Large amounts of the chemokine CCL5 was produced during the laparotomy. CCL5 is a chemoattractant for T cells, monocytes/macrophages, eosinophils, basophils, natural killer cells, granulocytes, and dendritic cells,[18] and therefore induce cascade reactions following immune responses in the peritoneal cavity together with CCL3, CCL4, and CXCL8, eventually leading to peritoneal adhesion. The exudate cells did not produce a significant amount of CCL5 during the overnight culture in the presence of LPS. This result suggests that peritoneal exudate cells may be not the source of CCL5 during operation. Indeed, human peritoneal mesothelial cells are reported to produce CCL5 in response to IL-1β or TNF-α, which results in significant up-regulation of CCL2 and CCL5 protein secretion.[19] These results indicate it is possible that CCL5 levels present in a sample may be indicative of mesothelial cell injury.

In our previous study using a mouse model, inhibition of macrophage migration and aggregation was an effective way of preventing peritoneal adhesion. In mice, we found approximately 0.5–1.5 ng/mL CCL1 in 2 mL peritoneal lavage fluid in mice with peritoneal adhesion, which was undetectable in our ELISA system in the peritoneal cavities of naïve mice.[9] This indicated that around 0.09 ng/g body weight (mouse body weight was estimated as 22 g) of CCL1 was secreted in mice when adhesion was induced. In contrast, in humans, only 0.00017 ng/g (body weight was estimated as 60 kg) of CCL1 was detected as mentioned above. Although we failed to detect a significant amount of CCL1 during the operation in humans, which needs further study, we did observe dramatic changes in CCL5 instead. CCL5 was produced at the level of 0.04 ng/g body weight in humans after laparotomy, which was comparable to the level of CCL1 in mice. Thus, we postulate that CCL5 may be a component of a chemokine system unique to the human peritoneal cavity. Importantly, both CCL1 in mice and CCL5 in humans are primarily produced by mesothelial cells. CCL5 induces recruitment of a wide range of cells including monocytes, T cells, eosinophils, mast cells, and basophils to sites of inflammation.[18] Indeed, we confirmed the chemoattractant activity of peritoneal lavage fluid, which was partially mediated by CCL5. In addition to its chemotactic activity, CCL5 promotes angiogenesis and cancer metastasis,[18] which may be related to the formation of postoperative adhesion. In addition, increase of CCL5 may promote postoperative cancer cell dissemination in the peritoneal cavity. Since major cytokines in the peritoneal cavity are produced by exudate cells, blocking their migration to mesothelial cells may be an effective way to prevent peritoneal adhesion. However, it is necessary to only block molecules that function specifically in peritoneal exudate and mesothelial cells, without affecting systemic immunity against infection, tissue repair, or coagulation and fibrinolytic systems. As such, inhibition of chemokines that specifically recruit immune cells may be more suitable

for inhibiting adhesion than blocking major cytokines and chemokines. Although further investigation is required, we propose that targeting CCL5 may prevent postoperative peritoneal adhesion as well as cancer metastasis.

CONCLUSION

During laparotomy, acute secretion of chemokines and cytokines, which are involved in the recruitment and activation of macrophages, was induced. IL-6 and CCL5 showed the most striking increase. Increase of CCL5 is the major primary response to surgical stress in the peritoneal cavity and is possibly involved in the mechanism of postoperative adhesion in humans.

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JLB

Review

The TWEAK/Fn14 pathway as an aggravating and perpetuating factor in inflammatory diseases; focus on inflammatory bowel diseases

Taeko Dohi and Linda C. Burkly[†,1]*

*Department of Gastroenterology, Research Center for Hepatitis and Immunology, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan; and [†]Department of Immunology, Biogen Idec, Cambridge, Massachusetts, USA

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ABSTRACT

The TWEAK/Fn14 pathway is a ligand/receptor pair of the TNFSF that has emerged as a prominent player in normal and pathological tissue remodeling. TWEAK/Fn14 pathway activation drives many processes relevant to autoimmune and inflammatory diseases. IBDs, including CD and UC, are chronic, relapsing inflammatory diseases of the GI tract. These diseases differ in their clinical, macroscopic, and histopathological presentation; however, pathological processes that prominently contribute, more or less in each case, include breakdown of the mucosal epithelial barrier, chronic inflammation, and tissue remodeling with fibrosis. TWEAK may promote the pathogenesis of IBD by signaling through Fn14, which can be up-regulated on IECs, thereby contributing to breakdown of the mucosal barrier; the induction of IEC-derived mediators that promote chronic inflammation and shape gut immunity against commensal flora; and delayed healing and fibrosis. TWEAK may also exert its action on endothelial and stromal cell types, including smooth muscle cells and fibroblasts, to promote chronic inflammation, dysregulated tissue repair, and fibrosis. Here, we review the data supporting an emerging role of the TWEAK/Fn14 pathway in autoimmune and inflammatory diseases, with a particular focus on IBD, and discuss how it interplays with other prominent pathways, including IL-13, TNF-α, and TGF-β, to aggravate and perpetuate the pathological processes underlying IBD. *J. Leukoc. Biol.* 92: 000–000; 2012.

TWEAK AND Fn14

In the past decade, the cytokine TWEAK (TNFSF12) and its receptor Fn14 (TNFRSF12a) have emerged as a ligand/receptor pair of the TNFSF that is prominently featured in normal and pathological remodeling of tissues. TWEAK, expressed primarily as a soluble cytokine by infiltrating leukocytes, mediates multiple activities through Fn14 up-regulated locally on epithelial and mesenchymal cell types in injured and diseased target tissues, including proinflammatory responses, angiogenesis, cell growth, cell death, and progenitor responses. As recently reviewed [1], a growing body of evidence supports the thesis that these TWEAK/Fn14 pathway-induced activities contribute to normal tissue regeneration and repair when appropriately orchestrated after acute injury. In contrast, these TWEAK/Fn14 pathway-induced activities contribute to progressive local tissue damage and maladaptive remodeling when exaggerated and dysregulated in injured and diseased target tissues.

TWEAK/Fn14-induced tissue remodeling is a mechanism that is broadly applicable across a variety of organs/disease target tissues. We and others have comprehensively reviewed this paradigm as it relates to several diseases, including RA, lupus nephritis, and other inflammatory kidney diseases, cardiovascular, neuroinflammatory/degenerative, and skeletal muscle diseases [1–6]. Given the role of the TWEAK/Fn14 pathway in regulating cell survival, growth, migration, and angiogenesis, implications for the role of this pathway in tumor biology have also been discussed [3, 7, 8].

A number of clues suggested a pathological role for TWEAK/Fn14 in the context of IBD. In recent years, a paradigm shift has occurred in the understanding of the role of tissue architecture cell types, such as epithelial cells and fibroblasts, in tissue responses. IECs, long viewed as principally a physical barrier, are now recognized as a critical innate immune cell type that responds to intestinal microbes and re-

Abbreviations: CD=Crohn's disease, cIAP1=cellular inhibitor of apoptosis 1, DSS=dextran sulfate sodium, Fn14=FGF-inducible molecule 14, GI=gastrointestinal, HMGB1=high-mobility group box 1, IBD=inflammatory bowel disease, IEC=intestinal epithelial cell, KO=knockout, MMP=matrix metalloproteinase, NOD=nucleotide-binding oligomerization domain-containing protein, Pam_3CysSK_4=palmitoyl-3-cysteine-serine-lysine-4, RA=rheumatoid arthritis, RIPK3=receptor-interacting serine/threonine-protein kinase 3, SMA=smooth muscle actin, TNBS=trinitrobenzene sulfate, TNFSF=TNF superfamily, TWEAK=TNF-like weak inducer of apoptosis, UC=ulcerative colitis

1. Correspondence: Department of Immunology, Biogen Idec, 12 Cambridge Center, Cambridge, MA 02142, USA. E-mail: linda.burkly@biogenidec.com

leases mediators that shape innate and adaptive immunity in health and disease [9, 10]. Thus, the focus has shifted from leukocytes to include IECs as central players in the pathogenesis of IBD. Whereas leukocytes as well as IECs may promote inflammatory and fibrogenic responses, fibroblasts and other nonhematopoietic cell types may be another prominent source of inflammatory mediators, thereby making critical contributions to the perpetuation of inflammatory responses, in addition to their well-established role as a source of cellular and ECM components. This is well exemplified by the role of fibroblast-like synoviocytes in the formation of ectopic pannus tissue, as well as in promoting the progression from acute to chronic inflammation in RA. In the intestine, α-SMA-expressing myofibroblasts have been reported to play roles in intestinal inflammation by secreting inflammatory mediators, including IL-8, MCP-1, MMPs, and IL-6 in response to TNF-α and bacterial components [11]. Furthermore, mesenchymal cells, including bone marrow-derived mesenchymal stem cells or hematopoietic stem cells, are recruited and participate in the repair of the damaged intestine of IBD [12]. Thus, the interplay among all of these cell types determines the balance between resolution of tissue injury/disease and chronic inflammation with fibrosis.

Dovetailing with these developments, clues suggesting a role for TWEAK/Fn14 in IBD came from the pattern of expression of Fn14, which was discovered initially as a FGF-inducible molecule in fibroblasts and then shown to be up-regulated dramatically in vivo in various contexts of tissue injury and disease and overexpressed in epithelial tumors, including colon carcinomas, in which TWEAK had been shown to induce cell death and inflammatory responses [8, 13]. Taken together, these observations suggested that Fn14 might be induced on IECs, fibroblasts, and other nonhematopoietic cell types in contexts of intestinal injury and in IBD, where it potentiates and perpetuates disease. Current data support the model, shown in **Fig. 1**, wherein TWEAK may act through Fn14, up-regulated on multiple nonhematopoietic cell types in the intestine to promote mucosal barrier breakdown, chronic tissue inflammation, dysregyulated tissue repair, and fibrosis, all of which are key mechanisms underlying IBD pathogenesis.

Here, we provide an overview of the TWEAK/Fn14 pathway and the cellular responses whereby persistent TWEAK/Fn14 pathway activation contributes to autoimmune and inflammatory disease pathologies. To illustrate this paradigm, we focus on IBD, where effective, new treatments are greatly needed to improve patient quality of life and prognosis, discussing key mechanisms underlying IBD pathogenesis and how the TWEAK/Fn14 pathway may contribute prominently as an aggravating and perpetuating factor.

