Exacerbated Experimental Colitis in TNFAIP8-Deficient Mice

Honghong Sun, Yunwei Lou, Thomas Porturas, Samantha Morrissey, George Luo, Ji Qi, Qingguo Ruan, Songlin Shi and Youhai H. Chen

*J Immunol* published online 6 May 2015
http://www.jimmunol.org/content/early/2015/05/06/jimmunol.1401986

**Supplementary Material**  
http://www.jimmunol.org/content/suppl/2015/05/06/jimmunol.1401986.6.DCSupplemental

**Subscription**  
Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  
Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  
Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Exacerbated Experimental Colitis in TNFAIP8-Deficient Mice

Honghong Sun,*† Yunwei Lou,*†,‡ Thomas Porturas,* Samantha Morrissey,* George Luo,* Ji Qi,* Qingguo Ruan,* Songlin Shi,*† and Youhai H. Chen*

The TNF-α–induced protein 8 (TNFAIP8 or TIPE) is a risk factor for cancer and bacterial infection, and its expression is upregulated in a number of human cancers. However, its physiologic and pathologic functions are unclear. In this study, we describe the generation of TIPE-deficient mice and their increased sensitivity to colonic inflammation. TIPE-deficient mice were generated by germ line gene targeting and were born without noticeable developmental abnormalities. Their major organs, including lymphoid organs and intestines, were macroscopically and microscopically normal. However, after drinking dextran sodium sulfate–containing water, TIPE-deficient mice developed more severe colitis than wild type mice did, as demonstrated by decreased survival rates, increased body weight loss, and enhanced leukocyte infiltration, bacterial invasion, and inflammatory cytokine production in the colon. Bone marrow chimeric experiments revealed that TIPE deficiency in nonhematopoietic cells was responsible for the exacerbated colitis in TIPE-deficient mice. Consistent with this result, TIPE-deficient intestinal epithelial cells had increased rate of cell death and decreased rate of proliferation as compared with wild type controls. These findings indicate that TIPE plays an important role in maintaining colon homeostasis and in protecting against colitis. The Journal of Immunology, 2015, 194: 000–000.

The TNF-α–induced protein 8 (TNFAIP8 or TIPE), also known as SCC-S2, GG2-1, NDED, and MDC-3,13, is the first member of the TNFAIP8 family that was cloned and studied (1–4). TIPE may prevent apoptosis in certain tumor cell lines and increase their oncogenic potential (1, 2). Overexpression of TIPE gene in tumor cells correlates with enhanced proliferation and tumorigenicity (5). By contrast, TIPE may promote glucocorticoid–induced apoptosis of normal thymocytes in culture (6). TIPE has been reported to interact with activated Gox to regulate cell death and transformation, and to interact with karyopherin alpha-2 in PC-3 cells (7). TIPE gene polymorphism or overexpression is associated with susceptibility to Staphylococcus aureus infection (8), cancer development (9–11), and psoriasis (12). TIPE might not only be involved in disease pathogenesis; it might also serve as a biomarker for certain inflammatory and neoplastic diseases. However, the precise roles of TIPE in health and disease remain to be established.

Human inflammatory bowel diseases, including ulcerative colitis and Crohn disease, are a major health problem in developed countries (13). These diseases are characterized by chronic colonic inflammation and are mediated by infiltrating inflammatory cells. The etiologic factors that trigger these diseases are not well understood. In mice, oral administration of dextran sodium sulfate (DSS) induces a form of colitis that shares many pathologic and clinical features with human inflammatory bowel diseases; therefore, DSS-induced colitis is considered to be a valuable experimental model for human inflammatory bowel diseases (14–16).

Increased colon epithelial cell death and decreased proliferation are associated with the development of colitis. Given the potential roles of TIPE in regulating these processes, we set out to test the in vivo effect of TIPE deficiency on the development of DSS-induced colitis using our newly generated Tipe-deficient mice. Our data indicate that TIPE plays a crucial role in maintaining colonic homeostasis during colitis.

