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**Trp53 Deficiency Protects against Acute Intestinal Inflammation**

Martina E. Spehlmann,*1 Carolin F. Manthey,*2 Sara M. Dann,*3 Elaine Hanson,* Sukhman S. Sandhu,* Linus Y. Liu,* Farid K. Abdelmalak,* Michaela A. Diamanti, † Kristin Retzlaff, ‡ Jürgen Scheller, §4 Stefan Rose-John, ‡ Florian R. Greten, ‡ Jean Y. J. Wang, § and Lars Eckmann*

The p53 protein has not only important tumor suppressor activity but also additional immunological and other functions, whose nature and extent are just beginning to be recognized. In this article, we show that p53 has a novel inflammation-promoting action in the intestinal tract, because loss of p53 or the upstream activating kinase, ATM, protects against acute intestinal inflammation in murine models. Mechanistically, deficiency in p53 leads to increased survival of epithelial cells and lamina propria macrophages, higher IL-6 expression owing to enhanced glucose-dependent NF-κB activation, and increased mucosal STAT3 activation. Blockade or loss of IL-6 signaling reverses the protective effects of p53 deficiency. Conversely, IL-6 treatment protects against acute colitis in a manner dependent on STAT3 signaling and induction of cytoprotective factors in epithelial cells. Together, these results indicate that p53 promotes inflammation in the intestinal tract through suppression of epithelium-protective factors, thus significantly expanding the spectrum of physiological and immunological p53 activities unrelated to cancer formation. *The Journal of Immunology*, 2013, 191: 837–847.

The multifunctional protein p53 acts as a tumor suppressor (1) that arrests cell growth at the G1/S checkpoint upon DNA damage, giving DNA repair enzymes time to correct the damage before resumption of DNA replication, and directly activates these enzymes (2). Furthermore, if DNA damage proves irreparable, p53 mediates programmed cell death and thus removal of mutated cells. In the absence of p53, certain forms of DNA damage fail to cause normal cell death of the afflicted cells. The p53 protein also acts as a transcriptional activator or repressor of multiple target genes, such as CD95/Fas and Puma (3, 4), and has an impact on cellular glucose metabolism. Tumor cells often switch from aerobic to anaerobic generation of ATP from glucose, which is in part mediated by p53 through differential expression of the key mitochondrial enzyme, cytochrome c oxidase 2, and the glucose transporter SLC2A3/GLUT3 (5). Enhanced glucose metabolism in the absence of p53 also leads to increased constitutive protein glycosylation, which can enhance signaling through the NF-κB signaling kinase, IKKβ (6). Owing to this impact on IKKβ activation and perhaps other mechanisms related to direct transcriptional activation, p53 deficiency is associated with increased NF-κB activation and expression of NF-κB target genes in macrophages (7), although the physiological consequences of these observations are not well understood.

The physiological functions of p53 have been extensively studied in gene-targeted mice. These animals are fertile and healthy when born and are grossly normal for the first few months, but then develop spontaneous tumors in the thymus and other sites. Before tumor formation, the knockout mice are more susceptible to severe joint destruction in collagen-induced arthritis due to increased T cell proliferation and release of cartilage-degrading enzymes (8–10). Mice lacking p53 also develop more severe experimental autoimmune encephalomyelitis (11) and enhanced chronic gastric inflammation upon infection with *Helicobacter pylori* (12). Together, these data suggest that p53 has anti-inflammatory functions in different organ systems.

Intestinal inflammation, which is associated with increased risk of colorectal cancer (13, 14), leads to p53 activation (15). Conversely, dysfunction of the protein plays a role in inflammation-associated colon cancer formation. For example, p53 mutations occur early in the development of ulcerative colitis–related colorectal cancers, whereas such mutations occur only late in the pathogenesis of sporadic colorectal cancers (16). Mutations of p53 have been identified in nondysplastic, inflamed tissues of ulcerative colitis patients without cancer (17), suggesting that p53 dysfunction may have an unrecognized physiological impact on mucosal inflammation. On the basis of findings in several organs and the role of p53 in inflammation-associated colon cancer, we set out to test the hypothesis that p53 antagonizes inflammatory processes in the intestinal tract. Surprisingly, our studies reveal the opposite,
a novel proinflammatory function of p53 related to limiting the production of survival factors for the intestinal epithelium. These results not only significantly expand the known physiological functions of p53 unrelated to tumor suppression, but also have important implications for the connection between inflammation and neoplastic transformation under chronic inflammatory conditions in the intestinal tract.

