TNF-related apoptosis-inducing ligand (TRAIL) regulates midline-1, thymic stromal lymphopoietin, inflammation, and remodeling in experimental eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EoE) is an inflammatory disorder of the esophagus defined by eosinophil infiltration and tissue remodeling with resulting symptoms of esophageal dysfunction. TNF-related apoptosis-inducing ligand (TRAIL) promotes inflammation through upregulation of the E3 ubiquitin-ligase midline-1 (MID1), which binds to and deactivates the catalytic subunit of protein phosphatase 2Ac, resulting in increased nuclear factor κB activation.
Objective: We sought to elucidate the role of TRAIL in EoE.
Methods: We used *Aspergillus fumigatus* to induce EoE in TRAIL-sufficient (wild-type) and TRAIL-deficient (TRAIL$^{-/-}$) mice and targeted MID1 in the esophagus with small interfering RNA. We also treated mice with recombinant thymic stromal lymphopoietin (TSLP) and TRAIL.
Results: TRAIL deficiency and MID1 silencing with small interfering RNA reduced esophageal eosinophil and mast cell numbers and protected against esophageal circumference enlargement, muscularis externa thickening, and collagen deposition. MID1 expression and nuclear factor κB activation were reduced in TRAIL$^{-/-}$ mice, whereas protein phosphatase 2Ac levels were increased compared with those seen in wild-type control mice. This was associated with reduced expression of CCL24, CCL11, CCL20, IL-5, IL-13, IL-25, TGFB, and TSLP. Treatment with TSLP reconstituted hallmark features of EoE in TRAIL$^{-/-}$ mice and recombinant TRAIL induced esophageal TSLP expression *in vivo* in the absence of allergen. *Post hoc* analysis of gene array data demonstrated significant upregulation of TRAIL and MID1 in a cohort of children with EoE compared with that seen in controls.
Conclusion: TRAIL regulates MID1 and TSLP, inflammation, fibrosis, smooth muscle hypertrophy, and expression of inflammatory effector chemokines and cytokines in experimental EoE. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Eosinophilic esophagitis, allergy, tissue remodeling, fibrosis, cytokine, TNF-related apoptosis-inducing ligand, midline-1, protein phosphatase 2Ac, nuclear factor κB, thymic stromal lymphopoietin, IL-25, CCL20, CCL24, CCL11*

Eosinophilic esophagitis (EoE) is characterized by eosinophil-dominant inflammation of the esophagus that is resistant to proton pump inhibitor therapy. Although an orphan disease, the prevalence of EoE is increasing in the Western world.[1,2] EoE can manifest at any age with a trend toward affecting atopic male children.[3-5] Patients classically present with esophageal or abdominal pain, vomiting, and dysphagia, and young children are at risk of significant weight loss as a result of prolonged esophageal inflammation.[6,7] Patients with EoE, particularly children, often have comorbid atopic disorders, such as food allergy or asthma.[3,8,9]

Dietary exposure to food allergens has been linked to the development of EoE, with dietary modifications shown to be a successful therapeutic approach in many patients.[9-11] Alternatively, topical administration of corticosteroids to the esophageal mucosa has also been shown to be effective in

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Abbreviations used
AAD: Allergic airways disease
ACTB: β-Actin
EoE: Eosinophilic esophagitis
MID1: Midline-1
NF-κB: Nuclear factor κB
PP2A: Protein phosphatase 2A
PP2Ac: Catalytic subunit of protein phosphatase 2A
siRNA: Small interfering RNA
STAT: Signal transducer and activator of transcription
TRAIL: TNF-related apoptosis-inducing ligand
TSLP: Thymic stromal lymphopoietin
WT: Wild-type

reducing esophageal eosinophilia.[6,12,13] However, neither dietary interventions nor steroid treatments are universally effective, with a subset of patients experiencing persistent EoE symptoms, refractory esophageal eosinophilia, or both. In cases where chronic inflammation of the esophagus is left untreated or patients do not respond to therapies, esophageal remodeling can lead to esophageal stricture formation and worsening food impaction.[14,15] Clinical guidelines suggest that patients with severe remodeling can receive endoscopic dilatation therapy to alleviate symptoms, with most patients responding well to this therapy in the short term.[16,17] However, up to 75% of patients experience dilatation complications, including pain, bleeding, and perforation,[18] highlighting the need for an effective pharmacologic alternative to treat elimination diet– and steroid-resistant EoE.