TWEAK/Fn14 PATHWAY

TWEAK expression

TWEAK is a typical TNFSF member, synthesized as a type II transmembrane protein in the ER and expressed as a ho-

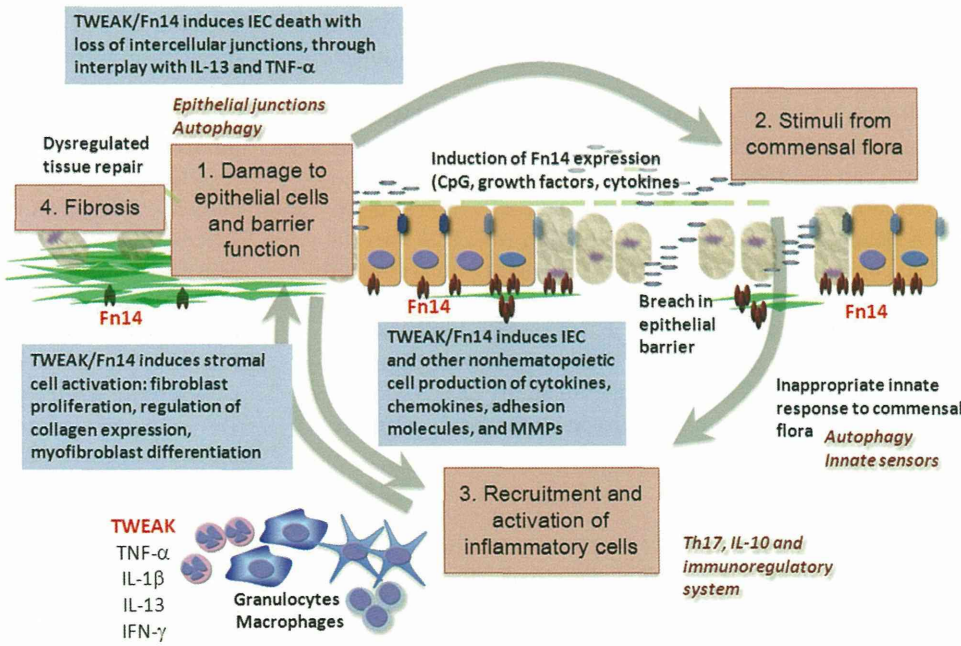

Figure 1. Model of pathological mechanisms for the TWEAK/Fn14 pathway in IBD. Scheme of the pathophysiology of IBD with key mechanisms underlying the pathogenesis of IBD shown in the pink boxes. Damage to epithelial cells and barrier function is a key underlying mechanism, leading to excessive stimulation from luminal bacteria, leading to the activation and recruitment of immune cells, resulting in a feed-forward loop, in which all of the above processes are further promoted, eventually also leading to tissue fibrosis. These processes are numbered 1–4, reflecting the described sequence. However, the loop (indicated by the gray arrows) may be initiated at any point, given individual differences in environmental and genetic factors. Corresponding processes affected by genetic susceptibility in IBD (where polymorphisms confer risk in IBD) are shown in italicized, red text. Inflammatory cells are a primary source of TWEAK. Inflammatory cells also secrete other inflammatory cytokines that activate IEC and other nonhematopoietic cells inducing the expression of Fn14. The blue boxes indicate the molecular and cellular activities, whereby the TWEAK/Fn14 pathway promotes processes 1, 3, and 4. Potential outcomes of TWEAK/Fn14 pathway signaling in this context are as follows: TWEAK signaling through Fn14 affects IEC integrity and function, promoting inflammatory cell recruitment/activation by production of inflammatory mediators, damage of the barrier and resulting defects in antibiotic peptide secretion, and immune homeostasis. Breach in the barrier further increases the inflammatory stimuli from commensal flora and subsequent production of inflammatory mediators from innate immune cells and induces/maintains the up-regulation of Fn14. Activation of mesenchymal cells and loss of IEC are involved in the delayed/dysregulated tissue repair and fibrotic changes. Thus, intestinal pathology is maintained and aggravated with the action of the TWEAK/Fn14 pathway.

motrimeric molecule. TWEAK is considered to be primarily a soluble cytokine as a result of efficient cleavage by members of the furin protease family located within the trans Golgi network [13]. Intracellular TWEAK protein is expressed by many types of leukocytes, including human resting and activated monocytes, DCs, and NK cells [14]. Consistent with leukocytes as a major source of TWEAK, TWEAK mRNA is expressed broadly by human peripheral blood-derived innate immune cells, including PMN leukocytes, macrophages, DCs, and NK cells, as well as in B and T cell subsets (Immunological Genome Project Consortium, Datagroup Human Immune Cells Garvan, Gene Symbol TNFSF12, Probe set 205611_at). TWEAK expression has also been reported in endothelial cells, astrocytes, and renal tubular epithelial cells [15–17], indicating that some nonhematopoietic lineage cells may provide an additional source of TWEAK.

TWEAK expression has been detected locally in the inflamed tissues in a variety of experimental models [3], which may reflect the contribution of leukocyte infiltrates, as well as nonhematopoeitic cell types. Notably, TWEAK levels in humans are also elevated locally within the end organs of autoimmune and inflammatory diseases, including IBD, as discussed below, supporting the potential contribution of TWEAK to human autoimmune and inflammatory pathologies [2].

Relevant to the role of TWEAK in IBD, we have recently found that TWEAK is expressed in IECs in a mouse model of colitis by immunohistochemistry [18], which was confirmed further by detection of up-regulated mRNA in inflamed IECs purified by flow cytometry (unpublished results). Given that the development of inflammation-associated colorectal cancer is also a significant complication of IBD, it is also relevant that TWEAK mRNA and protein have been reported in human colon carcinoma cell lines [19].

Whereas little is known about the regulation of TWEAK expression in cells, TWEAK protein can be up-regulated by IFN-γ or PMA but not LPS or IFN-α in cultured human peripheral blood monocytes, DCs, and NK cells [14, 20]. Interestingly, TWEAK was shown to act in combination with IFN-γ on the HT29 colon carcinoma to induce epithelial tumor cell death and IL-8 production [13]. In CD, there is an exaggerated mucosal Th1 response, where CD4 T cell express high levels of IFN-γ and Th1 response-related factors, including STAT4, T-bet, and IL-12Rβ2 [21, 22]. Thus, the ability of IFN-γ to induce TWEAK production may provide a means to promote TWEAK activity in IBD.

Fn14 expression

Fn14 is the smallest TNFRSF member and the only known signaling receptor for TWEAK [23]. Originally cloned as a bFGF-inducible molecule in fibroblasts [24], Fn14 can be highly induced by a variety of growth factors, including EGF, PDGF, and VEGF, and is principally expressed on the surface of epithelial, endothelial, fibroblasts, and other nonhematopoietic cells [8]. Of particular note, Fn14 is also expressed by many tissue progenitor cells, including epithelial, mesenchymal, and neural-lineage progenitors [3]. In contrast, Fn14 is not expressed by T or B lymphocytes and has been reported only in a few cases on monocytes/macrophages [25, 26].

Fn14 is normally expressed at relatively low levels in healthy tissues. Thus, activation of the TWEAK/Fn14 pathway is highly controlled by the inducible expression of this receptor. Fn14 up-regulation has been observed across numerous experimental systems, such as following chemical injury, hypoxia, mechanical injury, and oxidative stress, and in inflammatory disease models, likely occurring in response to growth factors produced in these contexts of tissue injury and disease [2]. This highly inducible pattern of Fn14 expression is well conserved between mouse and human, reflecting the evolutionarily conserved nature of this pathway [6]. Thus, Fn14 induction has also been observed in affected tissues from a broad range of human diseases [2], including RA, multiple sclerosis, inflammatory liver diseases, and IBD.

In addition to growth factors, a variety of other Fn14-inducing stimuli have been identified, including the cytokines TNF-α, IL-1β, IFN-γ, and TGF-β [8], which are also relevant in many autoimmune and inflammatory diseases. Thus, with respect to IBD, these cytokines may be inducers of Fn14 expression in the intestinal tissue, and as mentioned above, IFN-γ is also an inducer of TWEAK expression. Recently, Fn14 up-regulation has been shown to be induced by IL-13, TNF-α, or a TLR ligand, that is, an oligodeoxynucleotide containing CpG motifs, in intestinal explants [18, 27]. Fn14 expression is also up-regulated by IFN-γ in WiDr colon carcinoma cells (J. Michaelson, personal communication, January 2012).

The induction of Fn14 by other cytokines present in the injured/diseased tissue-specific microenvironment may explain how the TWEAK/Fn14 pathway is engaged to contribute to particular outcomes. For example, the induction of Fn14 expression by TGF-β, a major profibrotic mediator, may be a mechanism whereby the TGF-β activates the TWEAK/Fn14 pathway to contribute to fibrosis.

The induction of Fn14 by other cytokines present in the injured/diseased tissue microenvironment may also explain context-dependent differential outcomes of the TWEAK/Fn14 pathway, as TWEAK can act alone or in combination with other cytokines [3]. For example, TWEAK acts in combination with IL-13 and TNF-α but not alone to induce IEC death [27]. TWEAK alone induces renal cell proliferation but in combination with TNF and/or IFN-γ, promotes renal cell apoptosis [17, 28, 29], and TWEAK acts together with TNF-α to induce keratinocyte apoptosis [30] and synergizes with TGF-β for keratinocyte production of proinflammatory mediators [31].

TWEAK/Fn14 signaling

Fn14 has a short cytoplasmic tail, comprising only 28 aa, yet it has a diverse signaling capability [1]. Within this intracellular domain lies a TRAF-binding motif (PIEET) that facilitates functional interaction with TRAF2 and TRAF5. The ability of Fn14 to activate the NF-κB and MAPK pathways is generally attributed to this PIEET motif [32, 33]. Notably, Fn14 lacks a characteristic death domain found on many, although not all, TNFR family members.