Materials and Methods

Mice

Trp53<sup>−/−</sup> mice were obtained from The Jackson Laboratory. Ann<sup>−/−</sup> mice were kindly provided by Y. Xu (University of California, San Diego) (18) and Msh1<sup>−/−</sup> mice by W. Edelman (Albert Einstein College of Medicine, New York, NY) (19, 20). All three strains were backcrossed for at least 10 generations to a 129/SVJ background, and wild-type 129/ SVJ mice were used as controls. Wild-type C57BL/6 and Il6<sup>−/−</sup> mice were obtained from The Jackson Laboratory and crossed with Trp53<sup>−/−</sup> mice (on a C57BL/6 background) to obtain Trp53<sup>−/−</sup> x Il6<sup>−/−</sup> mice. Mouse with a floxed Trp53 allele, originally acquired from the National Cancer Institute, Bethesda, MD (21), were crossed with Villin-Cre transgenic mice or Msh1-Cre transgenic mice and neutrophils (Trp53<sup>IEC-KO</sup>) or macrophages and neutrophils (Trp53<sup>EPEC-KO</sup>) were used, respectively. Epithelial STAT3 knockout mice were generated as described before (24). Results from males and females were expressed as mean ± SEM. Histological and cell suspensions were passed through a 40-micron nylon mesh strainer and centrifuged, and the pellet was frozen in liquid nitrogen. The remaining tissue pieces were washed and incubated twice at 37°C for 30 min in RPMI 1640 medium containing 1 mg/ml collagenase D (Roche Applied Science) and 100 µg/ml DNase I (Worthington Biochemical). After vortexing for 30 s, cell suspensions were passed through a 40-µm cell strainer and collected by centrifugation.

Flow cytometry and cell sorting

Sensations of detached epithelial and lamina propria cells from the colon were stained for 10 min on ice with fluorochrome-conjugated annexin V (and/or Abs against mouse CD45, F4/80, CD11b, CD11c, or EpCAM (eBioscience), and propidium iodide (Molecular Probes). Sorting of viable cells was done on a Beckman Coulter MoFlo high-speed cell sorter. Epithelial cells were identified as CD45<sup>E</sup> EpCAM<sup>+</sup> cells, dendritic cells as CD45<sup>C</sup> CD11c<sup>+</sup> cells, and macrophages as CD45<sup>C</sup> CD11b<sup>+</sup> CD11c<sup>−</sup> cells. For analysis of antigen-macrophages, stained cells were fixed with 2% paraformaldehyde in PBS and analyzed on a BD Biosciences FACSCalibur flow cytometer by gating for CD45<sup>E</sup> and F4/80<sup>+</sup> cells.

Macrophage and dendritic cell cultures

Bone marrow macrophages were produced by culturing bone marrow suspensions in DMEM, 10% FCS, and 30% L cell–conditioned medium (containing M-CSF) for 7 d. Dendritic cells were generated by culturing bone marrow with 50 ng/ml murine GM-CSF for 7 d. Cells were stimulated with 100 ng/ml LPS from E. coli O127:B8 (Sigma-Aldrich), 10 ng/ml IFN-γ, 10 ng/ml TNF-α, 10 ng/ml IL-1β (all from PeproTech), or 200 µM H<sub>2</sub>O<sub>2</sub>. Cytokine levels were assayed by ELISA (R&D Systems).

Statistical analysis

Data were analyzed by t test, two-way ANOVA, repeated-measures ANOVA, or Wilcoxon rank sum test, as appropriate, with significance levels indicated for *p < 0.05, **p < 0.01, or ***p < 0.001. Survival data were plotted as Kaplan–Meier survival curves and were analyzed for statistical significance by the log-rank test.

