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IL-18 Production Downstream of the Nlrp3 Inflammasome Confers Protection against Colorectal Tumor Formation

Mohammad Hasan Zaki,* Peter Vogel,[†] Mathilde Body-Malapel,[‡] Mohamed Lamkanfi,^{§,¶} and Thirumala-Devi Kanneganti*

Colorectal cancer is a leading cause of cancer-related deaths worldwide. Chronic inflammation is recognized as a predisposing factor for the development of colon cancer, but the molecular mechanisms linking inflammation and tumorigenesis have remained elusive. Recent studies revealed a crucial role for the NOD-like receptor protein Nlrp3 in regulating inflammation through the assembly of proinflammatory protein complexes termed inflammasomes. However, its role in colorectal tumor formation remains unclear. In this study, we showed that mice deficient for Nlrp3 or the inflammasome effector caspase-1 were highly susceptible to azoxymethane/dextran sodium sulfate-induced inflammation and suffered from dramatically increased tumor burdens in the colon. This was a consequence of markedly reduced IL-18 levels in mice lacking components of the Nlrp3 inflammasome, which led to impaired production and activation of the tumor suppressors IFN- γ and STAT1, respectively. Thus, IL-18 production downstream of the Nlrp3 inflammasome is critically involved in protection against colorectal tumorigenesis. *The Journal of Immunology*, 2010, 185: 4912–4920.

Colorectal cancer is one of the leading causes of cancer-related deaths. Patients with inflammatory bowel diseases (IBDs), most commonly Crohn's disease and ulcerative colitis, are at increased risk of developing colorectal cancer (1–3). Indeed, IBD is considered the third most important risk factor for the development of colorectal cancer (4). Although the precise molecular mechanism of IBD-related colorectal tumor formation is not clearly understood, existing studies suggest that chronic inflammation primes the mucosal tissue in the gut for increased cell proliferation, angiogenesis, and tumor invasiveness (5). In this regard, proinflammatory cytokines such as IL-1 β , IL-6, IL-18, TNF- α , and IFNs have been demonstrated to exert key roles in inducing gut inflammation and colorectal tumor formation (6–8). The synthesis and secretion of these cytokines is controlled by transcription factors of the STAT, NF- κ B, and AP-1 families (6). Notably, recent evidence suggests that inhibition of NF- κ B reduces tumorigenesis (9, 10).

NF- κ B activation and induction of additional inflammatory signaling pathways is initiated by engagement of pathogen recognition receptors of the TLR and NOD-like receptor (NLR) families (11, 12). TLRs are membrane-bound receptors that detect pathogen-associated molecular patterns in the extracellular milieu

(13). The role of TLRs in the recruitment of immune cells at mucosal surfaces and in protection against tumorigenesis in the gut is well established. For example, TLR5 activation in a mouse xenograft model of human colon cancer elicited powerful antitumor activity (14). In addition to TLRs, several members of the cytosolic NLR family have been identified as key regulators of cytokine production (11). The NLR proteins NOD1 and NOD2 mediate activation of NF- κ B and MAPKs in response to the cytosolic presence of peptidoglycan fragments. In contrast, the NLR protein Nlrp3 (also referred to as Nalp3/CIAS1/cryopyrin) is involved in activation of the cysteine protease caspase-1 (15). Homotypic interactions between the pyrin domain in the N terminus of Nlrp3 and the bipartite adaptor protein apoptosis-associated speck-like protein containing CARD (ASC) bridge the association of caspase-1 to Nlrp3 in a large protein complex known as the “inflammasome” (16). Activated caspase-1 processes the cytosolic precursors of the related cytokines IL-1 β and IL-18, thus allowing secretion of the biologically active cytokines. Hence, mice lacking caspase-1 are defective in the maturation and secretion of IL-1 β and IL-18 (17, 18). IL-1 β participates in the generation of systemic and local responses to infection, injury, and immunological challenges by generating fever, activating lymphocytes, and by promoting leukocyte infiltration at sites of injury or infection. Binding of IL-18 to the IL-18R complex triggers many of the signaling pathways that are engaged by the IL-1R, including activation of NF- κ B, STAT1, and MAPKs (19, 20). IL-18 (previously known as IFN- γ -inducing factor) also promotes the production of IFN- γ in activated T cells and NK cells, thereby contributing to Th1 cell polarization (8, 21, 22). Finally, IL-18 was shown to induce Fas ligand production and the generation of multiple secondary proinflammatory cytokines, chemokines, cell adhesion molecules, and NO species (23, 24).

