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Pathological activation of canonical nuclear-factor κB by synergy of tumor necrosis factor α and TNF-like weak inducer of apoptosis in mouse acute colitis

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ABSTRACT

Tumor necrosis factor (TNF)-α is a major effector in various inflammatory conditions. TNF-like weak inducer of apoptosis (TWEAK) is a member of the TNF superfamily that promotes inflammatory tissue damage through its receptor, FGF-inducible molecule 14 (Fn14). Since both TWEAK and TNF-α have been shown to mediate pathological responses through inter-dependent or independent pathways by *in vitro*, the potential interplay of these pathways was investigated in a mouse colitis model. Acute colitis was induced by rectal injection of trinitrobenzene sulfonic acid (TNBS), with administration of control IgG, TNF receptor (TNFR)-Ig chimeric protein, anti-TWEAK monoclonal antibody, or the combination of TNFR-Ig and anti-TWEAK antibody. On day 4, disease severity was evaluated and gene expression profiling was analyzed using whole colon tissue. NF-κB activation was investigated with Western blot. Levels of transcript of TWEAK, Fn14 and NF-κB-related molecules were measured in purified colon epithelial cells (ECs). As a result, activation of the canonical (p50/RelA), but not noncanonical (p100/RelB)-mediated pathway was the hallmark of inflammatory responses in this model. Inflammation induced upregulation of Fn14 only in ECs but not in other cell types. Combination treatment of TNFR-Ig and anti-TWEAK antibody synergistically reduced disease severity in comparison with the control antibody or single agent treatment. Gene expression profile of the colon indicated downregulation of canonical NF-κB pathway with combination treatment. In conclusion, synergistic activation of canonical NF-κB by TWEAK and TNF-α is critical for the induction of inflammatory tissue damage in acute inflammation.

1. Introduction

TNF-like weak inducer of apoptosis (TWEAK) is a cytokine of the TNF ligand superfamily that is constitutively expressed by many innate and adaptive immune cell types [1,2]. In various animal models of inflammation, infiltrating leukocytes including macrophages and activated T cells produce TWEAK. On the other hand, the TWEAK receptor, fibroblast growth factor-inducible molecule 14 (Fn14), is a highly inducible molecule in mesenchymal cells, epithelial cells and endothelial cells in response to basic fibroblast growth factor [3], platelet-derived growth factor and vascular endothelial growth factor [4], indicating the activation of the TWEAK/Fn14 pathway in the context of tissue injury and inflammation occurs through induction of Fn14. Indeed, Fn14 expression is upregulated in many different organs systems in contexts of injury or disease, including the liver, heart, vasculature, skeletal muscle, kidney, intestinal epithelium, and spinal cord; and blocking or deficiency in the TWEAK/Fn14 pathway ameliorates disease in multiple models [5], including several models of colitis [6–8]. In a mouse hapten-induced colitis model [6], we found that Fn14 was

Abbreviations: ECs, epithelial cells; Fn14, fibroblast growth factor-inducible molecule 14; LPCs, lamina propria cells; LPS, lipopolysaccharide; mAb, monoclonal antibodies; MLN, mesenteric lymph node; NF, nuclear factor; PBS, phosphate-buffered saline; TLR, toll-like receptor; TNBS, trinitrobenzene sulfonic acid; TNF-a, tumor necrosis factor; TNFR2-Ig, TNF Receptor 2 fused with the murine IgG2a Fc region; TWEAK, TNF-like weak inducer of apoptosis.

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upregulated in the inflamed colonic epithelial cells (ECs), and TWEAK deficiency or TWEAK blocking mAbs significantly ameliorated this colitis, apparently by reducing inflammation, limiting chemokine and matrix metalloproteinase expression in EC and EC death [7]. Thus, TWEAK is involved in intestinal inflammation and tissue damage through inflammatory responses in EC.

Tumor necrosis factor (TNF)-α is a major cytokine that mediates inflammatory responses and tissue damage in various inflammatory conditions (reviewed in [9]). The source of TNF-α is mainly inflammatory cells type including macrophages and T cells. There are 2 cell surface receptors for TNF-α, TNFR1 and TNFR2, which are differentially expressed depending on cell types and normal/pathological conditions. TNFR1 and TNFR2 are both interact with TNF-α but generally TNFR1 is known to mediate major pathways for tissue damage by activating proinflammatory and the programmed cell-death related signals. Compared to TNFR1, TNFR2 signaling has not been well characterized, but shown to share some downstream pathways with TNFR1 and also promote tissue repair and angiogenesis. Activation of nuclear factor (NF)-κB is the key component of TNF-α signaling, leading to phosphorylation of IκB proteins to promote its ubiquitination and degradation, resulting in the nuclear translocation of NF-κB molecules, where NF-κB initiates gene transcription.