Materials and Methods

Animals

Wild type (WT) C57BL/6 (B6) and CD45.1+ B6 mice were purchased from The Jackson Laboratory. All mice used in this study were housed under pathogen-free conditions in the University of Pennsylvania Animal Care Facilities. All animal protocols used were preapproved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Generation and genotyping of Tipe−/− mice

The B6 ES cell line with a disrupted Tipe gene (NM_134131) was obtained from the Texas A&M Institute for Genomic Medicine (College Station, TX). The gene-trapping vector that disrupts the Tipe gene was inserted into the only intron of the gene. The ES cells were injected into mouse blastocysts to generate chimeras. Six chimeras, one female and five male, were produced. The germine transmission was obtained from one of the male chimeras. A common WT primer 1 (5′-CCCAAGGGTCAACATGCTCT-3′) was paired with 1) a reverse primer 3 (5′-CCAATAAACCCTCTTGCAG-3′) against the sequence on gene trap vector to produce a 190 bp PCR fragment from Tipe-deficient allele, and 2) a WT reverse primer 2 (5′-CCCATGTGTGCAAGTGAAAA-3′) to generate a 190 bp PCR fragment from the WT allele. Heterozygous mice for the disrupted Tipe locus were bred to produce homozygous Tipe−/− animals. Once identified, the mice were further tested with PCR using primers 4 (5′-ACCTGGCCGTTCAGGACCAAA-3′) and primer 5 (5′-TCACCGCGAGTGAGAGA-3′) to ensure that Tipe mRNA is not expressed in Tipe−/− animals.

RT-PCR

Total RNA was isolated from brain, spinal cord, lung, liver, heart, spleen, intestine, and mesenteric lymph nodes of WT and Tipe−/− mice with Trizol reagent (Sigma) according to the manufacturer’s instructions. The isolated RNA was further purified with RNeasy Mini kits (Qiagen) according to the manufacturer’s instructions. RNA samples (250 ng) were reverse transcribed with oligo(dT) and SuperScript II transcriptase (Invitrogen). The
generated cDNA was diluted with sterile Milli-Q water (1:4). Real-time quantitative PCR analysis was performed using specific Quantitect Primers for mouse GADPH, TIPe, TIPe1, TIPe2, TIPe3, IL-6, IL-1β, IL-17, and CXCL2 (Qiagen) in an Applied Biosystems 7500 system using Power SYBR Green PCR Master Mix (Applied Biosystems). Relative levels of gene expression were determined with GADPH as the control.

**DSS-induced colitis**

Experimental colitis was induced with 3% or 4% (w/v) DSS (m.w. 36,000–30,000; MP Biologials) dissolved in sterile, distilled water ad libitum for 5 d followed by normal drinking water until the end of experiment. For survival studies, 8–10 wk-old mice were treated with 4% DSS for 5 d that was replaced with normal drinking water until day 14. For histologic analysis and gene expression and cytokine production studies, mice were treated with 3% DSS for 5 d followed by regular drinking water for 2 d; they were euthanized at different time points after the DSS treatment.

**Determination of clinical scores**

Body weight, stool consistency, and rectal bleeding were monitored daily (17). Stool scores were determined as follows: 0, well-formed pellets; 1, soft and semiformal but not adhered to the anus; 2, semiformal but adhered to the anus; 3, liquid and adhered to the anus; 4, liquid stool. Bleeding scores were determined as follows: 0, no blood; 1, blood traces in rectum; 2, visible blood in whole rectum; 3, blood traces in both rectum and stool; 4, gross rectal bleeding. Weight loss scores were monitored as follows: 0, no body weight change; 1, body weight loss within 5%; 2, body weight loss within 10%; 3, weight loss within 20%; 4, body weight loss exceeding 20%. The disease activity index was the sum of these three scores.