A hallmark of TWEAK signaling through Fn14 is the induction of NF-κB pathway signaling leading to target gene transcription. Rapid activation of the canonical NF-κB pathway and stronger, delayed, and prolonged induction of the noncanoni-

cal NF-κB pathway were apparent after stimulation with a soluble CD8-TWEAK fusion protein [33]. In addition, it is now appreciated that the nature of NF-κB induction can vary with the form of TWEAK [34, 35]. Whereas TWEAK is primarily thought to function as a soluble cytokine (see above), membrane TWEAK has been described and efficiently induces the canonical NF-κB pathway, whereas a recombinant soluble form, likely reflecting the potential of the naturally occurring soluble homotrimer, induces canonical NF-κB modestly, if at all. Membrane and soluble TWEAK are potent inducers of the noncanonical NF-κB pathway. In an in vivo model, the administration of Fc-TWEAK, which is primarily a soluble hexameric form, resulted in canonical and noncanonical NF-κB signaling in various mouse tissues, including colon (J. Michaelson, personal communication, January 2012). The observation that TWEAK triggers prolonged, noncanonical NF-κB signaling is noteworthy and may reflect the role of the TWEAK pathway in mediating chronic inflammatory processes in a variety of autoimmune and inflammatory diseases, including IBD, as discussed further below. Whether some responses induced by TWEAK contribute to this delayed and prolonged induction of the noncanonical pathway has not been investigated. Evidence supporting a functional role for NF-κB signaling in response to the TWEAK has been demonstrated in numerous contexts, including mediating the proinflammatory effects of TWEAK [28, 36–39], as well as the effects of TWEAK on progenitor cell proliferation [40], cell atrophy and regeneration [41, 42], cell migration and invasion [43, 44], cell survival [45], and cell death [46] and inhibitory effects of TWEAK on differentiation [47]. The functional contribution of NF-κB signaling in vivo was confirmed by a blunted response to TWEAK administration in NF-κB signaling deficient mice [48].

Relevant to IBDs, the TWEAK/Fn14 pathway may contribute to the activation of IECs and other nonhematopoietic cell types via NF-κB, which has been shown to be markedly induced in patients and strongly influences the course of mucosal inflammation through its ability to promote the expression of various proinflammatory genes [49]. In a mouse model of chronic colitis, the NF-κB pathway is reported to mediate fibrotic changes [50]. The NF-κB signaling system is also involved in inflammation-associated tumor formation [51]. In an inflammation-related colorectal cancer model, selective deletion of a component of the canonical NF-κB pathway, IKKβ, in IEC resulted in decreased tumor incidence without affecting tumor size, with increased epithelial apoptosis during tumor promotion; whereas, deleting IKKβ in myeloid cells resulted in a significant decrease in tumor size [52]. Thus, given its role in canonical, as well as noncanonical NF-κB signaling, it is possible that TWEAK might be involved in the development of malignant tumors in IBD through augmentation of the NF-κB signaling pathway.

It is also well documented that TWEAK signals through MAPK, although whether activation is via the ERK, JNK, and/or p38 pathways is context-dependent. TWEAK-induced MAPK signaling has been reported in a wide range of cell types [1], including fibroblasts, endothelial cells, skeletal muscle cells, cardiomyocytes, renal cell types, osteoblasts, and astrocytes, reflecting the wide range of organs in which the TWEAK/Fn14 pathway signaling may contribute to outcomes in injury and disease. However, involvement of this pathway in human IBD has not been well established. More recently, there have also been reports of TWEAK signaling through other pathways, such as PI3K/AKT and Wnt/GSK3β. Induction of PI3K/AKT signaling by TWEAK was demonstrated in multiple cell types and shown to mediate TWEAK-induced responses, including proinflammatory and proliferative effects [29, 37, 53–55]. The ability of TWEAK to signal through Wnt, a pathway associated with epithelial cell development and differentiation, is supported by dephosphorylation of GSK3β in response to TWEAK in progenitor-like mesenchymal cell types [41, 55, 56]. As Wnt signaling plays significant roles in the differentiation and malignant transformation of IEC, it is of interest to investigate this pathway in the IEC in the context of regeneration after damage. Finally, there is also evidence that the JAK/STAT pathway, which has been implicated in the pathogenesis of IBD, can be induced by TWEAK in WiDr colon carcinoma cells (J. Michaelson, personal communication, January 2012).

The fact that Fn14 lacks a death domain has made it challenging to understand how the TWEAK/Fn14 pathway mediates cell death, an effect most widely described for tumor cells [57] but also observed in response to TWEAK in primary cortical neurons [46, 58], renal tubule and mesangial cells [17, 36], and keratinocytes [30], often with TWEAK acting together with other cytokines, such as IFN-γ and/or TNF-α. Although multiple mechanisms of cell death, including apoptosis, necrosis, and autophagy, may be at play, caspase cleavage in response to TWEAK has been documented in many cases [17, 58–62]. The upstream signals that mediate caspase cleavage are still not well understood, although in a subset of tumor cell lines, cell death appears to be mediated by a TWEAK-induced cIAP-TRAF2 complex with noncanonical NF-κB signaling leading to TNF-α synthesis and degradation of cIAP1, which precludes the TNF-α-induced NF-κB activation [63–65].

Relevant to IBD, we recently found that TWEAK/Fn14-induced IEC death was also dependent on TNF-α [27]. Although the precise molecular mechanism is not yet understood, IL-13 induces IEC death through TWEAK/Fn14-dependent shedding of latent TNF-α mediated by TACE, also known as ADAM17, a disintegrin and metalloproteinase domain 17, resulting in caspase-3, -8, and -9 activation (**Fig. 2**). In this case, de novo protein synthesis is not required, and also distinct from the mechanism above, cIAP1 degradation may not be involved in the IL-13-induced caspase activation, as cIAP1 protein levels were higher in the intestinal explants of WT mice after stimulation with IL-13 as compared with Fn14-deficient mice (unpublished results). We also found that exogenous TNF-α induces IEC death in mouse intestinal explants with activation of caspase 3, which was partially dependent on TWEAK/Fn14 and IL-13. Thus, TWEAK/Fn14 can function as a promoter of IEC death in the presence of IL-13 and TNF-α through a novel mechanism. TNF-α is also known to induce the necroptosis type of cell death, which is mediated by RIPKs, distinct from caspase-mediated apoptosis [66]. Up-regulation of RIPK3 in the Paneth cell of CD mucosa was reported, suggesting relevance to ileitis in CD [67]. However, deficiency of

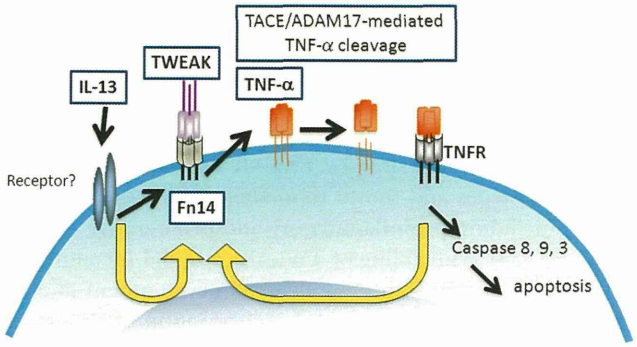

Figure 2. Scheme of the interplay of TWEAK, IL-13, and TNF-α in IEC damage. IL-13 engages the TWEAK/Fn14 pathway and thereby, induces TACE-mediated shedding of TNF-α, which then binds to the TNFR and induces caspase-dependent cell death. Black arrows indicate the pathway cascade. Yellow arrows indicate that IL-13 and TNF-α enhance Fn14 expression at the transcriptional level. Thus, IEC damage triggered by IL-13 is mediated by TWEAK/Fn14. TACE is also known as ADAM17, A disintegrin and metalloproteinase domain 17.

RIPK3 or inhibition of necroptosis by administration of necrostatin-1 did not protect mice from TNF-α-induced massive gut damage [68], indicating differential roles of apoptosis and necroptosis in IEC damage. Interplay between necroptosis and the TWEAK/Fn14 pathway is yet to be investigated.

TWEAK/Fn14 PATHWAY-DRIVEN PROCESSES

Consistent with the expression pattern and signaling potentials described above, TWEAK produced primarily by leukocytes engages Fn14 up-regulated on epithelial, endothelial, and other nonhematopoietic cells in injured and diseased tissues and promotes multiple cellular responses, thereby regulating tissue remodeling in health and disease.

General role of TWEAK/Fn14 in inflammatory responses

TWEAK-stimulated proinflammatory responses are commonly observed in epithelial, endothelial, and other nonhematopoietic cell types, indicating that TWEAK broadly affects these cell types to orchestrate the inflammatory response within tissues [3]. Through its ability to induce cytokines, chemokines, adhesion molecules, and MMPs in these cell types, the TWEAK/Fn14 pathway promotes the immune response locally within target tissues. This view provides a striking parallel to the modus operandi of the family of IL-17 cytokines and their receptors. Importantly, experimental data reviewed further below support the nonredundant contribution of TWEAK/Fn14 to the promotion of inflammatory responses.

In addition, TWEAK can cooperate with other immune stimuli in promoting the expression of inflammatory mediators. TWEAK cooperates with IL-17 to augment inflammatory mediator expression in HeLa cells (T. Hamilton, personal communication, January 16, 2009), analogous to the cooperation between TNF-α and IL-17 [69]. TWEAK also potentiates the proinflammatory effects of TLR ligands, synergizing with Pam_3CysSK_4 for the stimulation of IL-8 production by epithelial cells [70] and with oxidized-LDL for the production of MCP-1 and RANTES by vascular smooth muscle cells and a renal proximal tubule cell line [71]. In addition, TWEAK-induced RANTES was synergistically augmented by TGF-β in human keratinocyte cultures [31]. Thus, through its proinflammatory activity, the TWEAK/Fn14 pathway, alone or in concert with other mediators, may contribute to acute inflammation in tissue repair or excessive tissue damaging inflammation in contexts of diseases.

General role of TWEAK/Fn14 in cell survival, proliferation, fate, and catabolic pathways

TWEAK/Fn14 signaling mediates multiple other responses in nonhematopoietic cell types, including effects on cell proliferation, cell fate, cell death, and catabolic pathways. As a result, the immune system through TWEAK-mediated Fn14 signaling engages epithelial, endothelial, and stromal cells to make unique contributions to shaping tissue responses in injured and diseased target tissues. As this topic was comprehensively reviewed fairly recently [3], it is touched on only briefly here. TWEAK/Fn14 promotion of endothelial cell survival, growth, and migration results in angiogenesis, constituting a tissue regeneration response in acute injury, and a mechanism further promoting pathological inflammation in contexts of disease. Excessive expansion of stromal cell types, such as fibroblast-like synoviocytes, astrocytes, and renal mesangial cells may contribute to pannus formation in RA, glial scarring in neurodegenerative diseases, and glomerulosclerosis in kidney diseases, respectively. In other contexts, excessive TWEAK/Fn14-mediated cell death, as in the case of IEC, may contribute to loss of barrier function and IBD [27], as discussed further below. More recently, it has been shown that the TWEAK/Fn14 pathway induces catabolic pathways, as in the case of skeletal myocytes [41, 72], and thereby, contributes to muscle atrophy.

Of particular interest for tissue remodeling, the TWEAK/Fn14 induces expansion of progenitors, including liver, skeletal muscle, and bone, and this activity may promote tissue regeneration when the TWEAK/Fn14 pathway is transiently up-regulated after acute injury [3]. However, coupled with progenitor cell expansion, TWEAK/Fn14 signaling inhibits progenitor cell differentiation. Thus, tissue regeneration is likely impeded when TWEAK/Fn14 pathway signaling persists in contexts of severe or chronic tissue damage.