Results

p53 is activated by oxidative stress in acute colitis

Free radicals and oxidative stress can cause DNA damage and lead to p53 activation (31). To assess p53 activity during acute intestinal inflammation, we induced colitis in C57BL/6 mice by oral administration of the irritant DSS and determined the levels of total and Ser<sup>15</sup>-phosphorylated, activated p53 by immunoblotting. Under resting conditions, constitutive p53 activation was observed in the whole colon (Fig. 1A) and in isolated intestinal epithelial cells and lamina propria cells. DSS administration caused a marked increase in total and phospho-p53 (Ser<sup>15</sup>) levels by 7 and 10 d, times that coincide with peak inflammatory changes in this colitis model. Increased p53 activity was functionally relevant because expression of several p53 target genes, including Fas<sup>L</sup> and Puma, was increased in colitic mice (Fig. 1B). As expected, induction of these genes was abolished or strongly attenuated in mice lacking p53 (Fig. 1B). In contrast, a p53-independent inflammation-associated gene, Icam1, was induced in colitis but...
not affected by the absence of p53. Because oxidative stress is a major driver of p53 activation, we determined if it plays a role in colitis-induced activation. Treatment with the antioxidant N-acetylcysteine prevented the increase in total and phospho-p53 (Ser15) levels after colitis induction (Supplemental Fig. 1). Together, these results show that acute colitis causes marked oxidant-dependent p53 activation in the intestinal mucosa.

### p53 deficiency protects against acute colitis

To define the physiological function of p53 in acute colitis, we used mice deficient for p53 (Trp53<sup>−/−</sup>), mice heterozygous for the gene (Trp53<sup>+/−</sup>), and wild-type littermate controls (Trp53<sup>+/+</sup>). Controls exhibited maximal body weight loss 10–14 d after initiation of DSS feeding and regained weight over the subsequent 14 d (Fig. 1C). The disease was accompanied by modest mortality (∼30%) in the controls over the 4-wk period (Fig. 1D). In contrast, Trp53<sup>−/−</sup> mice showed significantly less weight loss in the acute stage and recovered more rapidly thereafter (Fig. 1C). In parallel, p53-deficient mice experienced significantly lower total mortality (∼10%) over the entire observation period (Fig. 1D). Mice heterozygous for Trp53 had an intermediate phenotype, showing weight loss and overall mortality at reduced levels compared with controls, but at higher levels than in Trp53<sup>+/−</sup> mice (Fig. 1C, 1D).

Histopathological analysis of colon sections confirmed and extended the clinical observations. Wild-type mice had marked ulcerations in the acute stage of colitis characterized by large areas of crypt loss and heavy infiltration of mucosa and submucosa with inflammatory cells, whereas Trp53<sup>−/−</sup> mice showed markedly less inflammation, as evidenced by significantly lower histological inflammation scores and smaller ulcerative lesions (Fig. 1E, 1F). Heterozygous mice exhibited an intermediate phenotype. Validation of the histological findings came from enzymatic assays of the neutrophil marker MPO in total colon extracts. MPO levels were significantly lower in Trp53<sup>−/−</sup> and Trp53<sup>+/−</sup> mice compared with controls (Fig. 1G). Thus, the clinical, histological, and biochemical data demonstrate that p53 deficiency markedly attenuates inflammation and mortality in the acute colitis model.

Because p53 plays a key role in protecting genome integrity, we questioned whether its loss would lead to increased DNA damage during acute intestinal inflammation. To assess DNA damage and repair processes, we assayed expression of the DNA repair enzyme Ogg1, which recognizes and excises 8-hydroxyl-deoxyguanosines that form in response to oxidative stress in inflammation and is an early marker of DNA damage. Ogg1 mRNA was increased by 9.1-fold in the colon of wild-type mice by 7 d of DSS administration, consistent with other observations that oxidant-related DNA damage activates expression of this enzyme (32). By comparison, Ogg1 mRNA levels were only 2.9-fold increased in the colon of DSS-treated Trp53<sup>−/−</sup> mice, suggesting that attenuated inflammation in the absence of p53 is associated with reduced production of DNA-damaging agents and decreased acute DNA damage.

