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Critical Roles of TIPE2 Protein in Murine Experimental Colitis

Yunwei Lou,*,†,1 Honghong Sun,*†,1 Samantha Morrissey,*, Thomas Porturas,*, Suxia Liu,† Xianxin Hua,‡ and Youhai H. Chen*

Both commensal bacteria and infiltrating inflammatory cells play essential roles in the pathogenesis of inflammatory bowel disease. The molecular mechanisms whereby these pathogenic factors are regulated during the disease are not fully understood. We report in this article that a member of the TNF-α–induced protein 8 (TNFAIP8) family called TIPE2 (TNFAIP8-like 2) plays a crucial role in regulating commensal bacteria dissemination and inflammatory cell function in experimental colitis induced by dextran sodium sulfate (DSS). Following DSS treatment, TIPE2-deficient mice, or chimeric mice that are deficient in TIPE2 only in their hematopoietic cells, lost less body weight and survived longer than wild-type controls. Consistent with this clinical observation, TIPE2-deficient mice exhibited significantly less severe colitis and colonic damage. This was associated with a marked reduction in the colonic expression of inflammatory cytokines, such as TNF-α, IL-6, and IL-12. Importantly, the ameliorated DSS-induced colitis in TIPE2−/− mice also was associated with reduced local dissemination of commensal bacteria and a weaker systemic inflammatory response. Combined with our previous report that TIPE2 is a negative regulator of antibacterial immunity, these results indicate that TIPE2 promotes colitis by inhibiting mucosal immunity to commensal bacteria. The Journal of Immunology, 2014, 193: 1064–1070.

Human inflammatory bowel diseases (IBDs), represented primarily by ulcerative colitis and Crohn’s disease, cause considerable morbidity and significantly increase the risk for cancer in the colon and rectum (1). Ulcerative colitis exhibits a characteristic profile of chronic, relapsing and remitting inflammation involving the distal colon and rectum, and it is generally considered an immune-mediated disorder resulting from abnormal interactions between colonic microflora and mucosal immune cells (2). The relapsing and remitting course of the disease varies greatly among patients, with ~15% developing an “acute severe” form some time in their lives (3). Although the precise etiology of ulcerative colitis is not well understood, it is widely accepted that both genetic and environmental factors are involved (4). Several animal models of experimental colitis have been developed to help investigate the molecular and cellular mechanisms of the disease. Of these, the dextran sodium sulfate (DSS)-induced experimental colitis model is one of the best studied. It is initiated by DSS-induced damage to the intestinal epithelial cells but is dependent on the presence of both commensal microflora and myeloid (but not lymphoid) cells (5–7). Similar to human IBD, DSS-induced colitis is limited to the colonic mucosa and is characterized by diarrhea, bloody feces, weight loss, colonic ulceration, and a histopathological picture of inflammation, consisting mainly of infiltrating macrophages and granulocytes (5, 6).

TIPE2, TNF-α–induced protein 8 (TNFAIP8) like-2, is a member of the TNFAIP8 family (8) and is preferentially expressed in hematopoietic cells (8–10). TIPE2 serves as a negative regulator of immunity that maintains immune homeostasis (8, 11). Abnormal expression of TIPE2 has been found in patients with systemic lupus erythematosus, hepatitis B, diabetic nephropathy, and childhood asthma (12–15). TIPE2 is also implicated in the development of atherosclerosis and experimental stroke (16–18). TIPE2 may control innate immunity to bacteria and dsRNA viruses by targeting the Rac GTPases (19, 20). However, the role of TIPE2 in IBDs has not been reported. To address this issue, we studied DSS-induced colitis in TIPE2-deficient mice. We report in this article that TIPE2 plays an important role in the development of acute colitis by promoting dissemination of commensal bacteria in the colon.

Materials and Methods

Mice

Wild-type (WT) C57BL/6 (B6) and CD45.1+ B6 mice were purchased from The Jackson Laboratory. B6 mice that carry a TIPE2 gene–null mutation were generated by backcrossing TIPE2−/− 129 mice to B6 mice for 12 generations, as described previously (8, 19). All mice used were male and 8–12 wk old and were maintained under pathogen-free conditions in the University of Pennsylvania Animal Care Facilities. All animal procedures were preapproved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Induction and evaluation of DSS-induced colitis

Experimental colitis was induced by adding DSS (m.w. = 36,000–50,000; MP Biomedicals, Solon, OH) to the drinking water to a final concentration of 4% (w/v). Subsequently, mice were switched to regular drinking water.
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Real-time quantitative PCR

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions. Two micrograms of total RNA were reverse transcribed using SuperScript II transcriptase (Invitrogen) as previously described (18, 24). Recipient mice received antibiotics in their drinking water, followed by a 5-wk enteral refeeding period. Six weeks after the transplantation, the degree of bone marrow reconstitution was determined by staining peripheral blood leukocytes with PE-conjugated anti-CD45.1 and PerCP-Cy5.5-conjugated anti-CD45.2 (BD Biosciences). As we reported, in the chimeric mice so generated, >90% of the hematopoietic cells were derived from donor bone marrow (18, 24).