**Histopathology and immunohistochemistry**

The entire colon was excised to measure the length. The distal colons were washed, fixed in 10% buffered formalin, and embedded in paraffin. Tissue sections were stained with H&E. For Ki-67 and active caspase 3 staining, mice were treated with DSS and colons were collected, washed, and fixed in 10% formalin. Colon sections were then stained with Ki-67 and active caspase 3 Ab (R&D Systems). For TUNEL, slides were deparaffinized and rehydrated, and then treated with proteinase K (DAKO S3004) for 20 min at 40˚C. Slides were then rinsed in Tris buffer, incubated with the Roche enzyme labeling mix for 60 min at 40˚C, counterstained with DAPI, and mounted with Prolong Gold (Life Technologies P36934). TUNEL slides were scanned on an fluorescence slide scanner (Leica Bioimaging) and images were analyzed using the Cyto-nuclear FL v1.3 algorithm (Indica Labs).

**Bone marrow chimeras**

Bone marrow cells were flushed from the femurs and tibias of donor mice. The RBCs were lysed with ACK solution (8.29 g NH₄Cl, 1 g KHCO₃, 37.2 mg Na₂ EDTA in 1 l of water). Colon samples were washed twice and resuspended in cold PBS. Recipient mice were lethally irradiated with 500 rad twice, separated by 24 h. The irradiated mice received a total of 10 x 10⁶ of donor bone marrow cells by tail vein injection 1 or 2 h after irradiation. Six to seven weeks later, peripheral blood leukocytes were collected and stained with CD45.1 and CD45.2 Abs to determine the reconstitution rate. In the chimeric mice so generated, more than 90% of the hematopoietic cells were derived from donor bone marrow (18).

**Isolation of colon epithelial cells**

Colonic epithelial cells were isolated as described (19, 20). Colons were dissected, washed with cold PBS, and cut into small pieces. Colon segments were incubated in PBS supplemented with 1 mM EDTA and 1 mM DTT for 30 min at 37˚C with gentle shaking. Cells in supernatants were filtered through a 70-μm cell strainer. The flow-through cells were collected and stained with CD45.1 and CD45.2 Abs to determine the reconstitution rate. In the chimeric mice so generated, more than 90% of the hematopoietic cells were derived from donor bone marrow (18).

**Isolation of colon leukocytes isolation**

Colon leukocytes were isolated using a modified version of a previously described protocol (21). Colon samples were washed three times and incubated in PBS with 5% FBS, 2 mM EDTA, and 1 mM DTT for 15 min at 37˚C with shaking (250 rpm). This process was repeated three times to remove epithelial cells. The colon samples without epithelial cells were digested in PBS with 5% FBS, 0.5 mg/ml collagenase (Sigma), 0.02 mg/ml DNase (Roche), and 0.1 mg/ml Dispase (Invitrogen), for 20 min at 37˚C with gentle shaking for an additional 45 min with shaking at 37˚C. The mixture then was filtered through a 70-μm cell strainer. The flow-through contains colon leukocytes.

**Plasmid DNA transfection and viral infection**

293T cells were transfected with plasmid DNA using Fugene 6 (Promega) reagent according to the manufacturer’s instructions. For virus production, pLKO.1 (with puromycin resistance) with shRNA-Tnfaip8 (purchased from Open Biosystems) or shScr (purchased from Addgene) fragments and packaging constructs were cotransfected into 293T cells. After 24 and 48 h, virus-containing medium was filtered and used to infect 3T3 cell lines in the presence of 6.5 mg/ml polybrene (Millipore). Infected cells were selected using puromycin (Sigma) to establish shRNA-Scr, shRNA-Tnfaip8-1, and shRNA-Tnfaip8-2 cell lines. Viral vectors were produced from the pLKO.1 plasmid containing shRNA-Tnfaip8-1 (5'-AAAGGGATTTGAT-CGAGGCAGC-3'), shRNA-Tnfaip8-2 (5'-TTGAAGTTGTTGTTCTTG-3'), or shRNA-scr (5'-CGAGGCGACTAACCTTAGG-3'). The shRNA-Tnfaip8-1 cell lines knock down all Tipe protein expression.

**ELISA**

The colon homogenates and serum were collected and stored at −80˚C. Abs used in ELISA were purchased from BD Pharmingen and eBioscience, including purified and biotinylated rat anti-mouse IL-6, IL-17A, and IL-1β. Quantitative ELISA was performed using paired mAbs specific for the corresponding cytokines according to the manufacturer’s instructions.