General role of TWEAK/Fn14 in fibrogenic responses

Growing evidence supports a role for the TWEAK/Fn14 pathway in promoting fibrogenic responses. This was shown by the Fn14 dependence of collagen deposition and TIMP-1 and -2 expression in a liver injury model [73]. Tissue fibrosis is an extreme version of the fibrogenic response, characterized by excessive ECM deposition with replacement of normal cellular components by scar tissue and impeded tissue repair, and is a common outcome in chronic inflammatory diseases. Fibrosis has very recently been shown to depend on TWEAK/Fn14 in a number of disease model systems [74–76] (unpublished re-

sults). There may be multiple mechanisms whereby TWEAK promotes fibrosis. As TWEAK/Fn14 promotes chronic inflammation, and fibrosis is a common outcome of chronic inflammation, TWEAK/Fn14 may promote fibrosis as a downstream consequence of its broad proinflammatory and tissue-damaging activity. However, as Fn14 is expressed by many stromal cell types and is likely also expressed on myofibroblasts, TWEAK/Fn14 may directly contribute to a fibrogenic response. Indeed, TWEAK treatment of 3T3 fibroblasts induced the mRNA expression of several collagen types (T. S. Zheng, unpublished results). In addition, TGF-β, a major profibrotic mediator, appears to cross-talk with the TWEAK/Fn14 pathway based on the observations that TGF-β can up-regulate Fn14 expression [24, 37].

TWEAK/Fn14 PATHWAY: ROLE IN TISSUE REGENERATION

The immune system appears to be critically involved in coordinating a productive regenerative response following tissue injury through a tightly coupled process of acute tissue inflammation and remodeling [77]. Its cellular and molecular components, such as macrophages and cytokines, actively participate in various phases of this process, which may include efficient removal of cellular and foreign debris, a beneficial fibrogenic response with controlled ECM production, and an endogenous regenerative program aimed at repairing damaged tissues through progenitor cell proliferation and differentiation.

We have recently reviewed the growing number of studies in the last few years that support an important role of the TWEAK/Fn14 pathway in contributing to the tissue regeneration response following acute injury [1]. Thus, this topic will only be covered in brief here. First, Fn14 expression is induced dramatically in numerous settings of tissue injury. Second, many in vitro studies have illustrated how TWEAK/Fn14 activation can contribute to the acute inflammatory response and to the activation of various progenitor cell types, including those of the liver, skeletal muscle, and other mesenchymal lineage progenitors, such as osteoblast, chondrocyte, and adipocyte progenitors, and neural progenitors. Finally, the contribution of the TWEAK/Fn14 pathway has now been directly demonstrated in several in vivo models of tissue injury. Specifically, the findings illustrated in models of acute injury in liver and skeletal muscle demonstrate how the innate immune system via TWEAK, produced by NK cells and macrophages, promotes tissue regeneration responses within the injured tissue, coordinating inflammation, progenitor expansion, and fibrogenic responses through its action on Fn14-expressing nonhematopoietic tissue cell types.

TWEAK/Fn14 PATHWAY DISEASE-DRIVING ACTIVITIES CONTRIBUTE TO AUTOIMMUNE AND INFLAMMATORY DISEASE PATHOLOGIES

The role of innate and adaptive immunity in contributing to autoimmune and inflammatory disease pathologies is well established. TWEAK is an important mediator, whereby leukocytes activate Fn14 on nonhematopoietic cells, thereby inducing the contributions of epithelial, endothelial, and other nonhematopoietic cell types to broadly affect tissue responses. Whereas TWEAK/Fn14 pathway activation appears to be beneficial in tissue regeneration after acute injury (discussed above), persistent TWEAK/Fn14 activation in the disease context can promote chronic inflammation, angiogenesis, pathological hyperplasia, tissue damage, and fibrosis. Although not yet shown, the ability of TWEAK/Fn14 to expand progenitors and inhibit differentiation may also contribute by impeding tissue repair.

An impressive body of evidence has emerged in recent years validating the role of the TWEAK/Fn14 pathway in models of autoimmune and inflammatory diseases. The wide range of studies derived from multiple independent laboratories is summarized in **Table 1**. Validation in the various disease model systems was achieved through one or several approaches, including pathway blockade using genetic models of TWEAK or Fn14 deficiency or administration of anti-TWEAK neutralizing antibodies, Fn14-Fc fusion protein, or anti-Fn14-blocking antibodies.

Importantly, also supporting our view that the immune system via TWEAK promotes pathology locally through its action on parenchymal and stromal cells in the affected tissues, a wealth of in vitro mechanistic studies has demonstrated disease-promoting activities of TWEAK acting directly on Fn14-expressing tissue cell types. These target cell types include endothelial cells, joint cell types (fibroblast-like synoviocytes, chondrocyte, and bone-lineage cells), CNS cell types (astro-

TABLE 1. TWEAK/Fn14 Pathway Mediates Pathological Outcomes in Animal Models of Injury/Disease

Injury/disease target tissue	Animal model (human injury/disease)	Primary evidence
CNS	Experimental autoimmune encephalomyelitis (multiple sclerosis)	[15, 78, 79]
	Cuprizone-induced demyelination (relevant to multiple sclerosis)	[80]
	Permanent middle cerebral artery occlusion (ischemic stroke)	[46, 48, 81, 82]
Kidney	Chronic graft-versus-host disease-induced nephritis (lupus nephritis)	[83]
	Acute kidney injury induced by folic acid (kidney injury)	[38, 39, 83, 84]
	Ischemia reperfusion injury (kidney injury)	[74]
Joint	Pristane-primed collagen-induced arthritis (RA)	[85]
	Collagen-induced arthritis (RA)	[86]
Skeletal muscle	Denervation-induced muscle wasting (muscle wasting diseases)	[76]
Vasculature	Atherosclerosis (cardiovascular disease)	[71]

cytes and neurons), and progenitor cells (all reviewed in ref. [3]), as well as renal cell types (mesangial cells, podocytes, and tubular cells) [28] and mature skeletal muscle myocytes [41, 72]. Also included are IECs and stromal cell types [18], both discussed within our focus on IBD further below. Consistent with the notion that the TWEAK/Fn14 pathway acts locally in disease target tissues to promote pathology, Fn14 is not expressed by T or B cells, and in general, there is generally no systemic effect of TWEAK/Fn14 pathway blockade or deficiency on adaptive immune responses [6].

Thus, these in vivo and in vitro studies have elucidated several general principles characterizing the role of TWEAK/Fn14 in pathological tissue remodeling. TWEAK appears to contribute universally to promoting pathogenesis in disease target tissues through Fn14 up-regulated on the parenchymal and stromal cells and may do so by coordinating multiple disease-driving processes. For the purpose of illustrating this paradigm, we focus on a particular area, in which data have emerged recently, namely, IBD, complementing our comprehensive reviews encompassing other end-organs [1–3, 6].

OVERVIEW OF UNMET NEEDS IN IBD TREATMENT

IBDs, including CD and UC, are chronic relapsing inflammatory diseases of the GI tract. IBD incidence has been rising in Europe and North America, but the recent increase in incidence in developed countries in Asia, such as Japan, has been striking [87, 88]. CD and UC affect patients across a wide range of ages but most commonly occur in young adults. As there is no curative treatment, the duration of morbidity in many patients is almost their lifetime. In the case of CD, which involves the small and large intestines, patients experience relapsing acute inflammatory illness. Fistula formation and intestinal stricture are a critical problem, necessitating surgical treatment, which is quite often repeated with relapse of the disease. In contrast to CD, in UC, which exclusively involves the colon, there are many cases with mild to moderate controllable inflammation. However, in severe disease, such as acute fulminant UC, there develops a wide extent of epithelial cell loss. The resulting life-threatening ulcers affect a large area of the colon, potentially causing perforation, toxic megacolon, and sepsis. Furthermore, development of inflammation-associated colorectal cancer is also a significant complication of IBD and a major concern in the long-term management of the disease. Total colectomy is often performed in UC patients, which eliminates the risk of cancer but frequently does not decrease the diarrheal symptoms. In addition to intestinal manifestations, osteoporosis is frequently observed in IBD patients even at a young age, especially in CD, with increased risk of bone fracture [89]. Thus, these clinical features affect patients' quality of life and prognosis and are still significant unmet needs in the treatment of IBD.

PATHOPHYSIOLOGY OF IBD

Aside from providing the essential functions of digestion and absorption, the GI tract is also the largest organ that regulates the immune system [90]. Interaction between the host GI tract and resident microbial flora establishes a balance between immunity and tolerance. Although the etiology of IBD remains to be clarified, it is generally accepted that intestinal immune responses to commensal flora are involved prominently. Recent major advances in genetics, including genome-wide association studies and understanding of the immunopathology of IBD support this notion, as discussed in extensive reviews [91–93].

Key mechanisms underlying the pathophysiology of IBD are depicted in Fig. 1. A layer of IECs provides a critical barrier protecting the host environment from bacteria. In addition to serving as a physical barrier, these IECs limit microbial invasion and maintain intestinal homeostasis by normally sensing microbes and responding with the production of mediators. IEC-derived defensins serve to limit microbial invasion, and IEC production of immunosuppressive molecules, including TGF-β, thymic stromal lymphopoietin, COX-2, and retinoic acid, regulate innate and adaptive immunity. Once IECs are damaged, barrier function is lost, and these immunoregulatory functions become dysregulated. The direct exposure of immune cells, namely macrophages and DCs, abundantly underlining the epithelia of the GI tract to luminal bacterial components, results in proinflammatory stimulation via TLRs and NOD-like receptor family. Inflammatory cytokines, including IL-1β, TNF-α, IL-6, IL-12, and IL-23 produced by activated macrophages and DCs, further activate IECs and myofibroblasts to express chemokines and cytokines, thereby exaggerating and promoting chronic inflammatory cell infiltration and activation. Continuous activation of macrophages and DCs also induces adaptive immune responses, the induction of Th1, Th17, and Th2-type T cell responses with excessive amounts of their respective hallmark effector cytokines. Inflammation-related local production of proteinases, including MMPs, may prevent tissue repair by degradating ECM and activating cytokines and growth factors that are associated with ECM and cell surfaces, promoting shedding of their active soluble forms. Dysregulated production of growth factors may stimulate fibroblast proliferation, leading to fibrosis and preventing the differentiation of IECs, thereby also disturbing normal tissue repair. All of these inflammatory responses are observed in IBD mucosa; therefore, IBD immunopathology reflects the presence of an uncontrolled inflammatory cycle with a feed-forward loop involving commensal flora to aggravate and perpetuate the tissue damage.