Assessment of intestinal permeability

Intestinal barrier permeability in vivo was measured using an FITC-labeled dextran method, as previously described (25). In brief, WT and TIPE2−/− mice were deprived of water and food overnight and were administered permeability tracer FITC-dextran (molecular mass 4 kDa; Sigma-Aldrich) at 400 mg/kg body weight by oral gavage. Blood was collected 4 h later by retro-orbital bleeding. Fluorescence intensity of the serum was measured using a fluorescence spectrophotometer with an excitation wavelength of 490 nm and an emission wavelength of 530 nm. FITC-dextran concentrations were calculated using standard curves generated by a serial dilution of FITC-dextran.

Results

Expression of TIPE family members in the murine colon

Unlike TIPE2−/− mice that develop systemic inflammation early in their lives, young TIPE2−/− mice of the B6 background are relatively healthy, with a normal gastrointestinal tract (Supplemental Fig. 1). TIPE2 mRNA was readily detected in the colon tissue homogenates from WT, but not TIPE2−/− mice (Fig. 1A). In contrast, similar levels of TNFAIP8, TIPE1, and TIPE3 were detected in WT and TIPE2−/− colon, indicating that TIPE2 deficiency did not affect the expression of these related genes (Fig. 1B).

TIPE2−/− mice are resistant to DSS-induced colitis

Experimental colitis, induced by oral feeding with DSS, is a well-established model for IBD (26–28). To determine the potential role of TIPE2 in colonic inflammation, WT and TIPE2−/− mice were administered 4% DSS for 5 d to induce acute colitis. TIPE2 deficiency did not affect water consumption (Supplemental Fig. 2), but it markedly reduced the severity of the colitis (Fig. 2A). Mortality was reduced from 80% in the WT group to 0% in the TIPE2−/− group. When a higher DSS dose (5%) was used, TIPE2−/− mice died as well, but with a significant delay (Supplemental Fig. 3). Body weight loss is considered a surrogate marker of morbidity.
Consistent with the mortality data, TIPE2−/− mice lost considerably less weight than did the WT controls, commencing on day 5. The body weight difference increased gradually until the end of the study (Fig. 2B). No weight loss was observed in mice drinking regular water (Fig. 2B). Consistent with these results, the clinical manifestation of the disease, as reflected by DAI, was significantly less severe for TIPE2−/− mice compared with WT mice (Fig. 2C). Differences in colon weight and colon length also were apparent between the two groups. Without DSS treatment, TIPE2−/− mice had colon weights and colon lengths similar to those of WT mice. After DSS treatment, the colon weight of TIPE2−/− mice was 23% more than that of WT mice (Fig. 2D), whereas the colon length of TIPE2−/− mice was 27% longer than that of WT mice (Fig. 2E; Fig. 2F, left panel).

Histological examination also was performed to validate the clinical data. DSS treatment induced significant histopathological changes in the colons of WT mice that were characterized by massive inflammatory infiltrates and disruption of mucosal structures (Fig. 2F, right panel), consistent with previous reports (29). However, TIPE2−/− mice displayed less severe injury compared with WT mice (Fig. 2F, right panel).

Reduced inflammatory cytokine expression and inflammatory cell infiltration in TIPE2−/− colon

DSS-induced colitis is an inflammatory disease mediated by many proinflammatory cytokines (30). To determine the effect of TIPE2 deficiency on the production of proinflammatory cytokines at the site of DSS-induced inflammation, we examined the expression levels of several cytokines by ELISA. We found that proinflammatory cytokines, such as TNF-α, IL-6, and IL-12, were markedly increased in WT mice after DSS treatment. However, TIPE2−/− mice produced significantly less of these cytokines relative to WT mice (Fig. 3A–C). In contrast, TIPE2−/− mice produced comparable amounts of IL-1β, IL-4, and IFN-γ to WT mice (data not shown).

To characterize the inflammatory cells involved, leukocytes isolated from lamina propria of the colon were analyzed by flow cytometry. Various inflammatory cell subsets were found in WT and TIPE2−/− colons after DSS feeding. In comparison with WT colon, the TIPE2−/− colon had markedly reduced neutrophils (8.89 ± 0.55 versus 2.92 ± 0.32, p < 0.001) (×10^6/g colon), macrophages (12.63 ± 0.63 versus 6.92 ± 0.69, p < 0.001) (×10^6/g colon), and dendritic cells (24.04 ± 3.65 versus 11.97 ± 0.27, p < 0.05) (×10^6/g colon) (Fig. 3D–F). Importantly, a significant proportion of the leukocytic infiltrate in both WT and TIPE2−/− mice consisted of Ly6G CD11b+ macrophages (Fig. 3D–F).

