDH (PrimerBank ID 83641890b1) forward, 5′-AAGGTGAAGGTCGGAGTCAAC-3′ and reverse, 5′-GGGGTCATTGATGGCAACAATA-3′ (102 bp). GAPDH gene expression was used as an endogenous control. Data were analyzed using the 7500 System SDS version 1.3.1 software (Applied Biosystems). Levels of specific mRNA in stimulated cells were presented as relative expression compared to the control culture using the $\Delta\Delta C_T$ method (Yuan et al., 2006).

Immunoblotting

Immunoblotting was performed as previously described (Yanagawa et al. 2010). The cells were washed with ice-cold phosphate-buffered saline, and whole cell lysates were prepared using a cell lysis buffer (Cell Signaling Technology). The cell lysates were separated by SDS-PAGE and then blotted onto a polyvinylidene fluoride membrane (Millipore, Bedford, MA). The membrane was probed with primary Ab and was developed using a HRP-linked secondary Ab by enhanced chemiluminescence.

Statistical analysis

The unpaired Student's *t*-test or Tukey's test was used to analyze data for significant differences. The data in the study with multiple time points were analyzed using two-way analysis of variance and