### ATM dependence of p53 activation in acute colitis

Activation of p53 requires phosphorylation of its N-terminal domain, which can occur upon activation of several signaling pathways. One of the major p53-activating kinases is the serine/threonine-specific protein kinase ATM, encoded by the Ataxia Telangiectasia Mutated (Atm) gene. ATM phosphorylates p53 at
of the Ser\(^{15}\) position (33). To determine whether ATM is important for p53 activation in acute colitis, we administered DSS to ATM-deficient (Atm\(^{-/-}\)) and wild-type (Atm\(^{+/+}\)) mice and examined the levels of total and Ser\(^{15}\)-phosphorylated p53 in the colon by immunoblotting. Atm\(^{-/-}\) mice exhibited constitutive p53 activation (presumably owing to ATM-independent p53 phosphorylation by kinases such as DNA-PK and ATR) but failed to induce further p53 activation and induction upon colitis induction, whereas wild-type littermates showed the expected p53 activation at 7 and 10 d after DSS administration (Fig. 2A). Furthermore, Atm\(^{-/-}\) mice, compared with Trp53\(^{-/-}\) mice, exhibited a similar colitis phenotype, as evidenced by less body weight loss in the acute stages of the disease (Fig. 2B), reduced mortality (Fig. 2C), decreased colon inflammation and ulceration (Fig. 2D, 2E), and lower MPO levels in colon extracts (Fig. 2F) compared with those in wild-type littermates. These findings indicate that ATM is required for inflammation-induced p53 activation and that its loss is protective against acute colitis, which, taken together, suggests that ATM and p53 are located in the same signaling pathway relevant to acute colon inflammation.

**DNA repair is not important in modulating acute colitis**

One of the major functions of p53 is sensing DNA damage and initiating DNA repair processes (34). Because inflammation-associated production of oxidants may cause DNA damage, we asked whether the DNA repair–related functions of p53 are important in mediating its activity in acute colitis. For this purpose, we used mice deficient for one of two critical DNA repair enzymes, MLH1 and MSH1, and subjected them to DSS challenge. In contrast to Trp53\(^{-/-}\) mice, we observed no differences in body weight loss, colon ulceration, or MPO levels in the colon of MLH1- or MSH1-deficient mice compared with their wild-type littermate controls (Supplemental Fig. 2). These data show that DNA repair processes mediated by MLH1 or MSH1 play no role in determining the extent of acute colitis injury or in the recovery from this injury. Thus, the observed inflammatory functions of p53 appear to be independent of DNA repair.

**Importance of p53 for colitis-associated apoptosis of epithelial and lamina propria cells**

Besides initiation of DNA repair, another key function of p53 is induction of cell cycle arrest and apoptosis (35–37). On this basis, we hypothesized that apoptosis control may be important in mediating the functions of p53 in acute intestinal inflammation. Induction of colitis by DSS feeding caused apoptosis in the colon of wild-type mice, as evidenced by increased levels of the cleaved, activated form of caspase 3, the central effector caspase responsible for integrating different proapoptotic signals and initiating a program of apoptotic cell death (Fig. 3A). By comparison, Trp53\(^{-/-}\) mice had reduced levels of cleaved caspase 3 after DSS administration (Fig. 3A). TUNEL staining of histological sections confirmed that wild-type mice had greatly increased numbers of apoptotic cells in the epithelium and lamina propria of the colon at 7 d, whereas Trp53\(^{-/-}\) mice showed only a mild increase in apoptotic cells (Fig. 3B). Furthermore, we found by flow cytometry of isolated lamina propria cells that the number of F4/80\(^{+}\) macrophages undergoing apoptosis (as defined by positive staining for annexin V and negative staining for propidium iodide) was 6-fold increased in colitic wild-type mice, but < 2.5-fold increased in Trp53\(^{-/-}\) mice (Fig. 3C). These results indicate that p53 deficiency protects against apoptotic cell death of epithelial cells and macrophages in the course of acute colitis, which may be partly responsible for protection against colitis-associated tissue damage.

![Figure 2](http://www.jimmunol.org/)
Intestinal epithelial cells are not responsible for p53 functions in colitis

Because epithelial cells were protected against apoptosis in the absence of p53 and because these cells are important in colitis induction (38), we next investigated whether they are responsible for the actions of p53 in acute colitis. To generate a model of selective p53 deficiency in intestinal epithelial cells, we crossed mice carrying floxed Trp53 alleles with Villin-Cre transgenic mice that express Cre recombinase specifically in the intestinal epithelium (22). The resulting Trp53<sup>flx<sup>/-</sup></sup>/Cre<sup>+</sup> mice, which were fertile and grossly normal, showed selective p53 ablation in the colon epithelium (Fig. 4A), but not in intestinal lamina propria cells or in the spleen. DSS challenge of the Trp53<sup>flx<sup>/-</sup></sup>/Cre<sup>+</sup> mice led to increased expression of the cytoprotective cytokines IL-6 and IL-22, as well as activation of STAT3 in epithelial and other cells in response to acute colitis (Fig. 5C). Together, these results indicate that the absence of p53 leads to increased expression of the cytoprotective cytokines IL-6 and IL-22, as well as activation of STAT3 in epithelial and other cells in response to acute colitis.