Although both TNF-α and TWEAK are thus profoundly involved in inflammatory tissue damage, interactions or independence between TNF-α and TWEAK signal pathway has not been fully elucidated. For example, TWEAK and TNF-α appear to act through independent pathways in mediating some pathological responses. The response of cultured synoviocytes, which produce inflammatory chemokines in response to TWEAK, is not inhibited by blocking TNF-α, and conversely, synoviocyte responses to TNF-α are not inhibited by anti-TWEAK mAb [10]. In addition, in the same study it was shown that combined TNF-α and TWEAK stimulation was synergistic for production of multiple proinflammatory mediators. It is also known that TNF-α induces rapid activation of the canonical (p50/RelA) pathway of nuclear factor (NF)-κB signaling. TWEAK signaling through Fn14 can activate canonical NF-κB, however in contrast to TNF-α TWEAK/Fn14 engagement reportedly induce the delayed and prolonged induction of the noncanonical (p100/RelB) NF-κB pathway [11–14]. These results suggest that TWEAK and TNF-α stimulations both yield inflammatory output but involve differential activation of NF-κB signaling pathways. In the case of intestinal epithelial cells, we recently found that in IL-13-induced apoptosis, presence of both TWEAK/Fn14 and TNF-α is indispensable, suggesting largely shared pathway of TWEAK and TNF-α in induction of programmed cell death [7]. In these intestinal explants, we also found that the process of TNF-α activation in EC, shedding from membrane of epithelial cells, was TWEAK/Fn14 dependent.

Based on these *in vitro* studies, we wished to explore the interdependency of TNF-α and TWEAK *in vivo* in an inflammatory setting. For this purpose, we chose hapten-induced colitis model in C57BL/6 strain, where acute inflammation can be stably induced. Our preliminary experiment indicated that full dose of TNF-α inhibitor was effective in prevention of colitis, while anti-TWEAK antibody administration was less effective, suggesting that other pathogenic pathways than TWEAK, especially TNF-α, are in play in this model. Thus to explore interplay between TNF-α and TWEAK and unveil the potential impact of a TWEAK-dependent pathway, we examined the effect of a suboptimal dose of TNF-α inhibitor in combination with TWEAK/Fn14 pathway blockade. This experimental system disclosed an unexpected synergy of TWEAK and TNF-α in acute inflammation.

2. Methods and materials

2.1. Mice

Wild type, 6-week C57BL/6 male mice for colitis studies were purchased from CLEA Japan and kept under specific pathogen-free conditions during experiments performed at 7–8 weeks old. All protocols were approved by the institutional animal care and use committees in the Research Institute, National Center for Global Health and Medicine.

2.2. Reagents

Trinitrobenzene sulfonic acid (TNBS) was purchased from Research Organics, (Cleveland, OH). For inhibition of endogenous TNF-α or TWEAK in mice, murine TNF Receptor 2 fused with the murine IgG2a Fc region (TNFR2-Ig) or murine anti-TWEAK IgG2a mAb (mP2D10) [15] were used, or mice were administered an isotype-matched control anti-human CD20 mAb that does not cross react with murine CD20, all prepared by Biogen Idec, Inc. (Cambridge, MA).

2.3. Induction and evaluation of colitis

TNBS colitis was induced by intrarectal administration of a 2% solution of TNBS in phosphate-buffered saline (PBS): ethanol (1:1) [16,17]. For acute inflammatory responses, 70 μg/g body weight of TNBS was given on day 0 and animals sacrificed on day 4. One hour prior to administration of TNBS, groups of mice were injected i.p. with the control IgG2a mAb (anti-human CD20) (10 mg/kg), TNFR-Ig (0.3 mg/kg), anti-TWEAK (mP2D10, 10 mg/kg), the combination of TNFR-Fc (0.3 mg/kg) and anti-TWEAK mP2D10 (10 mg/kg), or were untreated. For single agent and combination treatments groups, 0.3 mg/kg TNFR-Ig was employed, since 1 mg/kg of TNFR-Ig markedly ameliorated TNBS colitis but 0.3 mg/kg was much less effective as monitored by the effect on colon length and body weight. Open ulcer area was measured in captured macroscopic images using image J (NIH) software. Colon tissue was rolled and snap-frozen in liquid nitrogen. Specimens for RNA extraction or Western blotting were cut from the frozen rolled colon. Frozen sections, showing the whole length of the colon, were stained with hematoxylin and eosin. Histological scores were blindly assigned to each proximal, middle and distal segment as follows: 0 – normal, 1 – ulcer or cell infiltration limited to the mucosa, 2 – ulcer or limited cell infiltration in the submucosa, 3 – focal ulcer involving all layers of the colon, 4 – multiple lesions involving all layers of the colon or necrotizing ulcer larger than 3 mm in length. Thus the total possible histological score is 12. A set of experiments using 5–8 mice for each experimental group was performed 3 times. Total number of mice for each experimental condition was as follows; untreated TNBS colitis group, 12; control antibody, 20; TNFR-Ig, 24; anti-TWEAK mAb, 24; combination of TNFR-Ig and anti-TWEAK mAb, 24.

2.4. Western blotting

Snap-frozen colon tissue was crushed and total protein was extracted using standard methods. Extracts from individuals in a given treatment group pooled, resolved by SDS–PAGE and transferred to PVDF membranes (Bio Rad, Hercules, CA, USA) which were probed using the primary antibodies against IκBα (4812; Cell Signaling Technology), phospho-IκBα (9246; Cell Signaling Technology), phosho-p100 (4810; Cell Signaling Technology), p100/p52 (4882; Cell Signaling Technology), and GAPDH (AM4300; Applied Biosystems). Secondary antibodies were anti-mouse