The profound role of NLR-mediated inflammatory responses in shaping the microenvironment during colitis-associated colorectal tumorigenesis is starting to emerge. For instance, the NLR family member NOD1 is important for protection against colitis-associated colorectal tumor formation (25). Additionally, defective activation of the Nlrp3 inflammasome was linked to increased susceptibility to Crohn's disease in patients (26). Moreover, the

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Abbreviations used in this paper: AOM, azoxymethane; ASC, apoptosis-associated speck-like protein containing CARD; COX, cyclooxygenase; DSS, dextran sodium sulfate; IBD, inflammatory bowel disease; NLR, NOD-like receptor; WT, wild-type.

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Nlrp3 inflammasome was recently shown to confer protection against experimental colitis in mice (27–29). In this regard, mice lacking the inflammasome components Nlrp3, ASC, or caspase-1 all presented with more severe clinical manifestations of colitis and suffered from increased epithelial injury, bacterial invasion, and death rates (27–29). The increased susceptibility to colitis was correlated with defective IL-18 production in inflammasome-deficient mice (27, 29). Despite the role of the Nlrp3 inflammasome in controlling colitis-associated inflammation, its roles in controlling colitis-associated tumorigenesis and the relevant inflammasome effector pathways in this process have remained unclear. To resolve these issues, we determined the rate of colorectal tumor formation in *Nlrp3*^{-/-} and *Casp1*^{-/-} mice in the commonly used azoxymethane (AOM)/dextran sodium sulfate (DSS) model. IL-18 production downstream of the Nlrp3 inflammasome was found to exert a protective role against colorectal tumor formation. IL-18-mediated activation and induction of the respective tumor suppressors STAT1 and IFN- γ may represent a potentially critical mechanism for Nlrp3-mediated resistance against colitis-associated tumorigenesis.

Materials and Methods

Mice

Nlrp3^{-/-}, *ASC*^{-/-}, and *Casp1*^{-/-} mice backcrossed to a C57BL/6 background for at least 10 generations have been described before (30). *IL-18*^{-/-} mice were donated by Dr. Paul G. Thomas (St. Jude Children's Research Hospital). All mice were 8- to 10-wk-old males and were maintained in a pathogen-free facility, and the animal studies were conducted under protocols approved by the St. Jude Children's Research Hospital Committee on the Use and Care of Animals.

Induction of colorectal cancer

Mice were injected i.p. with 10 mg/kg AOM (Sigma-Aldrich, St. Louis, MO). After 5 d, 3% DSS (molecular mass, 36–40 kDa; MP Biologicals, Solon, OH) was given in drinking water over 5 d followed by regular drinking water for 2 wk. This cycle was repeated twice and mice were sacrificed 4 wk after the last DSS cycle.

Histopathological analysis

Formalin-preserved sections of cecum and colon (proximal, middle, and distal) were processed and embedded in paraffin by standard techniques. Longitudinal sections of 5 μ m thick were stained with H&E and examined by a pathologist blinded to the experimental groups. Colitis scores of each segment were assigned based on the extent and severity of inflammation, ulceration, and hyperplasia of the mucosa. Severity scores for inflammation were as follows: 0, normal (within normal limits); 1, mild (small, focal, or widely separated, limited to lamina propria); 2, moderate (multifocal or locally extensive, extending to submucosa); 3, severe (transmural inflammation with ulcers covering ≥ 20 crypts). Scores for ulceration were as follows: 0, normal (no ulcers); 1, mild (one to two ulcers involving up to a total of 20 crypts); 2, moderate (one to four ulcers involving a total of 20–40 crypts); 3, severe (more than four ulcers or >40 crypts). Mucosal hyperplasia scores were assigned as follows: 0, normal (within normal limits); 1, mild (crypts two to three times normal thickness, normal epithelium); 2, moderate (crypts two to three times normal thickness, hyperchromatic epithelium, reduced goblet cells, scattered arborization); 3, severe (crypts more than four times normal thickness, marked hyperchromasia, few to no goblet cells, high mitotic index, frequent arborization). Scoring for extent of lesions was as follows: 0, normal (0% involvement); 1, mild (up to 30% involvement); 2, moderate (30–70% involvement); 3, severe ($>70\%$ involvement). The individual scores from the four segments were summed such that the maximum colitis score for a given animal is 48 and the minimum score is 0. For immunohistochemistry, formalin-fixed paraffin-embedded tissues were cut into 4- μ m sections and slides were stained with Abs against the macrophage marker F4/80 (Caltag Laboratories, Burlingame, CA) and phospho-STAT1 (Cell Signaling Technology, Beverly, MA), respectively.