Importance of glucose-dependent NF-κB activation and IL-6 expression in p53-deficient macrophages

Because macrophages are a major source of many inflammatory cytokines, we next investigated how p53 deficiency might enhance expression of selected cytokines in these cells. Bone marrow-derived macrophages were generated from Trp53<sup>-/-</sup> and Trp53<sup>+/+</sup> mice and tested for IL-6 expression in response to different stimuli. Macrophages lacking p53 secreted significantly more IL-6 after stimulation with LPS, TNF-α, and H<sub>2</sub>O<sub>2</sub> than did p53-proficient cells (Fig. 5D). Similarly, p53-deficient bone marrow-derived dendritic cells also secreted significantly more IL-6 than did control cells after LPS stimulation (Fig. 5D). Enhanced IL-6 secretion was paralleled by increased mRNA levels in LPS-stimulated p53-deficient macrophages, whereas mRNA levels for the cytokines IL-10 and CCL5 were not altered by p53 loss (Fig. 5E). These results indicate that p53 deficiency enhances macrophage IL-6 production in a relatively selective manner. The data
further suggest that increased colon expression of IL-6 and perhaps other cytoprotective cytokines in the absence of p53 can be explained, at least in part, by a cell-autonomous function in macrophages and perhaps other cells, rather than by changes in the number of cells producing such cytokines (although the latter also occurs and may synergize with increased cellular cytokine production by increasing the number of cytokine-producing cells).

Because IL-6 is a target gene of NF-κB, whereas IL-10 and CCL5 are not, we explored the role of p53 in regulating NF-κB function in our macrophage system. As reported before in other...
cell models (7), p53 deficiency was associated with increased NF-κB activation upon agonist stimulation of macrophages (Fig. 5F).

Several mechanisms have been proposed that may account for enhanced NF-κB activation under these circumstances. For example, increased glucose uptake and metabolism in p53-deficient cells have been suggested to promote glycosylation of the central signaling kinase, IKKβ, which leads to increased cumulative NF-κB activity by preventing the normal inactivation of the kinase upon sustained stimulation (6). Consistent with this idea, we observed that p53 loss in macrophages was associated with increased glucose consumption after LPS stimulation (181 nmol glucose per 10^6 cells per h in p53^−/− cells versus 132 in p53^+/+ cells). Importantly, glucose consumption was critical for mediating the impact of p53 deficiency on cytokine expression, because addition of the glycosylation inhibitor 2-deoxyglucose completely abrogated the increase in IL-6 secretion and mRNA expression and NF-κB activation in p53-deficient macrophages (Fig. 5E). The inhibitor had no effect on LPS-stimulated mRNA expression of IL-10 and CCL5, proving that cell viability and general responsiveness were not compromised. Complementary studies revealed that addition of high levels of glucose to wild-type macrophages enhanced IL-6 secretion in response to LPS (315 pg/ml IL-6 with 6 g/l glucose versus 218 pg/ml in 1 g/l glucose after 4 h of LPS stimulation).

Taken together, these data support the concept that increased glucose consumption and metabolism, and secondarily protein glycosylation, in the absence of p53 can enhance NF-κB activation and IL-6 secretion in macrophages.

Bone marrow–derived cells mediate the functions of p53 in regulating colitis

Macrophages are central inflammatory cells and display increased cytokine production in the absence of p53, which prompted us to explore their role in mediating the protective effects of p53 deficiency. We crossed mice with floxed Trp53 alleles to LysM-Cre transgenic mice, which express Cre recombinase specifically in macrophages and neutrophils (23), to obtain mice with selective loss of p53 in these cells. Induction of colitis by DSS feeding showed that the conditional p53 knockout mice were not significantly different from p53-proficient controls in the severity of mucosal inflammation (Fig. 6A). These data suggest that p53 in macrophages (and neutrophils) is not sufficient to mediate the overall p53 functions in colitis.