Esophageal remodeling is thought to be the consequence of prolonged eosinophilic inflammation promoting collagen deposition and fibrogenesis, esophageal muscle hypertrophy, and angiogenesis.[14,19] T_H2 cytokine signaling plays a central role in EoE pathogenesis by driving the recruitment and proliferation of eosinophils to the esophagus.[20] In turn, eosinophil-derived proteins, including TGF-β, have been shown to drive profibrotic SMAD2/3 pathways.[14] IL-13 also plays a key role, with activation of the IL-4/IL-13 receptor inducing eotaxins (CCL11 and CCL24 in mice and CCL26 in human subjects) through signal transducer and activator of transcription (STAT) 6–mediated pathways.[21] However, recent studies have indicated that symptoms and remodeling can persist even when eosinophilia has been corrected,[22] suggesting that eosinophil-independent pathways might also be key drivers of esophageal remodeling in patients with EoE.[7] Mast cell and basophilic inflammation is also observed clinically and experimentally, with mast cells believed to contribute to a thickened muscularis externa through TGF-β, histamine, and tryptase.[23] A major EoE genetic susceptibility locus exists at the thymic stromal lymphopoietin *(TSLP)* gene (5q22)[24] and release of TSLP from esophageal epithelial cells promotes basophil infiltration[25] and has been demonstrated to induce cellular senescence and fibrosis in asthma models.[26] Upstream regulators of the remodeling and TSLP pathways in patients with EoE have yet to be elucidated; however, they might be promising therapeutic targets.

TNF-related apoptosis-inducing ligand (TRAIL) has been increasingly recognized as a proinflammatory cytokine.[27-29] We have shown previously in allergic airways disease (AAD) models and asthmatic patients that TRAIL is released by structural airway cells in response to allergen stimulation,[28] resulting in upregulation of the E3 ubiquitin ligase midline-1 (MID1).[29] In turn, MID1 monoubiquinates the α4 subunit of protein phosphatase 2A (PP2A), promoting proteosomal degradation of the catalytic subunit of PP2A (PP2Ac) and preventing the A and B subunits from forming an active complex.[30,31] Because of the central role of PP2A in the regulation of inflammatory cascades through dephosphorylation, including the nuclear factor κB (NF-κB) and mitogen-activated protein kinase pathways,[32,33] inhibition of PP2Ac permits activation of inflammatory cascades, primarily through T_H2-mediated mechanisms but also through early inflammatory factors, such as CCL11, CCL20, IL-25, and IL-33.[28,29,34] TRAIL-induced upregulation of MID1 has been shown to promote allergic inflammation and airways remodeling in the lung through inhibition of PP2A activity.[28,29,35]

Although EoE and allergic asthma remain distinct disorders, eosinophilic inflammation with subsequent remodeling is common to both diseases. Given the crucial role of TRAIL in the promotion of eosinophilic inflammation and remodeling in AAD, we hypothesized it would contribute to esophageal inflammation and remodeling in an allergen-induced murine model of EoE (eg, *Aspergillus fumigatus* induced).

METHODS

RNA sequencing of human biopsy specimens

Patient cohorts and methods for RNA sequencing and analyses have been described previously.[36] In brief, distal esophageal biopsy specimens from 6 healthy control subjects (no EoE diagnosis and 0 eosinophils per high-power field) and 10 patients with active EoE (EoE diagnosis and 163 ± 29 eosinophils per high-power field [mean ± SEM]) were subjected to RNA sequencing. Sequencing reads were aligned against the GRCh37 reference genome by using UCSC gene models. Raw expression data (fragments per kilobase of transcript per million mapped reads) were assessed for statistical significance by using the Welch *t* test with a Benjamini-Hochberg false discovery rate and *P* value threshold of less than .05 and a 2.0-fold cutoff filter, and cluster analysis was performed in GeneSpring GX (Agilent Technologies, Clara, Calif). These data were deposited into the Gene Expression Omnibus (GSE58640).

Mice

Wild-type (WT) and TRAIL-deficient ($TRAIL^{-/-}$) BALB/c mice (male, 8-12 weeks of age) were obtained from Australian Bioresources (Moss Vale, Australia) under a material transfer agreement with Amgen (Thousand Oaks, Calif). All experiments were approved by the Animal Care and Ethics Committee of the University of Newcastle.

A fumigatus mouse model of EoE

The *A fumigatus* mouse model of EoE, which was described previously by Mishra et al,[37] was used to investigate the role of TRAIL in EoE. Briefly, mice were intranasally challenged with 100 μg of *A fumigatus* extract (Greer Laboratories, Lenoir, NC) in 50 μL of sterile saline 3 times a week for 3 weeks after administration of isoflurane anesthetic. Control mice received 50 μL of saline only. Mice were killed for esophageal samples 24 hours after the final *A fumigatus* challenge by using pentobarbital sodium (Virbac, Milperra, Australia).

Small interfering RNA–mediated inhibition of MID1

ON-TARGET small interfering RNAs (siRNAs) were purchased from Dharmacon (Millennium Science, Mulgrave, Australia) at a concentration of