Cytokine measurements

To measure the cytokine levels in colon tissue, a part of the colon was homogenized mechanically in PBS containing 1% Nonidet P-40 and

a complete protease inhibitor mixture tablet (Roche Diagnostics, Indianapolis, IN). Mouse cytokines and chemokines in serum and colon homogenates were determined with Luminex (Bio-Rad, Hercules, CA) and ELISA (R&D Systems, Minneapolis, MN) assays.

Real-time RT-PCR

Total RNA from colon tissue was isolated with TRIzol (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized from 250 ng of RNA using SuperScript III (Invitrogen). Real-time PCR for cyclooxygenase (COX)-2 and IFN- γ was performed using SYBR Green Master mix (Invitrogen) on an ABI Prism 7500 real-time PCR system (Applied Biosystems, Foster City, CA). mRNA levels were determined by means of the standard curve method. A standard sample was serially diluted and used for constructing a standard curve. Simultaneous quantification of GAPDH mRNA was used as an internal control.

In situ intestinal proliferation assay

The number of proliferating cells in intestinal epithelium was determined using the immunoperoxidase staining protocol with the thymidine analog BrdU as described earlier (29). In brief, 1 mg/ml BrdU in PBS was injected i.p. Three hours later, colon tissue was collected, fixed in 10% neutral buffered formalin, and embedded in paraffin. Immunohistochemistry was performed using an in situ BrdU staining kit (BD Biosciences, San Jose, CA). Tissues were counterstained with hematoxylin.

Western blotting

Tissue homogenates were lysed in lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 0.1% Nonidet P-40, 0.25% sodium deoxycholate, supplemented with protease and phosphatase inhibitor cocktails; Roche Diagnostics), and membranes were removed by centrifugation at 11,000 \times g. Before separation by SDS-PAGE, protein samples were denatured with SDS plus 100 mM DTT and boiled for 5 min. Separated proteins were transferred to polyvinylidene difluoride membranes and immunoblotted with primary Abs against phospho-STAT1, STAT1, rabbit phospho-I κ B, I κ B (all from Cell Signaling Technology), and β -actin (Sigma-Aldrich).

Recombinant IL-18 and IFN- γ treatment

Recombinant IL-18 (MBL International, Woburn, MA) was injected i.p. at a concentration of 0.5 μ g/mouse on days 0, 2, and 4 and at 0.1 μ g/mouse on days 6 and 8 after DSS administration. Alternatively, *Casp1*^{-/-} mice were injected i.p. with recombinant mouse IFN- γ (R&D Systems) at a concentration of 200 IU/mouse on days 0, 2, 4, and 6 after DSS administration.

Statistical analysis

Data are represented as mean \pm SE. Statistical significance was determined by a Student *t* test or χ^2 test. The *p* values <0.05 were considered statistically significant.

Results

Increased susceptibility to colitis-associated colorectal tumor formation in *Nlrp3* inflammasome-deficient mice

Nlrp3-deficient mice were recently shown to be highly susceptible to the induction of inflammation and tissue damage in the acute DSS-induced colitis model (29). Similarly, Nlrp3-deficient mice developed more severe symptoms of chronic colitis when mice were administered multiple cycles of DSS (28, 29). To determine whether the increased and prolonged gut inflammation in inflammasome-deficient mice led to increased tumorigenesis, *Nlrp3*^{-/-}, *ASC*^{-/-}, and *Casp1*^{-/-} mice were administered a single dose of the DNA methylating agent AOM (10 mg/kg), followed by repeated cycles of a 3% DSS solution (31). Twelve weeks after AOM injection (Fig. 1A), the development of adenomatous polyps and well-formed tumors in the colons of Nlrp3 inflammasome-deficient mice was examined and compared with colons of treated wild-type mice. Mice in the wild-type, *Nlrp3*^{-/-}, *ASC*^{-/-}, and *Casp1*^{-/-} groups developed tumors after AOM and DSS administration, but tumor burdens were significantly increased in *Nlrp3*^{-/-}, *ASC*^{-/-}, and *Casp1*^{-/-} mice over wild-type mice (Fig. 1B, 1C). Nevertheless, statistically significant differences in tumor size could