TIPE2 deficiency in hematopoietic cells ameliorates colitis

Because both colonic epithelial and immune cells play important roles in the development of colitis, we next determined the cell populations that are critical for a TIPE2-mediated effect. We detected no significant difference in colonic epithelial cell apoptosis, proliferation, or permeability between WT and TIPE2−/− mice administered 4% DSS for 3 d (Y. Lou, H. Sun, and Y. Chen, unpublished observations), suggesting that TIPE2 may not have a primary effect on colonic epithelium in DSS-induced colitis.

**FIGURE 2.** Reduced experimental colitis in TIPE2−/− mice. (A) WT (n = 5) and TIPE2−/− (n = 5) mice were administered 4% DSS in the drinking water for 5 d and then switched to regular drinking water. Survival was monitored until day 14 after the initiation of DSS. This experiment was repeated three times with similar results. The differences between the two groups are statistically significant as determined by Kaplan–Meier analysis (p < 0.01). (B–G) WT (n = 5) and TIPE2−/− (n = 5) mice were administered 4% DSS or water ad libitum for 5 d, followed by recovery on regular drinking water for 2 d prior to euthanasia. (B) Body weight loss was measured daily. (C) DAI was scored daily. Mice were sacrificed on day 7, and colon weight (D) and colon length (E) were examined. (F) Representative colons (left panel) and their H&E-stained sections (right panel, original magnification ×100) from WT and TIPE2−/− mice on day 7. (G) Colonic histopathological scores of mice shown in (F, right panel). Data are mean ± SEM of one representative experiment of three. *p < 0.05, **p < 0.01, ***p < 0.001.

**FIGURE 1.** Expression of TNFAIP8 family of genes in murine colon. (A) mRNA levels of TNFAIP8 family members were determined by RT-PCR using total RNA isolated from the distal colon of WT and TIPE2−/− mice. GAPDH was used as a loading control. Samples lacking reverse transcriptase (-) were used as negative controls for the PCR. (B) Quantification of mRNA expression by quantitative real-time PCR. Data are mean ± SEM (n = 3).
To determine the potential roles of hematopoietic cells, we generated four groups of bone marrow chimeras. Six weeks after bone marrow transplantation, we found that >90% of the blood leukocytes were of donor origin, confirming successful engraftment (Supplemental Fig. 4). Following DSS treatment, knockout (KO) → KO chimeric mice developed the least severe colitis (Fig. 4A, 4B). Transplant of KO bone marrow to WT recipient mice (KO → WT) markedly reduced body weight loss (Fig. 4A), improved DAI (Fig. 4B), preserved the colon length (Fig. 4C), and reduced the histopathological score (Fig. 4D, 4E). These results indicate that TIPE2-deficient hematopoietic cells are responsible for the reduced colonic inflammation in TIPE2-KO mice.

Reduced local dissemination of commensal microflora and systemic inflammation in TIPE2 KO mice

It is well established that commensal microflora in the lumen of the colon play an essential role in the development of human IBD and murine experimental colitis (31–33). Our previous study showed that TIPE2-KO macrophages and neutrophils had enhanced phagocytic and bactericidal activities, and TIPE2-KO mice were resistant to *Listeria monocytogenes* and *Staphylococcus aureus*. 

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infections (19). Because we observed a reduced inflammatory response in DSS-induced colitis in TIE2−/− mice, we asked whether TIE2 deficiency affected commensal microflora dissemination in mice. As shown in Fig. 5A and 5B, significantly less bacteria were detected in the colon of TIE2−/− mice relative to DSS-treated WT mice. We also found a significant decrease in plasma FITC fluorescence in TIE2−/− mice after DSS-induced injury compared with WT mice (Fig. 5C), indicating less colon tissue disruption and less permeability (Fig. 2F). It is known that injury compared with WT mice (Fig. 5C), indicating less colon tissue disruption and less permeability (Fig. 2F). It is known that injury compared with WT mice (Fig. 5C), indicating less colon tissue disruption and less permeability (Fig. 2F). It is known that injury compared with WT mice (Fig. 5C), indicating less colon tissue disruption and less permeability (Fig. 2F). It is known that injury compared with WT mice (Fig. 5C), indicating less colon tissue disruption and less permeability (Fig. 2F). It is known that injury compared with WT mice (Fig. 5C), indicating less colon tissue disruption and less permeability (Fig. 2F). It is known that injury compared with WT mice (Fig. 5C), indicating less colon tissue disruption and less permeability (Fig. 2F). It is known that injury compared with WT mice (Fig. 5C), indicating less colon tissue disruption and less permeability (Fig. 2F).