Given that neither epithelial cells (Fig. 4) nor macrophages/neutrophils (Fig. 6A) were responsible for the protection that resulted from p53 deficiency, we asked more broadly whether any cells of hematopoietic origin were involved. To this end, we generated bone marrow chimeric mice, in which wild-type recipients were reconstituted with either p53-deficient or wild-type bone marrow. We could not test the reverse situation because Trp53^−/− mice are relatively radioresistant and thus not suitable as bone marrow recipients. Evaluation of acute DSS-induced colitis in the bone marrow chimeric mice showed that mice reconstituted with p53-deficient bone marrow, compared with mice reconstituted with wild-type bone marrow, lost less weight, had significantly lower histological inflammation scores, and showed reduced colon ulceration and mucosal MPO levels (Fig. 6B–D). The extent of these changes was comparable to those seen in total p53-deficient mice (Fig. 1C, 1F, 1G), suggesting that hematopoietic cells are responsible for mediating the overall functions of p53 in acute colitis, whereas nonhematopoietic cells in the lamina propria (such as endothelial cells and myofibroblasts) are unlikely to play a major role under these conditions.

IL-6 mediates protection attributable to p53 deficiency

Because IL-6 was increased in Trp53^−/− mice upon colitis induction, and in stimulated macrophages and dendritic cells from these mice, and this cytokine can be mucosa protective (26), we next examined its physiological role in protection against colitis in the absence of p53. Mice doubly deficient for p53 and IL-6 were not significantly different from p53-proficient mice in regard to histological inflammation scores and extent of colon ulceration, whereas mice lacking only p53 showed the expected protection against colitis (Fig. 7A). Similarly, administration of neutralizing Abs against IL-6 exacerbated colitis in Trp53^−/− mice and made them not significantly different from p53-proficient mice (Fig. 7B). These findings indicate that IL-6 is necessary to mediate the protective functions of p53 deficiency in acute colitis.

Certain functions of IL-6 can be mediated by cell-independent association of the cytokine with the soluble form of the IL-6R α-chain, followed by binding of the complex to the cell-bound coreceptor gp130 (28). We therefore tested whether IL-6 trans signaling had a role in colitis protection. A recombinant form of soluble gp130 (sgp130/Fc) interferes with binding of IL-6/IL-6R to membrane gp130 and thereby blocks IL-6 trans signaling, whereas

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Hematopoietic cells are responsible for colitis protection attributable to p53 deficiency. (A) Mice lacking p53 in macrophages and polymophonuclear neutrophils (Mac/PMN) were generated by crossing floxed Trp53 mice with LysM-Cre mice, and subjected to DSS-induced colitis. Histological inflammation scores were determined after 7 d. Analysis by rank sum test showed NS difference. (B–D) Bone marrow chimeric mice were generated with wild-type (Trp53^+/+) recipients and bone marrow from wild-type (closed bars) or p53-deficient donors (Trp53^−/−; open bars). After 8 wk, mice were subjected to DSS treatment. (B) Body weights are shown as mean ± SD of the percentage of the initial weight (n = 10 mice per group). The curves were significantly different (p < 0.05), as determined by repeated-measures ANOVA. (C) Histological inflammation scores and total ulcerations were determined on colon sections on days 7–10. The horizontal lines represent the means (n = 16–17 mice per group); significance was calculated by rank sum test (histology scores) or t test (ulcerations). (D) MPO levels were determined in colon lysates on days 0 and 10 of DSS treatment. Data are mean ± SD. n = 3, p value was determined by t test. *p < 0.05, **p < 0.01.
direct binding of IL-6 to cell-bound IL-6R and gp130 (leading to
classic IL-6 signaling) is not affected (28). Treatment of Trp53−/− mice with sgp130/Fc exacerbated colitis and made it indistinguishable from colitis in Trp53+/+ mice (Fig. 7B). Thus, IL-6 trans signaling contributes to the protection against acute inflammation.

**IL-6 administration protects against acute colitis through epithelial STAT3 activation**

Although IL-6 was necessary for protection against colitis, other cytoprotective cytokines were increased in the absence of p53 (Fig. 5A) and might contribute to protection under these conditions. To further address the importance of IL-6, we administered rIL-6 to wild-type mice and assessed the impact on acute colitis. Mice treated with IL-6 from the beginning of DSS administration lost significantly less body weight than did vehicle-treated mice (Fig. 7D). By comparison, delayed IL-6 administration after establishment of acute colitis had no significant effect on inflammation on day 14 (Fig. 7E). Furthermore, IL-6 administration induced expression of two cytoprotective factors, HSP70 and Bcl-XL, in the colon epithelium of STAT3-proficient littermates, but not in their STAT3-deficient counterparts (Fig. 7F). These data suggest that IL-6 protects against acute colitis through activation of STAT3 signaling and expression of cytoprotective factors in the intestinal epithelium.