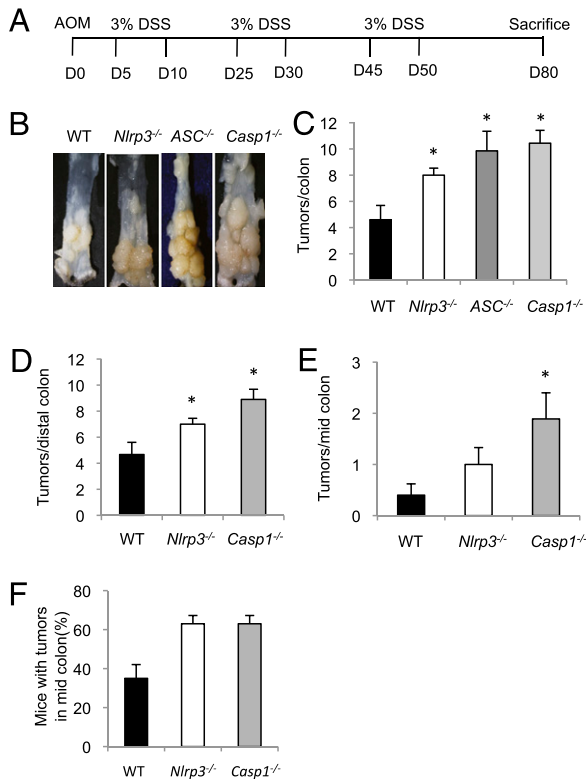


FIGURE 1. *Nlrp3*^{-/-}, *ASC*^{-/-}, and *Casp1*^{-/-} mice are hypersusceptible to colitis-associated colorectal tumorigenesis. *A*, WT ($n = 15$), *Nlrp3*^{-/-} ($n = 15$), *ASC*^{-/-} ($n = 8$), and *Casp1*^{-/-} mice ($n = 13$) were administered AOM on day 0 and were then given a 3% DSS solution during three 5-d cycles as described in *Materials and Methods*. *B*, Twelve weeks after AOM injection, mice were sacrificed to determine tumor development in the colon. *C–E*, Total tumor numbers observed in whole colon (*C*), distal colon (*D*), and middle colon (*E*) were determined. *F*, The percentage of mice containing tumors in the midcolon section was calculated. Data represent means \pm SE. * $p < 0.05$. WT, wild-type.

not be observed (data not shown). Most tumors were located in the distal area of the colon in all genotypes (Fig. 1*D*), although a fraction of the tumors were found in the midcolon section (Fig. 1*E*). Notably, the number of mice presenting with tumors in the midcolon region increased from ~40% of wild-type mice to >60% in the *Nlrp3*^{-/-} and *Casp1*^{-/-} cohorts (Fig. 1*F*). This may be due to the more severe inflammation and extended tissue damage that DSS induced in *Nlrp3*^{-/-} and *Casp1*^{-/-} mice (27–29).

Colons and ceca of representative tumor-bearing wild-type, *Nlrp3*^{-/-}, and *Casp1*^{-/-} mice were sectioned and stained with H&E to study mucosal dysplasia in more detail. Significantly more dysplastic cells and hyperplastic areas, adenomatous polyps, and well-formed tumors were visible in the distal colons of *Nlrp3*^{-/-} and *Casp1*^{-/-} mice relative to those of wild-type mice (Fig. 2*A*). Moreover, the number of mice presenting with dysplastic events was significantly higher in the *Nlrp3*^{-/-} and *Casp1*^{-/-} cohorts (Fig. 2*B*). In all three genotypes, tumors were mainly derived from dysplastic epithelial cells at the site of inflammation (Fig. 2*C*). The tumors appeared as sessile tubulovillous adenomas, and evidence for adenocarcinoma development was not observed. Histopathological scoring for severity of inflammation, inflamed area, ulceration, and hyperplasia in the colon and cecum was in agreement with significantly increased disease progression in *Nlrp3*^{-/-} and *Casp1*^{-/-} mice (Fig. 2*D*). As indicated before (Fig. 1*D*), most tumors were located in the distal area of the colon, but occasionally tumors were found in the

