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Multiple activating and repressive *cis*-promoter regions regulate *TNFSF15* expression in human primary mononuclear cells

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ABSTRACT

TL1A/*TNFSF15* has been associated with IBD (inflammatory bowel disease) in GWAS (genome-wide association study) and plays a role mediating mucosal inflammation in IBD. Higher TL1A expression is associated with disease severity in both patients and mouse models. Although TL1A has been studied extensively for IBD-associated SNPs, the *cis*/*trans*-regulatory regions are poorly defined. Herein we identify response elements regulating *TNFSF15* in primary human myeloid cells. Peripheral mononuclear cells transfected with *TNFSF15* promoter constructs displayed 30-fold enhanced promoter activity in a minimal −74 bp region. Transactivation was mediated partly by AP-1, since mutation of the AP-1 site resulting in loss of promoter activity. Monocytes transfected with c-Jun siRNA or treated with TAT-TI-JIP (JNK Inhibitor VII TAT-TI-JIP) demonstrated reduced TL1A mRNA and protein levels. Surprisingly, constructs larger than −74 bp did not increase promoter expression (expression of −1275 bp construct was 25% of −74 bp activity), suggesting the presence of both activating and repressing TL1A promoter elements. In fact, mutation of the −210 bp NFκB site enhanced promoter activity (60-fold) suggesting a repressive role for this site. DNA–protein binding to the TL1A AP-1 and NFκB elements was inhibited by excess consensus or TL1A oligonucleotides and binding and confirmed by chromatin immuno-precipitation analysis. Yet, despite the fact that the −210 bp NFκB site acts as a suppressor element, overall mRNA and protein expression were inhibited in monocytes treated with MG132 (NFκB/proteasome inhibitor) or SN50 (NFκB-p50 blocking peptide), suggesting that NFκB acts as both an activator and silencer of TL1A expression. These data suggest that modulation of TL1A expression involves a complex interplay between positive and negative signals, binding to distinct regulatory regions.

1. Introduction

TL1A (*TNFSF15*) is a tumor necrosis factor family member expressed by monocytes, macrophages, dendritic cells, T cells and endothelial cells [1–3]. Expression of TL1A is initiated via signaling of different molecular pathways depending on the cell type. TL1A was first reported to be expressed in human endothelial cells in response to TNFα or IL-1 [1]. In innate immune cells, such as monocytes, macrophages, and dendritic cells, TL1A expression is induced following stimulation by immune complexes and through interaction with enteric microorganisms [2,3]. In T cells, expression of TL1A occurs as a consequence of inflammation or T cell receptor stimulation [4]. We have previously shown that TL1A augments inflammatory cytokine secretion in PB T cells through IL-12/IL-18-mediated production of IFN-γ and, augments both IFN-γ production and cytotoxicity in IL-12/IL-18-activated NK cells [5,6]. Recently, a role for TL1A was identified in regulation of Th17 responses [7–10].

Although TL1A has been implicated in the inflammatory process of several diseases, such as renal inflammation, rheumatoid arthritis, asthma and irritable bowel syndrome [10–14], its role in the context of inflammatory bowel disease is probably best established. Expression of TL1A is upregulated in macrophages and T cells of inflamed tissue from the colon and small bowel of CD (Crohn's Disease) patients compared to uninvolved areas, and in serum of patients with active UC (ulcerative colitis) [15–17]. TL1A is currently under development as a potential therapeutic target for treating IBD, since two different murine models of colitis have demonstrated the potential for anti-TL1A blocking antibodies to effectively reduce the severity of inflammation [10,18].

TNFSF15 has been identified and confirmed in GWAS as an IBD-associated gene [19]. *TNFSF15* is one of the few genes that is associated with IBD in all ethnic populations studied. Although the functional significance of these polymorphisms remains unknown, expression of TL1A in monocytes from patients carrying disease-associated SNPs is enhanced and more rapid [20]. Furthermore, risk SNPs within the *TNFSF15* locus are associated with the

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development of severe, medically-refractive UC [21], as well as fibrostenotic CD [22,23]. In transgenic mice, constitutive TL1A expression in lymphoid or myeloid cells spontaneously leads to intestinal inflammation and fibrosis, and disease severity positively correlates with transgene expression levels [18,22,24]. Likewise, protein expression is associated with IBD disease severity in both patients and mouse models, and is believed to play a key role in driving the inflammatory process.

Although *TNFSF15* has been studied extensively for IBD-associated SNPs, the *cis*-and *trans*-regulatory regions remain poorly defined. Two studies of TNFα mediated regulation of TL1A expression in murine and human endothelial cell lines identified NFκB (−210 bp) and AP-1 binding (+12 bp) sites [25–27] within the *TNFSF15* promoter region. However, TNFα transactivation of the *TNFSF15* promoter resulted in a modest 1.5–5-fold increase of promoter activity. Likewise, activation of the human monocytic U937 cell line with 5 μg LPS (lipopolysaccharide), resulted in less than 2-fold enhancement of promoter activity. Although the authors proposed a role for NFκB in LPS-mediated activation of TL1A, the biological significance of this finding remains to be determined [27]. In the present study we identified regulatory response elements and pathways that mediate primary human myeloid expression of TL1A. In view of the importance of myeloid expression of TL1A as a modulator of inflammation in IBD, understanding the molecular mechanisms involved in transcriptional regulation of TL1A may help elucidate molecular pathways involved in the pathogenesis of disease.