**Discussion**

The findings reported herein indicate that p53 has a novel inflammation-promoting function in the intestinal tract, which is opposed to its inflammation-limiting activity in the joints and CNS (8, 11). The underlying mechanism of intestinal p53 function is primarily indirect, as it could be explained by a suppression of factors that protect the epithelium. Direct p53-dependent induction of proinflammatory cytokines may also occur (9) but is not
likely to be primarily responsible for p53 function in acute colitis. Furthermore, the proinflammatory action of p53 is mediated by leukocytes in the lamina propria, which contrasts with the importance of p53 in epithelial cells in protecting against neoplastic transformation (13). Nonetheless, our data suggest that leukocytic p53 can contribute to protection against the development of intestinal neoplasms by limiting the production of paracrine factors that promote survival and outgrowth of transformed cells.

Chronic intestinal inflammation is associated with accumulation of p53 mutations in the mucosa, even in the absence of adenomas or cancers (17). Our findings raise the intriguing possibility that functional p53 loss might act as a delayed mechanism of limiting inflammation severity in the intestinal tract. This idea may have evolutionary implications, as colon cancer formation involving p53 inactivation is a late consequence of long-standing disease, whereas acute inflammatory damage can be life threatening in acute flare-ups of inflammatory bowel disease. In any case, the present data provide further support for the concept that p53 has physiologically important inflammation-controlling functions that are independent of its role in DNA damage repair and prevention of cancer formation (9).

Activation of p53 can occur in response to DNA double-strand breaks, but also by mechanisms not involving DNA damage. In particular, reactive oxygen radicals lead to p53 phosphorylation and activation (41). Consistent with this observation, we observed Ser15-phosphorylation of p53 in acute colitis, a condition associated with radical production (42), and administration of the radical scavenger N-acetylcysteine prevented this phosphorylation event. Oxygen radicals activate the upstream serine kinase ATM, which can phosphorylate p53 at Ser15 (33). Loss of ATM prevented Ser15-phosphorylation in acute colitis, the physiological importance of which is suggested by the finding that ATM deficiency caused a colitis phenotype similar to that of p53 deficiency. However, it must be noted that p53 activation is a complex process that involves multiple phosphorylation sites and protein stabilization, depending on the specific cellular stresses (43). Nonetheless, our data suggest that p53 activation may be a new mechanism by which reactive oxygen radicals can promote inflammation (44). In variance to our findings, another study found that ATM-deficient mice had more severe acute colitis (45). The reasons for this discrepancy are not immediately apparent, but the genetic mouse background may be important or the approach to disease assessment, in which we relied on actual histological changes in the colon rather than a more indirect disease activity index, may be a factor (45).

Acute inflammation causes DNA damage, which can lead to cell loss by apoptosis. Because epithelial cell damage is a key pathogenetic feature of acute ulcerating colitis, we had initially pursued the idea that adequate DNA repair is necessary for preventing epithelial loss and thus limiting mucosal damage in acute colitis. Our data argue against this notion. Neither total deficiency in MLH1 or MSH1, key components of the DNA repair complex, nor epithelium-selective ablation of p53, which plays a role in initiating DNA repair in a cell-autonomous manner in epithelial cells (46), had a significant impact on epithelial ulceration or the severity of mucosal inflammation. Although it remains possible that other DNA repair processes independent of MLH1 and MSH1 have a role in attenuating acute inflammation, our results are consistent with the notion that any inflammation-associated DNA damage during acute colitis is not sufficiently extensive to interfere with survival and proliferation in the vast majority of epithelial cells and perhaps other cells required for limiting and overcoming acute mucosal damage. However, the long-term consequences of loss of DNA repair under chronic inflammatory conditions are likely to be detrimental because accumulation of DNA-damaged cells can contribute to tumorigenesis.