In the case of CD, events in stromal cells, in addition to inflammatory reactions by hematopoietic cells, appear to contribute to the signature pathological changes. An imbalanced, prolonged reaction of MMPs and overgrowth of fibroblasts and myofibroblasts may prevent closure of deep fissuring ulcers involving all layers of the intestine, which eventually results in the formation of fistula and fibrotic strictures even after inflammation is subdued. In CD, such severe fibrotic changes of the intestine cause critical symptoms with requirement for surgical or mechanical intervention. On the other hand, IEC loss, characteristic in UC, suggests that major target cells of the inflammatory response are IECs, and activated inflammatory infiltrating cells and stressed residual IECs further aggravate acute IEC damage. Thus, in addition to inflammatory cells, GI tract cells of nonhematopoietic origin, i.e., IEC and stromal cells, may significantly participate in the patho-

physiology of IBD, especially in perpetuating and aggravating the cycle.

TWEAK AND Fn14 EXPRESSION IN MOUSE MODELS OF COLITIS AND IBD

As mentioned above, Fn14 expression is generally low in normal, healthy tissues and can be up-regulated dramatically in contexts of tissue injury and disease. Relevant to IBD, the expression of TWEAK and Fn14 has been investigated in multiple models of intestinal injury and colitis (**Table 2**). Multiple experimental models for IBD were used, as these is no single model that represents all of the pathophysiological features of IBD. Among them, TNBS-induced colitis and colitis in IL-10 KO mice well reflect the abnormal excess immune response to the commensal flora, a hallmark of IBD pathophysiology. In case of TNBS-induced colitis, all luminal components are haptenized with barrier breakdown induced by intracolonic injection of TNBS with ethanol. Therefore, the host mounts an excess immune response to the luminal antigens, including flora. In IL-10 KO mice, the defect in immunoregulation causes inflammation in the lower intestinal tract. In these models, up-regulation of Fn14 was obvious, and colitis was ameliorated in the absence of the TWEAK/Fn14 pathway. In a model of acute TNBS-induced colitis, characterized by IEC damage associated with granulocyte and macrophage infiltration, the expression of TWEAK is relatively unaltered, but Fn14 protein is strongly up-regulated in the IEC of the inflamed colon [18]. This expression pattern was also observed in a chronic type of TNBS colitis (unpublished results). Of note, the TWEAK/Fn14 pathway promotes acute [18] and chronic TNBS colitis (unpublished results).

TWEAK and Fn14 expression were also examined in the context of tissue damage after γ-irradiation, a model system relevant to IBD. The expression of TWEAK mRNA was constitutive in the small and large intestine and tended to increase at 24 h after γ-irradiation, whereas Fn14 mRNA, also detected in naïve small and large intestine, was up-regulated significantly at 6–24 h in the jejunum and colon [27]. Tissue damage after γ-irradiation is mainly triggered by oxidative stress-induced DNA damage, followed by activation of the p53 pathway and cell cycle arrest. Increased oxidative stress associated with epithelial cell injury [94] and DNA damage [95] in UC has also been reported. Thus, in UC, Fn14 may enhance DNA damage as a consequence of TWEAK/Fn14-induced proinflammatory mediators, which recruit ROS-producing macrophages and neutrophils. Following DNA damage, the induction of p21 and cell cycle checkpoint arrest may result in accelerated IEC cell death. Notably, IEC cell death is reduced after γ-irradiation in TWEAK or Fn14 KO mice.

In IL-10 gene-deficient mice that develop spontaneous colitis as a result of a defect in immunoregulatory function, TWEAK mRNA levels in the colon did not change significantly; however, Fn14 mRNA expression was up-regulated, especially in old mice with chronic colitis as compared with young mice at the onset of inflammation. Of note, deficiency of TWEAK or Fn14 in IL-10 KO mice was protective from the development of colitis, suggesting a critical role of the TWEAK/Fn14 pathway in pathogenesis in this IBD model [27].

Consistent with up-regulation of the TWEAK/Fn14 pathway expression in multiple mouse models relevant to IBD, the TWEAK/Fn14 pathway is up-regulated in human IBD. In two raw, unfiltered datasets made publicly available through the National Center for Biotechnology Information Gene Expression Omnibus datasets, Fn14 was shown to be up-regulated in colonic biopsies from patients with IBD as compared with healthy subjects, and particularly, in those with UC, there was a trend toward increased Fn14 expression in patients with CD only involving the colon [2]. In an independent study in surgi-

TABLE 2. Pathological Contribution of the TWEAK/Fn14 Pathway in Models of Intestinal Injury and Colitis

	Animal models relevant to IBD			
Lines of evidence	Acute TNBS-induced colitis [18]	γ-irradiation-induced intestinal injury [18]	IL-10 KO colitis [27]	Chronic TNBS-induced colitis
TWEAK expression	Present	Up-regulated	Present	Up-regulated
Fn14 expression	Up-regulated	Up-regulated	Up-regulated in older mice	Up-regulated
Effect of TWEAK or Fn14 pathway deficiency on injury/disease	TWEAK KO, Fn14 KO, and anti-TWEAK mAb treatment reduce clinical and histopathology scores[a]	TWEAK KO and Fn14 KO reduce IEC death	TWEAK KO and Fn14 KO reduce histopathology scores[a]	Not done
Effect of TWEAK or Fn14 pathway deficiency on molecular mediators	Reduced array of proinflammatory cytokines, chemokines, and MMPs	Reduced cell cycle arrest and IL-13	Reduced IL-13, TNF-α, and IL-17	Not done
Effect of TWEAK on adaptive immunity	No effect on T or B cell hapten- specific responses	N/A	N/A	Not done

[a]Histopathology scores for acute TNBS-induced colitis were a composite of the severity of ulcers (indicating IEC death) and cell infiltration and for IL-10 KO colitis, were a composite of the severity of loss of goblet cells, crypt elongation, and cell infiltration.

cally resected UC mucosal samples that were obtained from severe or fulminant cases, TWEAK and Fn14 mRNA were up-regulated in UC in comparison with healthy mucosa in an inflammation-dependent manner [27]. In the latter study, only a limited number of four CD mucosa was examined, and although TWEAK transcripts were up-regulated, up-regulation of Fn14 expression was not seen. Taken together, these data strongly suggest that the TWEAK/Fn14 pathway is involved in the pathogenesis of UC, particularly in patients with severe disease, and that further investigation of TWEAK and Fn14 expression should be studied in CD.

The stimuli for Fn14 expression in the mouse models relevant to IBD and human IBD tissue are, as mentioned above, likely to be mediators expressed in injured and diseased tissues, including growth factors and cytokines, such as IL-13, TNF-α, IL-1β, IFN-γ, and TGF-β, as well as the TLR ligand CpG, a bacterial component, all of which are featured in IBD. In particular, TNF-α is highly up-regulated in CD and a well-validated drug target for CD and UC treatment. IL-13 is a cytokine produced by Th2 cells and innate helper cells [96] and a prominent mediator of IEC damage in models of GI injury and inflammatory disease. We [27] and others [97] have found that IL-13 is up-regulated in the UC mucosa, and growing evidence supports its role in IEC damage, especially in UC [97, 98]. Thus, the factors present in IBD tissue support mechanisms, whereby Fn14 is up-regulated in the IBD mucosa, associated with severity of the inflammation.

ROLE OF TWEAK PATHWAY IN IBD PATHOGENESIS

The TWEAK/Fn14 pathway potentially contributes to the aggravation and perpetuation of IBD by TWEAK signaling through Fn14 up-regulated on multiple nonhematopoietic cell types in the intestine, including IECs and stromal cell types, to promote: mucosal barrier breakdown, chronic tissue inflammation, and delayed/dysregulated tissue repair and fibrosis. As such, the involvement of the TWEAK/Fn14 pathway in the pathophysiology of IBD is depicted in Fig. 1. The following sections are devoted to evidence supporting the contribution of the TWEAK/Fn14 pathway to each of these three major pathological mechanisms, with in vivo data summarized in Table 2.

Barrier breakdown

IBD may be caused by genetic risk and/or environmental factors, such as diet, stress, or injury. Regardless, breach of the mucosal epithelial barrier is a key mechanism underlying excessive inflammatory stimulation from luminal bacteria and dysregulated immune function in the GI tract in IBD. As mentioned above, once IECs are damaged, direct exposure of immune cells, namely, macrophages and DCs, abundantly underlining the epithelia of the GI tract to bacterial components, results in proinflammatory stimulation via TLRs and NOD-like receptor family and consequently, dysregulated adaptive immunity. In addition to disruption of the physical barrier, IEC damage and death disrupt the normal innate sensing function of the mucosal epithelium, thereby also impairing their production of protective and immunoregulatory mediators.

TWEAK/Fn14-mediated IEC death may be a key mechanism underlying a pathogenic role for the TWEAK/Fn14 pathway in IBD. Fn14 is highly up-regulated in contexts of intestinal injury and inflammatory disease (Table 2) and was shown to be expressed by IEC in acute and chronic TNBS colitis, as mentioned above. In vivo evidence for a role of TWEAK/Fn14 in mediating IEC death was shown in the model of γ-irradiation-induced IEC death in mice, where IEC death was reduced in TWEAK or Fn14 KO as compared with WT mice [18]. Crypt loss was also reduced in the models of acute and chronic TNBS-induced colitis and IL-10 KO colitis, although in these inflammatory disease models, it is difficult to discern whether the IEC death is directly induced by TWEAK/Fn14 signaling, or secondary to pathway-enhanced inflammation which promotes tissue damage, or a result of contributions from both mechanisms.

Another frequently used colitis model is that induced by DSS, fed in the drinking water of mice. DSS results in IEC damage, more extensive and diffuse than that observed with TNBS, which is characterized by focal penetrating ulcers. Although the mechanisms underlying DSS versus TNBS-induced IEC damage are not clear, it seems that DSS is primarily, directly toxic to IEC, likely triggering massive IEC damage as a result of DNA damage resulting from ROS production. The contribution of the inflammasome as another possible IEC-damaging mechanism was addressed in a recent study showing that DSS colitis was ameliorated by the absence of the inflammasome [99], although others show that inflammasome functions protect IEC in DSS colitis [100, 101]. In our studies, there was a trend to less-severe, acute DSS-induced colitis in TWEAK KO as compared with WT mice on the BALB/c strain background; however, the difference was not robust, and no consistent difference was found in acute or chronic DSS-induced colitis between TWEAK WT and KO mice on the C57BL/6 strain (unpublished results). We speculate that DSS-induced colitis is not affected significantly by the TWEAK/Fn14 pathway, as IECs are a major site of action of TWEAK, and their wide range loss in this model precludes their contribution to the colitis outcome.