2. Materials and methods

2.1. Study subjects

Human subjects were recruited through the IBD Center at Cedars-Sinai Medical Center. All controls subjects were healthy individuals, free of medication, and with no known personal or family history of autoimmune disease or IBD. Informed consent (approved by the Institutional Review Board at Cedars-Sinai Medical Center) was obtained from all participating subjects.

2.2. Isolation of monocytes

PBMC (peripheral blood mononuclear cells) were isolated from healthy volunteers by separation on Ficoll-Hypaque gradients. Monocytes were isolated using negative selection by depletion with magnetic beads (Stemcell Technologies, Vancouver, BC, Canada) and were at least 95% pure.

2.3. TL1A ELISA assay

TL1A was measured by an amplified ELISA. ELISA plates (Greiner Bio-One, Longwood, FL) were coated overnight with 100 μL of 5 μg/mL monoclonal anti-TL1A (Clone 04H08, Teva Pharmaceuticals, Irvine, CA). Samples and standards were added for 24 h followed by addition of 100 μL of 2.5 μg/mL biotinylated anti-TL1A (Clone 16H02, Teva) for 2 h. This was followed by addition of 100 μL of 1/1000 diluted alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 h. Substrate, 0.2 mM NADP (Sigma–Aldrich, St. Louis, MO) was added for 30 min followed by addition of amplifier (3% 2-propanol, 1 mM iodonitrotetrazolium violet, 75 μg/mL alcohol dehydrogenase, and 50 μg/mL diaphorase; Sigma–Aldrich) for 30 min. Plates were read at 490 nm using an E max plate reader (Molecular Devices, Sunnyvale, CA).

2.4. Gel Mobility electrophoretic shift assay (EMSA)

Nuclear extract protein (3–6 μg) or human recombinant c-Jun protein (Promega) was incubated at 25 °C with 0.25 mg/ml poly (dI-dC), in 20% glycerol, 5 mM $MgCl_2$, 2.5 mM EDTA, 2.5 mM DTT, 250 mM NaCl, 50 mM Tris pH 7.5 for 10 min. Oligonucleotides 5′-IRD700-labeled (Integrated DNA Technology, Coraville, Iowa) were then added (250 fmol) and the binding reactions incubated for an additional 30 min. Specificity was determined by the addition of 50–100-fold excess unlabeled oligonucleotide as competitor. The DNA–protein complexes were separated from unbound probe on a pre-run native 6% polyacrylamide gel in low ionic strength buffer (22.3 mM Tris pH 7.4, 22.3 mM Borate, 0.5 mM EDTA pH 8.0) and analyzed with Odyssey infrared imaging system (Li-Cor Biosciences). The elements used were:

- *TNFSF15* NFκB 5′-TCCTTCAGGGACTTTCCTAACTTC-3′,
- mut *TNFSF15* NFκB 5′-TCCTTCAcaGACgcTCCTAACTTC-3′,
- *TNFSF15* AP-1 5′-GGTGACTTTAATCACTCAGTCTCC-3′,
- mut *TNFSF15* AP-1 5′-GGTtgctTTAATCACTCAGTCTCC-3′.

Consensus AP-1 and NFκB oligonucleotides were obtained from Santa Cruz Biotechnology (Santa Cruz, California).

2.5. Promoter cloning and reporter constructs

The *TNFSF15* promoter–reporter (Firefly luciferase) constructs were a generous gift from the laboratory of Steinberg et al. [26]. Constructs, originally numbered from first translated sequence, have been re-labeled to reflect their position relative to the transcriptional start site.

2.6. Transfection

Freshly isolated PBMC were transfected following overnight culture in RPMI 1640 medium containing 10% fetal calf serum. Cells were then washed and resuspended in 250 μL fresh medium at 2×10^7 cells/ml and electroporated in the presence of 50 μg of Firefly reporter construct (600 V, for 9 pulses of 500 μs, with 100 μs between pulses) using 4 mm (gap width) cuvettes in a BTX Electro Square Porator ECM 830 (Genetronics, Inc., San Diego, CA). A control plasmid (2 μg) containing the β-actin promoter driving Renilla luciferase (provided by Dr. Christopher Wilson, University of Washington) was co-transfected as an internal standard and values were normalized to correct for transfection efficiency:

$$\text{Normalized Fold Increase} = \frac{\text{Test}_{\text{FF}} \times \text{pGL3}_{\text{Ren}}}{\text{Test}_{\text{Ren}} \times \text{pGL3}_{\text{FF}}} \quad (1)$$

After electroporation, the cells were diluted in fresh medium, allowed to rest for 1 h prior to plating, and then stimulated with 40 ng/mL PMA (phorbol 12-myristate 13-acetate, Sigma–Aldrich, St. Louis, MO) plus 1 μg/mL ionomycin (Sigma–Aldrich) for 4 h. Luminescence was measured using a Promega (Madison, WI) luciferase assay kit and counted on a 6-detector Perkin Elmer Life Sciences (Gaithersberg, MD) 1450 Microbeta liquid z deactivated.

2.7. siRNA

Monocytes were transfected with siRNAs for c-Jun, TL1A or non-target scramble control siRNA using two transfection modalities. Transductin (15 μM, Integrated DNA Technologies) was incubated with 0.5 μM siRNA (Integrated DNA Technologies) in PBS for 30 min at 4 °C. Complexed siRNA was incubated with 1×10^5 monocytes/well in 48-well cell culture plate for 4 h at 37 °C in RPMI 1640 with 10% Q-depleted serum (Integrated DNA