FIGURE 5. Reduced local dissemination of commensal microflora and weakened systemic inflammatory responses in TIE2−/− mice during colitis. WT (n = 5) and TIE2−/− (n = 4) mice were administered 4% DSS or water ad libitum for 5 d, followed by recovery on regular drinking water for 2 d before sacrifice. Bacterial counts in colonic tissues of control and DSS-fed WT and TIE2−/− mice were determined by colony-forming assay using brain heart infusion agar (A) and blood agar (B). (C) Control and DSS-fed mice were administered FITC-dextran (0.4 mg/g) by oral gavage, and serum FITC-dextran concentrations were determined 4 h later. (D) Serum concentrations of IL-6 in WT and TIE2−/− mice were determined by ELISA at the end of the experiment. (E) WBC counts of WT and TIE2−/− mice were determined using a Drew Hemavet 950FS on day 7. Data are mean ± SEM of one representative experiment of three. *p < 0.05, **p < 0.01, ***p < 0.001.

Discussion

The results reported in this article indicate that TIE2 is an important regulator of DSS-induced colitis. TIE2−/− mice are resistant to DSS-induced colitis, and enhanced bacterial clearance after DSS treatment may be responsible. TIE2 function has been studied in several diseases, including autoimmune diseases, bacterial infection, chronic inflammatory diseases, and cancer (12, 18, 19, 34). TIE2 is constitutively expressed at high levels in immune cells, especially in macrophages and neutrophils (8, 19, 20), which play crucial roles in acute ulcerative colitis. To our knowledge, little is known about the function of TIE2 in intestinal disorders. Acute ulcerative colitis induced by DSS is characterized by massive infiltration of inflammatory cells, such as macrophages, neutrophils, and CD4+ T cells, within the colonic walls, which destroy epithelium and shorten the colon length (35). These infiltrated inflammatory cells are major producers of inflammatory mediators, such as macrophages, neutrophils, and CD4+ T cells, within the colonic walls, which destroy epithelium and shorten the colon length (35).

Our previous studies showed that TIE2-deficient macrophages produced significantly more IL-6 and IL-12 upon stimulation with LPS, and TIE2-deficient mice were more susceptible to LPS-induced septic shock (8). We also found that TIE2-deficient mice were more susceptible to polyinosinic-polycytidylic acid lethality (20). In addition, TIE2 deficiency exacerbates cerebral ischemia/reperfusion injury (16) and atherosclerosis in Ldlr−/− mice (18). Based on these findings, we expected TIE2−/− mice to be more susceptible to colitis development than WT mice. On the contrary, TIE2 deficiency rendered mice resistant to DSS-induced colitis. Adoptive transfer of TIE2-deficient bone marrow cells was capable of rescuing colonic injury phenotype in WT mice, suggesting that TIE2 expression in hematopoietic cells may play an important role in the development of colitis. Our previous studies also showed that TIE2 serves as a negative regulator of phagocytosis and oxidative burst during infection. TIE2-deficient mice exhibited resistance to bacterial challenge, and TIE2-deficient macrophages and neutrophils exhibited enhanced bacterial clearance (19). Indeed, significantly fewer bacteria were found in the colon of TIE2−/− mice relative to DSS-treated WT mice. The number of leukocytes and serum concentrations of proinflammatory cytokine IL-6 were markedly reduced in TIE2−/− mice, consistent with a reduced bacterial dissemination in these mice. Thus, in colitis, enhanced bacterial clearance in TIE2−/− mice likely ameliorated the disease.
In summary, we discovered that TIPE2 deficiency reduces inflammatory responses in a murine acute colitis model through enhancement of immune responses to commensal bacteria. Our data suggest a potential role for TIPE2 in the pathogenesis of IBD, especially in patients with impaired protection against commensal bacteria. This finding advances our understanding of the mechanisms of IBD, and it may lead to the development of TIPE2-based strategies for treating the disease.

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Disclosures

The authors have no financial conflicts of interest.

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FIGURE 6. Analysis of microbiota composition in naive WT and TIPE2−/− mice. Stool genomic DNA of 6-wk-old (A) and 8-wk-old (B) WT (n = 3) and TIPE2−/− (n = 3) mice was extracted using the QIAamp DNA Stool Mini Kit. Quantitative real-time PCR was performed to quantify the numbers of total bacteria, Enterobacteriaceae (Ent), the E. rectale–C. coccoides (Eub) group, and the Bacteroides (Bac) group per gram of stool. Data are mean ± SEM of one representative experiment of three.


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