**Phenotyping of Tipe−/− mice**

Six-to-eight-week-old, age-matched WT and knockout mice were sacrificed and their immune organs were collected and weighted. Single-cell suspensions were prepared, and total cell numbers from each organ were determined using the Coulter counter (Beckman Coulter). Flow cytometry was performed after staining cells with anti-mouse CD4, CD8, B220, Gr-1, CD11b, CD11c, NK1.1, CD25, CD44, Foxp3, CD62L, and CD69. All the Abs used were purchased from BD Bioscience.

**Bacterial culture**

Colon samples were collected and processed as described above and then homogenized in Cell-Lytic M buffer (Sigma) and serially diluted. Different dilutions of the tissue homogenates were plated in triplicates on blood agar

**FIGURE 1.** Generation of Tipe-deficient mice by gene targeting. (A) The WT Tipe (Tnfaip8) gene locus and the knockout allele. Arrows indicate the locations of PCR primers. (B) Genomic DNA was extracted from mice tails. PCR was performed using primers shown in (A) (primers 1, 2, and 3). WT band: 334 bp. Tipe knock out band: 190 bp. (C) Total RNA was isolated from the indicated organs of WT and Tipe−/− mice. RT-PCR was performed using the Tipe primers shown in (A) (primers 4 and 5). LTR, viral long terminal repeat; Neo, neomycin-resistant gene; pA, polyadenylation sequence; SA, splice acceptor.
(BD Bioscience) and BHI agar. The bacterial colonies formed were counted after incubating at 37˚C for 24 h.

**Cell death and proliferation**

Colon samples were processed, and epithelial cells were obtained from control and colitis WT and Tipe\(^{−/−}\) mice. The isolated epithelial cells were collected and the cell death was determined by 7-aminoactinomycin D (7-AAD) and Annexin V staining. Alternatively, Tipe knockdown 3T3 cells were treated with 3% DSS for 16 h and the cells were harvested for 7-AAD and Annexin V staining. The in situ colonocyte proliferation was assessed by Ki67 staining, and cell death was determined by active caspase 3 and TUNEL staining after the colon tissues were collected from DSS-treated mice and fixed in 10% buffered formalin and embedded in paraffin.

**Blood cell counts**

Blood was collected retro-orbitally, and whole blood cell counts were determined using Drew Hemavet 950FS (Drew Scientific, Oxford, U.K.).

**Statistical analysis**

Quantitative data are presented as means ± SEM of two or three experiments. The survival curves were plotted according to the Kaplan–Meier method and compared using the log-rank test. Mann–Whitney \(U\) test was used to compare the body weight data. Two-tailed Student \(t\) test was used for all other cases, and \(p < 0.05\) was considered statistically significant. All statistical analyses were performed with the Prism Software.

**Results**

**Generation and phenotyping of Tipe-deficient mice**

Tipe\(^{−/−}\) mice were generated using gene trap technology to target Tipe gene in B6 ES cells. The gene-trapping vector is composed of a viral long terminal repeat, a splice acceptor site, a Neomycin-resistant gene, and a polyA tail that stops Tipe gene transcription (Fig. 1A). This disrupted Tipe gene allele results in a complete loss of the short isoform of Tipe mRNA and a truncated long isoform of Tipe mRNA that encodes the first N-terminal 24 aa. Homozygous offspring were identified with PCR (Fig. 1B). They were further tested to ensure the lack of the full-length Tipe mRNA in a variety of organs (Fig. 1C).

Tipe gene mutation did not affect the gross growth and development of mice. Homozygous Tipe\(^{−/−}\) mice were born with the expected Mendelian ratios with no detectable developmental abnormalities. The weight, structure, and cellular compositions of lymphoid organs are normal (Supplemental Figs. 1, 2A–2C) in Tipe\(^{−/−}\) mice. Downregulation of TIPE was reported to protect thymocytes against glucocorticoid-mediated apoptosis. However, no significant differences between WT and Tipe\(^{−/−}\) groups were detected in thymocyte death induced by dexamethasone (Supplemental Fig. 2D).