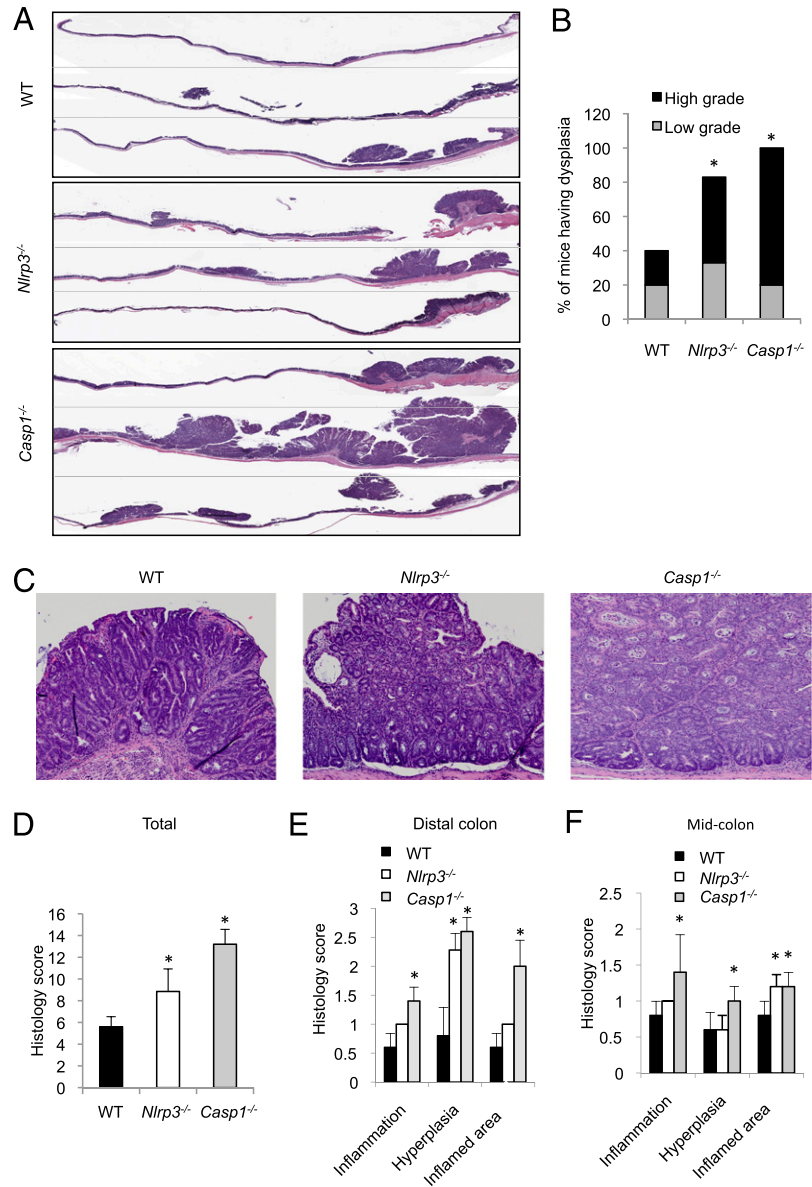
midcolon area. Separate histopathological scorings for the distal and midcolon regions were performed to determine whether spatial differences in pathology could be observed. In both the distal (Fig. 2*E*) and midcolon (Fig. 2*F*) regions, read-outs for inflammation severity, inflamed area, and hyperplasia were significantly increased in *Nlrp3*^{-/-} and *Casp1*^{-/-} mice over wild-type controls. Collectively, these results demonstrate that a functional Nlrp3 inflammasome is critical for protection against colitis-associated dysplasia and tumorigenesis in the gut. These observations are in agreement with a recent report showing increased AOM/DSS-induced colon tumorigenesis in mice lacking Nlrp3 or the inflammasome effectors ASC and caspase-1 (28).