Whereas TWEAK induces IEC death after γ-irradiation in mice, TWEAK alone is not a potent inducer of IEC death in intestinal explants. Thus, the mechanism of TWEAK-induced IEC death was unclear. Interestingly, IL-13 is also up-regulated in the intestine after γ-irradiation in mice, and blockade of IL-13 reduces IEC damage [98]. Furthermore, IL-13 induces detachment of the IEC intercellular junctions by disturbing the arrangement of β-catenin after addition to intestinal explants [98] or a colon carcinoma cell line HT29/B6 and induces apoptosis of this cell line [102]. The interplay between IL-13 and the TWEAK/Fn14 pathway was investigated further in mouse intestinal explants, and a novel mechanism of TWEAK/Fn14-induced IEC death was elucidated [27]. Notably, IL-13 strongly induces IEC cell death and activation of caspase 3 but not anoikis-induced caspase 2 in naïve mouse intestinal explants at a comparable level with that induced by TNF-α. Loss of intercellular adherence occurs as a result of the apoptosis. Importantly, this IL-

13-induced apoptosis was not seen in TWEAK or Fn14-deficient tissue. Furthermore, this process did not require de novo protein synthesis but did require secretion of endogenous TNF-α activation mediated by TACE, distinguishing it from a previously described, TNF-α-dependent mechanism(s) [63–65] (also discussed above). Further studies are needed to delineate the signaling events downstream of IL-13 that lead to TWEAK/Fn14-dependent, TACE-mediated TNF-α shedding. However, as this series of experiments was performed using the intestinal tissue of naïve mice, the results indicate that IL-13 can trigger IEC damage with endogenous levels of TWEAK/Fn14 and TNF-α. Once the process is started, these pathways act interdependently to promote IEC apoptosis, destroying the mucosal barrier and increasing exposure to bacterial stimulation from the lumen, thereby promoting the recruitment and activation of inflammatory cells. This is a powerful mechanism to perpetuate and aggravate intestinal inflammation, which may play a critical role in UC, where IEC damage is the major pathological finding, especially in the acute fulminant type. Efficacy of anti-TNF-α therapy in UC has been reported in spite of the fact that up-regulation of TNF-α in UC mucosa is not as prominent as seen in CD. Given that IL-13 triggers TWEAK/Fn14-dependent cell death in the context of endogenous levels of TNF-α, it is understandable. Furthermore, a report that TACE activity is up-regulated in UC mucosa but not CD also supports the relevance of our findings [103].

Chronic inflammation

Chronic inflammation is a hallmark of IBD. Leukocytes infiltrating the damaged intestinal tissue respond to bacterial components, resulting in proinflammatory stimulation and promotion of dysregulated adaptive immunity. In addition, IECs are an important innate-sensing cell type, responding to luminal bacteria, as well as inflammatory cytokines produced by activated macrophages and DCs. IECs thereby participate in a feed-forward loop, which aggravates and perpetuates chronic inflammatory cell infiltration and tissue damage, as well as shapes the Th-type adaptive response through their production of immunoregulatory molecules [92, 104]. In addition, other nonhematopoietic cell types, such as myofibroblasts, respond to and express chemokines and cytokines, thereby promoting proinflammatory as well as fibrotic disease pathologies [11].

The TWEAK/Fn14 pathway appears to be involved in the persistence of intestinal inflammation. This is supported by observations of TWEAK/Fn14-induced proinflammatory activity downstream of NF-κB activation that has been documented in many different cell types [3], including epithelial and endothelial cells and many stromal cell types that are present in the intestine. In addition, TWEAK or Fn14 deficiency or pathway blockade has been shown to reduce end-organ inflammation in multiple models relevant to IBD (Table 2).

In the context of IBD, IECs may respond to TWEAK that is up-regulated on their cell surface and thereby substantially contributes to the proinflammatory responses, as evidenced by the TWEAK-induced IEC secretion of well-known NF-κB targets, such as the inflammatory cytokine IL-6 and keratinocyte-derived chemokine, as well as MMP-9 in vitro [18]. In addition, a wide array of chemokines, cytokines, and MMPs was strikingly reduced in acute TNBS-induced colitis in TWEAK-deficient mice as compared with WT mice [18], and the prominent cytokines IL-13, TNF-α, and IL-17 were all reduced in IL-10 KO colitis by TWEAK or Fn14 deficiency [27]. TWEAK/Fn14-induced chemokines may recruit inflammatory cells, such as macrophages and granulocytes, and the TWEAK/Fn14-induced inflammatory cytokines may further activate leukocytes and nonhematopoietic cell types to promote their own production of inflammatory cytokines and chemokines. In addition to IEC, Fn14 is likely to be expressed on α-SMA-expressing myofibroblasts, as it is generally inducible on mesenchymal cell types. Myofibroblasts appear to play roles in the intestinal inflammation by secreting inflammatory mediators, including IL-8, MCP-1, MMPs, and IL-6 in response to TNF-α and bacterial components [11] and therefore, may also contribute to inflammatory cytokine production in response to TWEAK. Thus, TWEAK may act locally through Fn14 up-regulated on IEC and nonhematopoietic cell types to promote the production of proinflammatory mediators and thereby, perpetuate and aggravate colitis.

There are additional mechanisms, whereby the TWEAK/Fn14 pathway may aggravate and perpetuate the inflammatory response in colitis. The ability of bacterial components and inflammatory cytokines to induce the expression of Fn14 is another important aspect of the feed-forward loop, promoting the accumulation of immune effector cells and substances. In addition, it has been reported that TWEAK augments stimulation with TLR ligands, synergizing with Pam_3CysSK_4 for stimulation of IL-8 production in the case of urogenital epithelial cells [70]. Further, TWEAK cooperates with IL-17, an inflammatory cytokine up-regulated in CD, to augument inflammatory mediator expression in Hela cells (T. Hamilton, personal communication, January 16, 2009), analogou to the cooperation between TNF-α and IL-17 [69, 105].

Fn14 is primarily inducible on epithelial and other nonhematopoietic cell types and has only rarely been detected on macrophages. In one such report of a macrophage cell line THP-1, TWEAK was shown to stimulate secretion of HMGB1 [106], which can function as an extracellular DNA sensor and also a proinflammatory cytokine. As HMGB1 is reported to be actively produced and secreted into the feces at high levels in IBD patients but not detectable in normal subjects [107], TWEAK may be involved in its up-regulation mechanism in IBD.

Adaptive immunity evolves in colitis as a result of activated DCs, which act as APCs, leading to the generation of T cell-mediated and humoral responses. As Fn14 is not expressed in T or B lymphocytes and has rarely been observed on other hematopoietic cell types, including APCs, the TWEAK/Fn14 pathway does not appear to be directly involved in adaptive immunity, and in studies reported thus far, neither TWEAK nor Fn14 deficiency has a prominent effect on systemic adaptive immunity [6]. However, the TWEAK/Fn14 pathway may promote the infiltration of adaptive immune cell types into the intestinal tissue through stimulation of chemokine production in IEC locally in the intestinal tissue, thereby also contributing to the severity and chronicity of colitis. In a mouse model of

chronic inflammation as a result of adaptive immune dysregulation, IL-10 gene deficient (KO) mice develop spontaneous colitis 4–8 weeks after birth. In contrast, mice deficient in IL-10 and TWEAK or IL-10 and Fn14 were almost completely free from colonic inflammation [27]. Histologically, infiltration of monocytes/macrophages, a hallmark of IL-10-deficient colitis, was reduced in the colons of double KO mice, even at >20 weeks of age. Interestingly, in IL-10 KO colitis, TNF-α and IL-17 mRNA levels were up-regulated (over tenfold) as early as 8 weeks of age, whereas up-regulation of IL-13 (>100-fold) and Fn14 (threefold) are not seen until the mice are 15 weeks old. Furthermore, IL-13, TNF-α, and IL-17 expression was normalized in IL-10 KO colitis by TWEAK or Fn14 deficiency. Thus, based on studies of IEC death described above, we speculate that TNF-α-induced IEC death, supported by the TWEAK/Fn14 pathway, is a major effector mechanism at the onset of colitis in young mice and that the IL-13-TWEAK/Fn14 axis is engaged in the chronic phase, further destroying the epithelial integrity, which results in increased bacterial translocation. In addition, this may explain why splenomegaly was also decreased in the double KO mice as compared with IL-10 KO mice. Notably, the TWEAK/Fn14 pathway promotes the expression of IL-17 in IL-10 KO colitis, and as mentioned above, TWEAK can cooperate with IL-17 to augment inflammatory mediator expression. Thus, the TWEAK/Fn14 pathway likely contributes to IL-10 KO colitis through its ability to promote inflammatory responses and IEC death.

The contribution of the TWEAK/Fn14 pathway to the pathogenesis of colitis does not appear to involve its direct regulation of systemic adaptive immunity. However, the TWEAK/Fn14 pathway may indirectly affect adaptive immunity through its activity locally on IECs in the disease target tissue. For example, IECs can promote Th2-type adaptive immunity through their production of mediators that condition DC within the intestinal tissue—the DC subsequently migrating to draining lymphoid tissues to shape Th2-type immune responses [9, 10]. As the TWEAK/Fn14 pathway signals in IECs, the possibility remains that TWEAK may affect adaptive immunity through its local action on Fn14-expressing IECs. This additional mechanism warrants investigation.

Thus, TWEAK is profoundly involved in the induction of a wide array of proinflammatory mediators, including chemokines, cytokines, and MMPs, by signaling through Fn14, which is up-regulated on IECs and other nonhematopoietic cell types locally in the intestine, thereby promoting the infiltration of innate and adaptive inflammatory cell infiltration and perpetuation of colitis.

Impaired healing and fibrosis

Tissue fibrosis is characterized by excessive ECM deposition with replacement of normal cellular components by scar tissue and impeded tissue repair and is a common outcome in chronic inflammatory diseases. Fibrosis can occur in IBD as a consequence of the attempts to repair ongoing tissue damage. Fibrosis in UC is limited to the mucosal layer but affects all layers of the intestine in CD where fistula formation and intestinal stricture/obstruction are a critical problem, often necessitating surgical treatment, which may need to be repeated with relapse of the disease. Peritoneal adhesions also occur with severe fibrosis.