---

**FIGURE 2.** Tipe\(^{−/−}\) mice are more susceptible to DSS-induced colitis. (A) WT \((n = 5)\) and Tipe\(^{−/−}\) mice \((n = 5)\) were fed with water containing DSS for 5 d, followed by regular drinking water. Survival was monitored for 2 wk after the start of DSS treatment. (B-E) WT and Tipe\(^{−/−}\) mice were fed with DSS water for 5 d, followed by regular drinking water for 2 d. Body weight (B) and disease score (C) were recorded daily. Mice were euthanized on day 7; colon length and weight (D, E, and F) were measured, and histopathologic changes (G) in colon tissues were examined using microscopy and H&E staining. Data shown are representatives of at least three experiments. Scale bar in (F), 1 mm; scale bar in (G), 50 \(\mu\)m. *\(p < 0.05\).
**TIPE-deficiency in nonhematopoietic cells exacerbates colitis**

Although enhanced inflammatory responses were detected in the DSS-challenged Tipe$^{-/-}$ mice, both hematopoietic and nonhematopoietic compartments could contribute to the exacerbated colitis phenotype observed above. To assess which compartment contributes directly to the exacerbation of colitis in Tipe$^{-/-}$ mice, we reconstituted lethally irradiated WT and Tipe$^{-/-}$ mice with bone marrow cells from WT or Tipe$^{-/-}$ mice. After recovering for 8 wk, mice were then subjected to 5 d of DSS treatment followed by 2 d of normal drinking water. As shown in Fig. 5, after DSS treatment, the WT mice receiving WT or Tipe$^{-/-}$ bone marrow cells demonstrated similar changes in body weight, disease index, and colon length (Fig. 5), indicating that hematopoietic cells are not major contributors to the exacerbation of colitis observed in Tipe$^{-/-}$ mice. By contrast, when WT bone marrow cells were injected into Tipe$^{-/-}$ or WT recipient mice, after DSS treatment, Tipe$^{-/-}$ recipients developed more severe colitis than the WT recipients indicating that the nonhematopoietic compartment plays a major role in the disease exacerbation (Fig. 5).

**FIGURE 3.** Increased leukocyte numbers in the blood and colon of Tipe$^{-/-}$ mice. Mice ($n = 4$) were treated and sacrificed as described in Fig. 2. (A and B) Increased blood neutrophil counts (A) and decreased spleen weight (B) were detected in Tipe$^{-/-}$ mice. (C and D) The colonos from WT and Tipe$^{-/-}$ mice were harvested, and lamina propria immune cells were isolated. After counting in a hemocytometer, the cells were stained with Abs to T, B, and myeloid cell markers and analyzed using flow cytometry. Increased total (C) and CD11b, CD11c, and B220 cells (D) were identified. Data shown are representatives of at least three experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

**FIGURE 4.** Increased inflammatory cytokines in serum and colon of Tipe$^{-/-}$ mice. WT and Tipe$^{-/-}$ ($n = 5$) mice were treated with DSS water for 5 d, followed by regular drinking water for 2 d. Mice were then sacrificed, and their blood was collected retro-orbitally. Colonos were harvested, and half of them were homogenized in lysis buffer (w/v = 1/10). The cytokines in sera and colon homogenates were measured with ELISA. (A) IL-6 and IL-17 in sera. (B) IL-6, IL-17A, and IL-1β in colon homogenates. (C) Total RNA was isolated from the other half of the colon using Trizol reagent. The levels of IL-17, IL-1β, and CXCL2 mRNAs were examined using quantitative real-time PCR. Data shown are representatives of two experiments. *$p < 0.05$, ***$p < 0.001$. 

