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Critical Roles of TIPE2 Protein in Murine Experimental Colitis

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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.

H uman 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−/− 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.
until the end of the experiment. Mice were examined daily to determine their clinical Disease Activity Index (DAI), which was based on the degree of body weight loss, stool consistency, and fecal blood (ranging from 0 to 12), as described previously (21). Briefly, DAI was scored as follows: weight loss (no change = 0; <5% = 1; 6–10% = 2; 11–20% = 3; >20% = 4), stool normal = 0; soft, well-formed = 1; soft without pellets = 2; diarrhea = 4), and blood (no blood = 0; visible blood in rectum = 1; gross bleeding in rectum = 2; visible blood on fur = 4). For histological analysis, the distal colonic specimens were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with H&E, and pathological scores, ranging from 0 to 6 (combining inflammatory cell infiltration score and tissue damage score), were determined as follows (22): inflammatory cell infiltration in the lamina propria (occasional inflammatory cells = 0; increased inflammatory cells = 1; confluence of inflammatory cells extending to the submucosa = 2; transmural extension = 3) and tissue damage (no mucosal damage = 0; lymphoepithelial lesions = 1; surface mucosal erosion = 2; extensive mucosal damage and extension into deeper structures of the bowel wall = 3).

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). Real-time quantitative PCR was performed out in an Applied Biosystems 7500 System with Power SYBR Green PCR Master Mix (Applied Biosystems). Quantitect Primers for mouse GAPDH, TIPE1, TIPE2, and TIPE3 were purchased from QIAGEN. TIPE primer sequences used were as follows: forward, 5'-AGCAGTCCAACTCCGGGGAACAG-3' and reverse, 5'-TGTGACATGCGTGGTGCGGATG-3'. Each sample was run in triplicate. The relative changes in gene expression were calculated using GAPDH as the loading control. For interrogating gut microbial diversity, triplicate. The relative changes in gene expression were calculated using

Bacterial culture

After euthanizing the mice, the entire colon was removed under aseptic conditions. The terminal 3-cm segment of distal colon was washed, weighed, homogenized, and serially diluted. Different dilutions of the suspensions were plated in triplicate on brain heart infusion agar and blood agar (BD Biosciences) plates and incubated at 37°C for 24 h to quantify the bacterial colonies.

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.

Blood cell counts

Before euthanizing the DSS-treated mice, blood was collected, and whole-blood cell counts were determined using a Drew Hemavet 950FS (Drew Scientific, Oxford, U.K.).

Statistical analysis

Quantitative data are presented as mean ± SEM of two or three experiments. The survival curves were plotted according to the Kaplan–Meier method and compared using the log-rank test. Two-tailed Student’s t test was used for all other cases, and p < 0.05 was considered statistically significant. All statistical analyses were performed with Prism 5.0 for Windows (GraphPad, San Diego, CA).

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, 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). The histopathological score of TIPE2−/− mice was significantly lower than that of WT mice (2.6 ± 0.4 versus 4.6 ± 0.6, respectively) (Fig. 2G). Taken together, these results demonstrate that TIPE2−/− mice may be significantly less susceptible to DSS-induced colitis.

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).

**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).

**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 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 colon sections (left panel) and their H&E-stained sections (right panel, original magnification ×100) from WT and TIPE2−/− mice on day 7. (G) Colon histopathological scores of mice are shown in (F, right panel). Data are mean ± SEM of one representative experiment of three. *p < 0.05, **p < 0.01, ***p < 0.001.
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−/− 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*.
infections (19). Because we observed a reduced inflammatory response in DSS-induced colitis in TIPE2−/− mice, we asked whether TIPE2 deficiency affected commensal microflora dissemination in mice. As shown in Fig. 5A and 5B, significantly less bacteria were detected in the colon of TIPE2−/− mice relative to DSS-treated WT mice. We also found a significant decrease in plasma FITC fluorescence in TIPE2−/− 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 systemic dissemination of bacteria and bacterial components leads to increased peripheral blood leukocyte counts, as well as triggers tissue disruption and less permeability (Fig. 2F). It is known that injury compared with WT mice (Fig. 5C), indicating less colon dissemination in mice. As shown in Fig. 5A and 5B, significantly less bacteria were detected in the colon of TIPE2−/− mice relative to DSS-treated WT mice (Fig. 5E). Although there appeared to be a trend toward decreased neutrophils and lymphocytes in the KO mice, the differences were not statistically significant (Fig. 5D). Furthermore, serum concentrations of the proinflammatory cytokine IL-6 also were lower in TIPE2−/− mice compared with WT mice (Fig. 5D). Taken together, these results indicate that the reduced DSS-induced morbidity and mortality in the absence of TIPE2 may be caused by enhanced immunity to commensal bacteria.

To interrogate gut microbial diversity, we performed quantitative real-time PCR amplification of 16S rRNA gene sequences. The total DNA in stool was extracted, and real-time PCR was conducted using specific 16S rRNA primers for the following major groups: E. rectale–C. cocoides, Bacteroides spp., and Enterobacteriaceae. In addition, the total bacterial (eubacteria) numbers were determined using standard curves constructed with reference bacteria specific for each group. We found no significant differences in the numbers of total bacteria or the three representative bacterial groups between naive WT and TIPE2−/− mice at 6–8 wk of age (Fig. 6), indicating that bacterial composition was not affected by TIPE2 deficiency.
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.

References

28. Ghia, J. E., F. Galeazzi, D. C. Ford, C. M. Hogaboam, B. A. Vallance, and S. Collins. 2008. Role of M-CSF-dependent macrophages in colitis is driven by...
Fig. S1. Young *TIPE2*-/- B6 mice display normal colonic morphology. Representative images of HE-stained colonic cross-sections (A) and longitudinal sections (B) of unmanipulated six-week-old WT and *TIPE2*-/- B6 mice. Original magnifications for A, X50; original magnifications for B, X100. Data are representative of two independent experiments (n=3).
Fig. S2. *TIPE2*−/− mice have normal water consumption. WT and *TIPE2*−/− mice (n=5) were fed with 4% DSS for 5 days. Water consumption was monitored daily. Data shown are means ± SEM of water consumption per day per mice, pooled from four independent experiments.
Fig S3. TIPE2 deficiency prolongs the lives of mice with DSS-induced colitis. WT and TIPE2−/− (n=5) mice were fed with 5% DSS in the drinking water for 5 days and then switched to regular drinking water. The difference in survival between the two groups is statistically significant as determined by Kaplan-Meier analysis (P<0.05).
Fig. S4. Confirmation of reconstitution of bone marrow chimeric mice. Representative flow cytometric profiles of peripheral blood leukocytes of the chimeric mice stained with anti-CD45.1-PE (for WT cells) and anti-CD45.2-Percp-Cy5.5 (for Tipe2−/− cells).