The link between the TWEAK/Fn14 pathway and fibrosis is an emerging theme. Although it has not yet been reported in the context of IBD, studies from multiple other systems support a potential role of TWEAK/Fn14 in driving fibrosis, which may also be relevant in the context of colitis. The link between the TWEAK/Fn14 pathway and fibrogenic responses was shown by the Fn14 dependence of collagen deposition and TIMP-1 and -2 expression in a liver injury model [73]. In addition, fibrosis has recently been shown to depend on the TWEAK/Fn14 pathway in a number of injury/disease model systems, including in kidney after ischemia reperfusion injury [74], unilateral ureteral obstruction (A. Ucero and A. Ortiz, personal communication, January 12, 2012), and nephrotoxic serum transfer nephritis (C. Putterman, personal communication, February 1, 2012); in skeletal muscle in a model of denervation-induced skeletal muscle atrophy [76]; in the heart in the context of dilated cardiomyopathy [75]; in the right ventricle in a model of pulmonary arterial hypertension (T. S. Zheng, personal communication, August 3, 2011); and in a model of liver injury (Y. Popov, personal communication, May 11, 2011).

There are multiple mechanisms whereby TWEAK promotes fibrosis. As TWEAK/Fn14 promotes chronic inflammation, and fibrosis is a common outcome of chronic inflammation, TWEAK/Fn14 may promote fibrosis as a downstream consequence of its broad proinflammatory and cell death/tissue-damaging activity. These proinflammatory mechanisms also result in the stimulation of fibroblast expansion and matrix deposition, which replace normal tissue. The TWEAK/Fn14 pathway may also regulate fibrosis through its direct action on fibroblasts/myofibroblasts and their progenitors. Evidence supporting each of these mechanisms is discussed in more detail below.

Proinflammatory mediators induced by TWEAK/Fn14 signaling, including cytokines, chemokines, and MMPs, promote leukocyte infiltration, as well as directly damage tissue (discussed in section on Pathophysiology of IBD). MMPs are of particular note with respect to fibrosis, given their critical role in balancing pathological tissue remodeling and tissue repair. The degradation of excessive ECM deposition by MMPs is necessary for normal wound healing and tissue repair. Therefore, the activity of MMPs is tightly regulated by their natural inhibitors, α-macroglobulin and TIMP. In IBD mucosa, MMPs (MMP-1, -2, -3, -8, -9, and -12 and membrane type 1-MMP-1) are reported to be up-regulated, and some of these are surprisingly associated with fistula formation in CD, in addition to ulcers, and epithelial dysfunction [108]. MMP up-regulation may also be related to the delayed anastomosis or wound closure in CD patients [109]. Fistula formation in CD and subsequent fibrotic strictures are thought to be formed by an imbalanced, prolonged interaction of MMPs with fibroblasts that prevents closure of deep fissuring ulcers even after inflammation is subdued. The TWEAK/Fn14 pathway may contribute to this up-regulated MMP expression in IBD through its ability to directly induce MMP secretion by IECs [18]. The TWEAK/Fn14 pathway may also support excess expression of MMPs by cooperating with TNF-α, as TNF-α is known to induce MMP expression, and TWEAK and TNF-α have previously been shown to cooperate with each other for the induction of other proinflammatory mediators [110]. Another mecha-

nism, whereby TWEAK/Fn14 might promote fibrosis, is through its effect on the regulation of collagen deposition. Given that the TWEAK/Fn14 pathway induces the expression of MMPs and TIMPs, as was observed in the model of acute TNBS colitis [18], it may be involved in the dysregulation of collagen deposition by affecting the balance of enzymes that regulate ECM deposition and degradation, as in the context of liver injury [73].

The TWEAK/Fn14 pathway may also regulate fibrosis through its direct action on stromal cells. As Fn14 is up-regulated by TGF-β on fibroblasts [24, 37] and is likely also expressed on myofibroblasts, the TWEAK/Fn14 pathway may contribute directly to a fibrogenic response by regulating the proliferation of these fibroblasts and their differentiation to myofibroblasts and ECM production. Indeed, emerging data indicate that TWEAK treatment of fibroblasts induces their proliferation, collagen expression, and myofibroblast differentiation (T. S. Zheng, personal communication, August 3, 2011). TWEAK treatment of 3T3 fibroblasts also induced the expression of mRNA of several collagen types (T. S. Zheng, personal communication). In yet another independent study, it was found that TWEAK promotes fibroblast proliferation and regulates ECM expression (A. Ucero and A. Ortiz, personal communication). Recently, we also found that TWEAK synergizes with IL-13 or TGF-β to promote fibroblast proliferation (unpublished results). Thus, TWEAK may make a significant contribution to fibrosis, particularly in the fibrogenic milieu.

In addition, the TWEAK/Fn14 pathway may regulate fibrosis through its effect on fibroblast/myofibroblast progenitors, given the precedence for its regulation of progenitor cell fate, including mesenchymal lineage progenitors [3]. In the intestinal lamina propria, mesenchymal cell types include fibroblasts, myofibroblasts, and vascular pericytes. Histologically, these cell types show distinct localization forming layers and subepithelial sheath adjacent to IECs. In the inflamed mucosa, these cells proliferate and are activated to accumulate ECM. Their origin may be local resident stromal cells; however, in addition, there is evidence to support the recruitment of precursor cells of myofibroblasts and pericytes from the circulation during the inflammatory process, but it is unclear whether the critical pool is originally from a hematopoietic stem cell origin or a mesenchymal stem cell origin or both [12]. Expression and function of TWEAK and Fn14 expression in these precursor cells also warrant future study.

Thus, fibrosis and impaired tissue healing are critical features of IBD. Whereas not explored directly in models of IBD, there is growing evidence for a role of the TWEAK/Fn14 pathway in promoting fibrosis from other organ systems. Future investigations are warranted to elucidate the potential and mechanisms whereby TWEAK-induced fibrogenic responses may contribute to fibrosis in IBD.

Other tissue remodeling

In addition to colitis, osteoporosis is observed frequently in IBD patients, even at a young age, especially in CD [89], and multiple factors have been suggested as causes, including steroid treatment and inflammation. A direct relation between TWEAK levels and osteoporosis in IBD has not been explored yet. However, in a model of RA, the TWEAK/Fn14 pathway was shown to promote bone erosion [85, 86]. TWEAK/Fn14-induced proinflammatory mechanisms may contribute, as well as direct effects of TWEAK on osteoblasts, including the up-regulation of RANKL, which promotes bone erosion and induction of sclerostin, impeding bone repair [53, 56]. Thus, the TWEAK/Fn14 pathway is likely to be involved in the enhancement of gut inflammation and in osteoporosis in IBD.

PERSPECTIVE

The TWEAK/Fn14 pathway has emerged in recent years as an important factor, contributing significantly to the pathogenesis of autoimmune and inflammatory diseases, based on the amelioration of disease in multiple animal models by pathway deficiency or blockade. CD and UC are chronic relapsing forms of IBD, whose pathophysiology prominently features epithelial damage, chronic inflammation, and tissue remodeling with impaired healing and fibrosis. CD and UC patients are afflicted with significant morbidities, despite standard of care treatment. In CD, delayed healing of ulcers and severe fibrotic changes may result in the formation of fistula and fibrotic strictures, whereas UC may feature severe EC loss, leading to life-threatening ulcers. Emerging evidence supports a role for the TWEAK/Fn14 pathway as a potential aggravating and perpetuating factor in IBD. TWEAK, acting through Fn14, up-regulated on IECs and other nonhematopoietic cell types, promotes the pathophysiology of IBD through its ability to induce IEC death, proinflammatory mediators, and multiple mechanisms contributing to fibrosis. Importantly, the TWEAK/Fn14 pathway has been integrated into the framework of molecular mechanisms underlying IBD, based on recent data demonstrating that Fn14 can be up-regulated by a variety of other mediators that are well-known players in IBD, including IL-13, TNF-α, IL-1β, IFN-γ, TGF-β, and the TLR ligand CpG; and TWEAK can act together with other mediators, such as with IL-13 and TNF-α, to induce IEC death and with IL-17 or TGF-β, to promote inflammatory responses. However, we and others have also shown that TWEAK can act independently from TNF-α in the induction of proinflammatory mediators using dermal fibroblasts and fibroblast-like synoviocytes [110] and (T. S. Zheng and J. S. Michaelson, personal communication, January, 2012). Indeed, we are just beginning to understand how the TWEAK/Fn14 pathway interplays with other prominent mediators. Thus, there are many outstanding questions, and the contribution of TWEAK to pathophysiology in IBD and other autoimmune and inflammatory diseases will be enlightened by future studies dissecting such networks.

Based on this emerging understanding, targeting the TWEAK/Fn14 pathway may be a promising new approach to IBD treatment. However, outstanding questions remain as to whether the promising effects observed in animal models of colitis will translate to the clinic and which type(s) of IBD patients may most benefit from pathway blockade. In UC, TWEAK and Fn14 are up-regulated in association with inflammation as compared with healthy mucosa. Given the characteristically marked IEC loss in UC and novel role for TWEAK in IEC death, targeting TWEAK may be a well-suited treatment strategy. As stated above, as TWEAK and TNF-α can act independently in the promotion of proinflammatory mediators, blocking TWEAK may address an

unmet need in those patients with an inadequate or unsustained response to the TNF inhibitor. Of note, given that Fn14 is not expressed on T or B cells and has rarely been reported on other hematopoietic cell types, including APCs, blocking TWEAK is not expected to have a prominent impact on systemic adaptive immunity and therefore, may not confer an increased risk of infection. Targeting TWEAK may also be a well-suited treatment strategy for CD, given the predilection in CD for development of fistula and strictures and the exciting new data supporting the role of the TWEAK/Fn14 pathway in promoting fibrosis. However, the studies of TWEAK/Fn14 pathway expression in CD are limited to date, and thus, future studies are required to investigate further whether the TWEAK/Fn14 pathway is indeed up-regulated in CD patients.

Another exciting future direction is suggested by our understanding of the role of the TWEAK/Fn14 pathway in the GI tract, which implies a potential pathogenic role of TWEAK/Fn14 in other diseases in which epithelial injury is prominent, such as the respiratory tract and skin. The interplay of TWEAK with IL-13 further supports a potential role of TWEAK in respiratory diseases, such as allergic asthma and fibrotic pulmonary diseases, as well as skin diseases, such as atopic dermatitis. However, we expect that the precise effects of TWEAK/Fn14 signaling will be different in the context of different cell types and tissue microenvironments. Further advances in the identification of TWEAK and Fn14-expressing cell types and clarification of how TWEAK/Fn14 signaling interplays with other signaling pathways in a given tissue microenvironment will inform our broader knowledge of disease pathophysiology, of TWEAK as a target for treatment of different diseases and patient segments and thereby, expand these fields of research.