Tipe$^{-/-}$ mice are hypersensitive to DSS-induced colitis

Tipe$^{-/-}$ mice did not appear to develop more spontaneous diseases than WT littermates. However, after drinking DSS-containing water, despite a similar amount of water consumption (Supplemental Fig. 3), the mice developed more severe colitis than WT mice did. They began to die on day 6 after the DSS treatment. By day 10, all the Tipe$^{-/-}$ animals had died, but 40% of WT animals were still alive (Fig. 2A). The Tipe$^{-/-}$ mice suffered greater body weight loss (Fig. 2B) and increased overall disease manifestations (Fig. 2C). To assess the severity of colitis further, colon length was also measured. The DSS-fed Tipe$^{-/-}$ mice had significantly shorter and lighter colons (Fig. 2D–F). Consistent with these findings, a histopathologic examination of H&E-stained colons of knockout mice revealed more severe colitis characterized by crypt loss and infiltrating leukocytes than in the controls (Fig. 2G).

Enhanced inflammatory responses in Tipe$^{-/-}$ mice with colitis

To identify further the roles of TIPE in DSS-induced colitis, we examined the hematologic and immunologic aspects of the disease in age- and sex-matched Tipe$^{-/-}$ mice and WT controls. As shown in Fig. 3A, significantly increased neutrophil counts were detected in Tipe$^{-/-}$ blood samples. At the same time, the spleens of Tipe$^{-/-}$ mice became smaller (Fig. 3B), whereas more leukocytes were found in the colons of these mice (Fig. 3C, 3D). Enhanced levels of IL-17A and IL-6 were also found in the sera of Tipe$^{-/-}$ mice (Fig. 4A). Similarly, enhanced levels of IL-6, IL-17A, and IL-1β proteins (Fig. 4B) and mRNAs (Fig. 4C) were detected in the Tipe$^{-/-}$ colons. Increased CXCL2 mRNA expression was also detected in Tipe$^{-/-}$ colons (Fig. 4C). These observations point to enhanced inflammatory responses in the Tipe$^{-/-}$ colon after DSS treatment.
TIPE deficiency in nonhematopoietic cells contributes to the enhanced colitis in Tipe<sup>−/−</sup> mice. Bone marrow chimeric mice (n = 5) were generated as described in Materials and Methods and treated with DSS for 5 d, followed by regular drinking water for 2 d. (A) Body weight. (B) Disease activity index calculated based on the degrees of weight loss, stool consistency, and rectal bleeding. (C) Colon length. Data shown are representatives of two experiments. *p < 0.05, **p < 0.01, ***p < 0.001.

**FIGURE 5.** Increased bacterial invasion of the colonic tissue in Tipe-deficient mice

The disruption of mucosal barrier following gastrointestinal epithelial cell injury can lead to commensal bacterial infection and potent inflammatory responses against them, which is believed to be part of the pathogenic mechanisms of DSS-induced colitis (22–24). We next checked bacterial numbers in the colons of WT and Tipe<sup>−/−</sup> mice with colitis. We found significantly more bacteria in the colons of Tipe<sup>−/−</sup> mice (Fig. 6) than in WT mice, indicating enhanced dissemination of the commensal bacteria.

**TIPE-deficiency in epithelial cells affects their survival and proliferation**

TIPE is expressed in a large number of cell types, including epithelial cells (Supplemental Fig. 4). The increased mortality in Tipe<sup>−/−</sup> mice could be explained by increased cell death, decreased proliferation of colon epithelial cells during colitis, or both (25). To test whether TIPE deficiency affects epithelial cell death and proliferation, epithelial cells were isolated from normal and DSS-fed WT and Tipe-deficient colons, and the cell death was determined with 7-AAD and Annexin V staining. Live epithelial cells were 7-AAD and Annexin V double-negative cells (Fig. 7A). Increased cell death was detected in the Tipe<sup>−/−</sup> group as compared with WT controls. PI3K-AKT pathway is important for regulating cell death and immunity (26). A significantly reduced level of activated AKT was detected in the Tipe<sup>−/−</sup> colon samples, indicating that the increased cell death might be due to the downregulation of the PI3K-AKT signaling (Fig. 7B). Consistent with these findings, Tipe knockdown in the NIH3T3 cell line significantly increased cell death induced by 3% DSS (Fig. 7C). K67 is an endogenous marker for cell proliferation. Significantly reduced Ki67 staining (Fig. 7D, 7E) was found in DSS-fed Tipe<sup>−/−</sup> colons compared with those of the WT, suggesting that Tipe protein is required for epithelial cell proliferation after DSS-induced colon damage. Significantly increased active caspase 3 staining and TUNEL staining (Fig. 7F–I) were also observed in the Tipe<sup>−/−</sup> colons, demonstrating augmented cell death. Increased death of Tipe<sup>−/−</sup> epithelial cells, combined with compromised proliferation, may be responsible for the increased mortality in Tipe-deficient mice treated with DSS.