Several prototypic target genes of p53, including Fasl and Puma, were induced in acute colitis and could contribute to its overall proinflammatory activity, although little is known in this regard. Loss of Fasl is associated with the development of autoimmune diseases (47), and Fas ligand deficiency causes more severe colitis (48), so decreased Fas expression in the absence of p53 might be expected to increase colitis susceptibility, but the opposite was observed. PUMA deficiency blocks epithelial apoptosis following radiation (49), but an impact on acute inflammation has not been reported. It is possible that one or several of the hundreds of direct target genes of p53 (3) may mediate its overall function in acute colitis, or that loss of p53-dependent transcriptional repression of particular target genes, such as IL-6 (50), may be important. However, our data suggest that p53 acts indirectly during colitis by regulating other transcriptional circuits. In particular, p53 attenuates the transcriptional activity of NF-κB (51), which controls expression of multiple inflammation-associated and cytotoxic factors. Although NF-κB can promote inflammation under certain circumstances (52), it also protects against cell death (53). The latter is the dominant activity in acute colitis, as inhibition of total NF-κB in the mucosa causes exacerbation of inflammation (38). Our data suggest that the same overall concept applies, albeit in reverse, in the context of p53 deficiency, because the increase in NF-κB activity in the absence of p53 is consistent with the observed colitis-associated enhanced expression of selected NF-κB target genes, such as IL-6 and IL-1β, both of which can protect against acute inflammation in the intestine (26, 54). Importantly, neutralization or loss of IL-6 reversed the protection, strongly arguing that the impact of p53 deficiency on this NF-κB–dependent pathway is responsible for protection, rather than any effects on classical p53 target genes.

Consistent with the IL-6–mediated protection of p53-deficient mice against acute colitis, direct IL-6 administration to wild-type mice also conferred mucosal protection when given in the early stages. This effect was dependent on STAT3 signaling in epithelial cells and was likely due to cytoprotection against epithelial apoptosis and ulceration (55). Although IL-6 played a central role in our acute colitis model, other cytotoxic factors were increased in the absence of p53 and could contribute to mucosal protection (39, 56). IL-6 is a multifunctional cytokine that can suppress or stimulate inflammation, depending on the underlying pathophysiological mechanisms. For example, IL-6 protects against mucosal inflammation upon infection with an epithelium-adherent enteric pathogen and after DSS challenge (26). IL-6 trans signaling is an important mechanism responsible for promoting wound healing via trefoil factor activation (57), which is consistent with the observation that intestinal epithelial cells express only low levels of membrane-bound IL-6Rs and require soluble IL-6Rs for effective IL-6 signaling (58). In contrast, IL-6 signaling promotes activation and survival of T cells, and can thereby promote the development of adaptive immune responses (29). Consequently, in models of chronic inflammation in the intestine and other organs that are driven by aberrant T cell responses, IL-6 blockade can attenuate disease (29). Furthermore, IL-6 may not have any major functions under particular conditions, as suggested by our observations in mice treated with IL-6 only late in established colitis. The dual, seemingly opposing roles of IL-6 in protecting against acute mucosal injury but also promoting potentially colitogenic T cell responses may explain why therapeutic IL-6 blockade has not been effective in inflammatory bowel disease (59). Nonetheless, the efficacy of exogenously administered IL-6 in protecting against acute, T cell–independent colitis in the current study suggests that this
cytokine, or others with similar cytotoxic properties in the mucosa, may have therapeutic potential for alleviating acute inflammatory bowel disease upon initial presentation or on relapses with acute disease exacerbation.

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Disclosures
The authors have no financial conflicts of interests.

References


**Supplementary Information**

*Trp53 deficiency protects against acute intestinal inflammation*

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**FIGURE S1.** Role of oxidants in p53 activation in acute colitis. Wild-type mice were given DSS for the indicated times, with and without co-treatment with N-acetylcysteine (NAC) in the drinking water, and p53 levels and activation state in the colon were examined by immunoblotting. Data from one representative out of two experiments are shown.
FIGURE S2. DNA repair mediated by MLH1 or MSH1 has no impact on acute colitis. Mice lacking MLH1 (Mlh1−/−, open circles, n=21), MSH1 (Msh1−/−, open triangles, n=21), and the matching wild-type littermate controls (WT, closed circles, n=19) were treated with 3% DSS for 6 days and returned to normal drinking water. (A) Body weights were recorded daily and are shown as mean ± SEM of the percentage of the initial weight. No significant differences were found between the groups. (B) Total colon ulceration on day 7 was determined morphometrically on hematoxylin and eosin-stained paraffin sections. Each data point represents one mouse, with medians shown as horizontal lines. No significant differences were observed between the groups by rank sum test. (C) MPO levels were assayed enzymatically in whole colon lysates obtained 10 days after initiation of DSS feeding. Data are mean ± SEM (n=6 mice/group). No significant differences were observed between the groups (t-test).