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DISCLOSURES

L.C.B. is an employee and stockholder in Biogen Idec, Inc.

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KEY WORDS:
Crohn's disease · ulcerative colitis · TNF superfamily · epithelial death · inflammation · tissue remodeling

TWEAK/Fn14 pathway promotes a T helper 2-type chronic colitis with fibrosis in mice

A Son[1], T Oshio[1], YI Kawamura[1], T Hagiwara[1], M Yamazaki[1], K Inagaki-Ohara[1], T Okada[1], P Wu[2], M Iseki[3], S Takaki[3], LC Burkly[2,4] and T Dohi[1,4]

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK), a TNF superfamily member, induces damage of the epithelial cells (ECs) and production of inflammatory mediaters through its receptor Fn14 in a model of acute colitis. In our current study of chronic colitis induced by repeated rectal injection of a hapten, we found that inflammation, fibrosis, and T helper 2 (Th2)-type immunity were significantly reduced in Fn14 gene knockout (KO) mice when compared with wild-type (WT) control mice. Expression of thymic stromal lymphopoietin (TSLP) was lower in Fn14 KO colon ECs than in WT ECs. TWEAK potentiates the induction of TSLP by interleukin-13 (IL-13) in colon explants from WT but not in Fn14 KO tissue. TSLP receptor KO mice exhibit milder chronic colitis, similar to that in Fn14 KO mice. TWEAK and IL-13 synergistically promote fibroblast proliferation. Thus we propose an IL-13-TWEAK/Fn14-TSLP axis as a key mechanism underlying chronic colitis with fibrosis.

INTRODUCTION

Crohn's disease and ulcerative colitis are chronic relapsing inflammatory diseases of gastrointestinal tract. In Crohn's disease, which involves both the small and large intestines, relapsing acute inflammatory illness causes significant tissue remodeling and deformity of the intestine, such as intestinal fibrotic stricture. These legions start as inflammation involving all layers of the intestinal wall, frequently associated with cleft ulcers and fistula, followed by severe fibrosis and stenosis even after inflammation has subdued. However, the mechanism of the pathogenic intestinal fibrosis has just recently been recognized as an important target for the treatment.[1,2]

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a cytokine of the TNF ligand superfamily that is constitutively expressed by many immune cell types, including monocytes/macrophages, dendritic cells (DC), neutrophils, natural killer (NK) cells, and also T cells,[3–6] although TWEAK expression has also been reported in some non-hematopoietic cell types, including renal cells.[7] The only known signaling receptor for TWEAK, FGF (fibroblast growth factor)-inducible molecule 14 (Fn14), is a highly inducible molecule in epithelial cells (ECs), endothelial cells, and other mesenchymal cell types wherein TWEAK signals through Fn14 to promote the canonical and noncanonical nuclear factor (NF)-κB, mitogen-activated protein kinase, and potentially other pathways.[8,9] Fn14 is induced by various growth factors and proinflammatory cytokines[9] and as well as by the bacterial component CpG-DNA[10] and cytokines TNF-α and interleukin-13 (IL-13) in the intestinal ECs.[11] Fn14 expression appears to be universally upregulated in contexts of tissue injury and disease (reviewed in Winkles,[9] Burkly and Dohi,[12] and Zheng and Burkly[13]), including in intestinal epithelium after hapten-induced injury and γ – irradiation injury.[10] TWEAK significantly contributes to driving tissue pathology, likely by acting on Fn14-expressing tissue cell types to directly promote tissue damage, potentiate inflammation locally in the disease target tissue, including the infiltration of innate and adaptive immune cell types, and pathological remodeling, including fibrogenic responses.[8,12] However, the contribution of TWEAK/Fn14 signaling to systemic acquired immunity seems to be minimal. In the model of collagen-induced arthritis,[14,15] hapten-induced acute colitis,[10] and autoantibody-mediated nephritis induced by chronic graft-versus-host disease,[16] TWEAK/Fn14 pathway blocking or deficiency ameliorated inflammation and tissue

[1]Department of Gastroenterology, Research Center for Hepatitis and Immunology, Research Institute, National Center for Global Health and Medicine, Chiba, Japan. [2]Departments of Immunobiology and Drug Discovery, Biogen Idec, Cambridge, Massachusetts, USA and [3]Department of Immune regulation, Research Center for Hepatitis and Immunology, Research Institute, National Center for Global Health and Medicine, Chiba, Japan. Correspondence: T Dohi (dohi@ri.ncgm.go.jp).
[4]These authors share senior authorship.

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damage locally within the disease target tissue, with no effect on systemic antigen-specific humoral or cellular immune responses. Consistent with this, Fn14 expression is absent in T and B cells and immune responses to systemic neo-antigen immunization are intact in TWEAK or Fn14 gene–deficient mice (Burkly, unpublished).

We recently found that there is crosstalk between the IL-13, TWEAK/Fn14 and TNF-α signaling pathways in mediating intestinal EC death.[11] Therefore, we hypothesized that in disease models where IL-13 has major role, TWEAK/Fn14 may also be significantly involved. IL-13 is a pathogenic profibrotic cytokine in the respiratory tract[17] and the major effector of lung tissue remodeling. IL-13 stimulates the proliferation of lung fibroblasts from human subjects with asthma, by induction of platelet-derived growth factor (PDGF)-AA and PDGF-CC.[18] IL-13 also mediates fibrosis in a model of chronic intestinal damage induced by the weekly intracolonic injection of hapten, trinitrobenzene sulfonic acid (TNBS) with ethanol.[19,20] This model features increased collagen and tissue architecture remodeling of the colon along with increased expression of transforming growth factor (TGF)-β1, collagen (Col1a2), matrix metalloproteinases (MMPs), and tissue inhibitor of metalloproteinases TIMP-1.[21] These pathological changes were partially dependent on NF-κB.[20] Further, this model induced in T helper type 2 (Th2)-prone BALB/c mice was shown to be mediated primarily by IL-13 followed by the induction of TGF-β.[19] In this study, we used this model to investigate the potential contribution of the TWEAK/Fn14 pathway to the pathology of the Th2-type chronic intestinal inflammation and fibrosis and interplay with IL-13 in mediating its effects.

RESULTS

The TWEAK/Fn14 pathway promotes chronic colitis with fibrosis in chronic TNBS-induced colitis in mice

Chronic colitis was induced by rectal injection of TNBS with ethanol weekly for 6 consecutive weeks in wild-type (WT) and Fn14 or TWEAK knockout (KO) BALB/c mice. Colons from naive WT and KO mice were comparable, as previously reported.[10]

There were no macroscopic open ulcers in the WT mouse colon after chronic TNBS administration, in contrast to that frequently seen in the acute model. However, histologically, the crypts were lost and replaced by leukocyte infiltration along with fibroblasts and connective tissue. Accumulated collagen was detected in the mucosal as well as in the submucosal layer as previously reported in this model (**Figure 1a**). The degree of crypt loss and accumulation of collagen was much reduced in Fn14 KO as compared with WT mice (**Figure 1a**). Weight gain of WT mice with colitis was reduced, whereas the Fn14 KO mice gained weight normally (**Figure 1b**). In agreement with histological findings, when the ratio of collagen/non-collagen protein was measured in colon sections, we found an increased ratio in WT mice with chronic colitis, with a significantly lower value in Fn14 KO (**Figure 1c**). Total histological scores as well as scores for the degree of crypt loss were significantly higher in WT as compared with that in Fn14 KO mice (**Figure 1d**). Infiltration of $CD45^+$ leukocytes, $F4/80^+$ macrophages, $Gr1^+$ granulocytes, and $CD11c^+$ DC was observed and infiltration of these cell types was attenuated in Fn14 KO as compared with that in WT mice (**Figure 2a**). Lymphocyte infiltration in the LP was not as obvious (data not shown); however, enlargement of colonic patches and lymphoid follicles were more severe in WT mice than in Fn14 KO mice (**Figure 2a**). In addition, infiltration of eosinophils was seen in WT mice but was not seen much in Fn14 KO mice, likely reflecting the reduction in granulocytes (**Figure 2a**). Total leukocyte infiltration area was significantly reduced in the Fn14 KO colon (**Figure 2b**). These results indicate that Fn14 deficiency ameliorated colonic damage, inflammation, and fibrosis in the chronic colitis induced by repeated injection of TNBS.

Differential gene expression in chronic TNBS-induced colitis in WT and Fn14 KO mice

In order to elucidate the mechanisms underlying protection by Fn14 deficiency in chronic TNBS-induced colitis, gene expression profiling was conducted using whole-colon tissue after 6 weekly TNBS administrations (**Figure 3**). In WT colon, mast cell and eosinophil-related molecules are highly increased in mice with colitis as compared with the naive condition, reflecting the local infiltration of these cell types. In addition, molecules expressed in macrophages and DC, including the expression of innate sensors and associated molecules, are upregulated, and these cells are apparently also activated because MHC (major histocompatibility complex) class II molecules are upregulated. Interestingly, we also found strong upregulation of signature molecules of alternatively activated macrophages, which are induced by Th2-type cytokines,[22] namely, arginase 1 (*arg1*), chitinase 3-like 3 (*chi3l3* also known as YM1), and dectin-1 (*Clec7a*). Intelectin (*Itlna*), which was reported to be induced in ECs by parasite infection[23] and IL-13,[24] is also upregulated. In addition, genes related to NK cells and neutrophils and B-cell- and T-cell-related molecules were increased in the inflamed WT colon. An array of chemokines that mediate leukocyte recruitment, as well as a variety of effector cytokines, MMPs, and inflammation-related adhesion molecules, were also upregulated. Also, of note, TGF-β and IL-13Rα2, both of which are molecular mediators of the fibrotic changes in this colitis model,[19] were upregulated. Importantly, collagen and matrix molecule expression were upregulated matching the result of the histology.

By contrast, in the Fn14 KO colon, mast cell and eosinophil-related molecules were upregulated to a lesser degree than in WT mice. Macrophage and DC-related molecules as well as MHC molecules, which were highly induced WT mice, mostly did not change from the naive condition in Fn14 KO mice. These findings may reflect the lower cell infiltration in Fn14 KO mice. Interestingly, the expression pattern of genes related to B and T cells suggests that a different type of adaptive response is induced in WT and in Fn14 KO mice. For example, immunoglobulin G1 (*Igh-4*), which is characteristic of Th2-type responses, is increased in WT colon, whereas immunoglobulin G2a (*Igh-1a*), which