**Discussion**

In the current study, we generated TIPE-deficient mice and studied the role of TIPE in development and DSS-induced colitis. We showed that Tipe<sup>−/−</sup> mice were significantly more susceptible to DSS-induced colitis, and the loss of TIPE expression in the nonhematopoietic compartment played a major role. The knockout mice suffered from a greater body weight loss, more severe diarrhea and rectal bleeding, and enhanced mortality, indicating a key role for TIPE in protecting against DSS-induced colon damage.

TIPE has been reported as an antiapoptotic molecule in tumor cells (5), but knocking down its expression by RNAi protects thymocytes from glucocorticoid-mediated apoptosis (6). Although we did not observe significant differences in apoptosis between knockout immune cells and WT controls under physiologic conditions, increased cell death was observed in Tipe<sup>−/−</sup> epithelial cells under inflamed conditions, indicating an important role for TIPE protein in inflammatory diseases.

The mammalian TIPE family consists of four members—TIPE1, TIPE2, and TIPE3—that are highly conserved in their amino acid sequences. TIPE2 regulates the function of the innate immune system by targeting PI3K-AKT-Rac axis (26), whereas TIPE3 promotes AKT activation by acting as a PtdIns(4,5)P<sub>2</sub> and PtdIns(3,4,5)P<sub>3</sub> transfer protein (27). In this study, we found decreased AKT activity in Tipe knockout colon, indicating that Tipe may regulate epithelial cell death by targeting AKT. PI3-kinase generates PtdIns(3,4,5)P<sub>3</sub>, which in turn activates AKT. AKT is
a serine/threonine protein kinase with oncogenic and antiapoptotic activities. It phosphorylates a number of proteins, including cell death regulators such as BAD, CREB, MDM2, and NF-κB, leading to diminished cell death. PI3K p110α mutant mice of the C57BL/6 background develop spontaneous colitis (28), and AKT1-deficient mice of the 129 background have increased sensitivity to DSS-induced colitis (29). In addition, protein kinase-R–deficient mice develop more severe clinical and histologic manifestations of DSS-induced colitis, likely because of reduced activation of STAT3 and AKT (30). Therefore, the roles of AKT in the death of Tipe-deficient epithelial cells during colitis need to be studied further.

The epithelial barrier integrity in the gastrointestinal system is crucial for protecting against environmental insults, including toxins, Ags, and microbes (31). Increased epithelial cell death and decreased proliferation are associated with DSS-induced colitis (20, 32, 33). In our study, we showed that the loss of epithelial integrity caused by increased epithelial cell death in Tipe knockout mice can lead to increased dissemination of commensal bacteria and enhanced leukocyte infiltration and inflammatory responses. Inflammation is closely linked to the development of cancer. Tipe dysregulation is associated with the development of a number of human cancers. Results reported here may also help to advance our understanding of the pathogenic mechanisms of cancer. Besides its function in cell death and proliferation, Tipe may also play a role in cell migration and metastasis. The knockout mice described in this study provide a useful tool to study the broad biology of the Tipe protein in health and disease (see also the companion article, Ref. 34).

**Acknowledgments**

We thank Drs. Jean Richa, Ping Jiang, and members of the Transgenic Cores of the University of Pennsylvania and Wister Institute for technical supports in generating Tipe-deficient mice; the Pathology Core at Children’s Hospital of Philadelphia for H&E and immunohistological staining; and Drs. Terry Cathopoulis, Derek Johnson, Xiaohong Liang, Svetlana Fayngerts, and George Buchlis for reagents and advice.

![FIGURE 7. Tipe in gut epithelial cell death and growth and AKT activation.](image-url)
Disclosures

The authors have no financial conflicts of interest.

References