Enhanced tumorigenesis in *Nlrp3* inflammasome-deficient mice is associated with deregulated IL-18 production and increased macrophage infiltration

The Nlrp3 inflammasome is required for maturation and secretion of the inflammatory cytokines IL-1 β and IL-18 (32). To determine whether the absence of a functional Nlrp3 inflammasome affects local IL-1 β and IL-18 production in the gut during early stages of tumorigenesis, the levels of these cytokines were measured in colon homogenates of *Nlrp3*^{-/-} and *Casp1*^{-/-} mice 5 d after completion of the first DSS cycle (day 10 after AOM treatment). IL-1 β amounts in the colon homogenates of AOM/DSS-treated wild-type, *Nlrp3*^{-/-}, and *Casp1*^{-/-} mice remained low and barely rose above those of untreated animals (Fig. 3*A* and data not shown). In contrast, significant levels of IL-18 were measured in colon homogenates of AOM/DSS-treated wild-type mice (Fig. 3*A*). However, IL-18 levels in colon homogenates of *Nlrp3*^{-/-} and *Casp1*^{-/-} mice were nearly 50% lower than those of treated wild-type controls (Fig. 3*A*). Unlike IL-18, the levels of the cytokines IL-6, IL-12, and TNF- α did not differ significantly from those found in wild-type controls (data not shown), demonstrating the specificity of these results. Moreover, we measured significantly higher levels of the chemokines MIP-1 α and eotaxin in colon homogenates of *Nlrp3*^{-/-} and *Casp1*^{-/-} mice (Fig. 3*B*, 3*C*), suggesting that deregulated IL-18 production triggered an increased recruitment of inflammatory cells in colons of *Nlrp3*^{-/-} and *Casp1*^{-/-} mice. In agreement, colon sections of the latter genotypes contained significantly more F4/80-positive cells than did wild-type colons (Fig. 3*D*), indicating a dramatically increased infiltration of macrophages in colons of *Nlrp3*^{-/-} and *Casp1*^{-/-} mice. Macrophages exert a regulating role in the colorectal tumor microenvironment through the production of a variety of tumorigenic factors including COX-2, which promotes tumor development through the synthesis of its enzymatic product PGE₂ (33). Consistent with the increased macrophage infiltration in colons of *Nlrp3*^{-/-} and *Casp1*^{-/-} mice, real-time PCR analysis demonstrated significantly higher COX-2 mRNA levels in colon tissue of *Nlrp3*^{-/-} and *Casp1*^{-/-} mice (Fig. 3*E*).

We next sought to determine the effect of deregulated IL-18 production, increased macrophage infiltration, and COX-2 production on epithelial cell proliferation in the colons of AOM/DSS-treated *Nlrp3*^{-/-} and *Casp1*^{-/-} mice. To this end, epithelial cell proliferation was examined by BrdU staining at the early and late time points of 10 d and 12 wk after AOM injection, respectively. Interestingly, the number of proliferating cells located in dysplastic regions of *Nlrp3*^{-/-} and *Casp1*^{-/-} colons was significantly higher than in tumor tissue of AOM/DSS-treated wild-type mice at both analyzed time points (Fig. 4). In contrast, no significant differences in proliferation were noted between the three genotypes in regions of the normal mucosa (Fig. 4). AOM-induced mutagenic events are likely to be critical for inducing neoplasia in the context of the colonic microenvironment of *Nlrp3*^{-/-} and

FIGURE 2. Histopathological examination of tumor and colon tissue of WT, *Nlrp3*^{-/-}, and *Casp1*^{-/-} mice. WT, *Nlrp3*^{-/-}, and *Casp1*^{-/-} mice (*n* = 10/genotype) were injected with AOM and then received three cycles of a 3% DSS solution as described in *Materials and Methods*. Twelve weeks after AOM injection, colons were collected and sections were stained with H&E for histopathological analysis. **A**, Low magnification (original magnification ×20) scanning of distal colon after H&E staining. **B**, Overall grading of dysplasia in each genotype was performed as described in *Materials and Methods*. **C**, Representative high magnification images of H&E staining showing dysplasia in colon tissue of WT (low-grade dysplasia), *Nlrp3*^{-/-} (low-grade dysplasia), and *Casp1*^{-/-} (high-grade dysplasia) mice. Original magnification ×20. **D–F**, Semiquantitative scoring of inflammation, hyperplasia, and inflamed area in total (**D**), distal (**E**), and midcolon (**F**) sections. Data represent means ± SE. **p* < 0.05. WT, wild-type.



Casp1^{-/-} mice because DSS administration alone failed to induce increased cell proliferation in colonic crypts of *Nlrp3*^{-/-} and *Casp1*^{-/-} mice (29). Taken together, these results suggest a criti-

cal role for *Nlrp3* inflammasome-mediated production of IL-18 in protection against colitis-associated immune cell invasion and neoplasia.

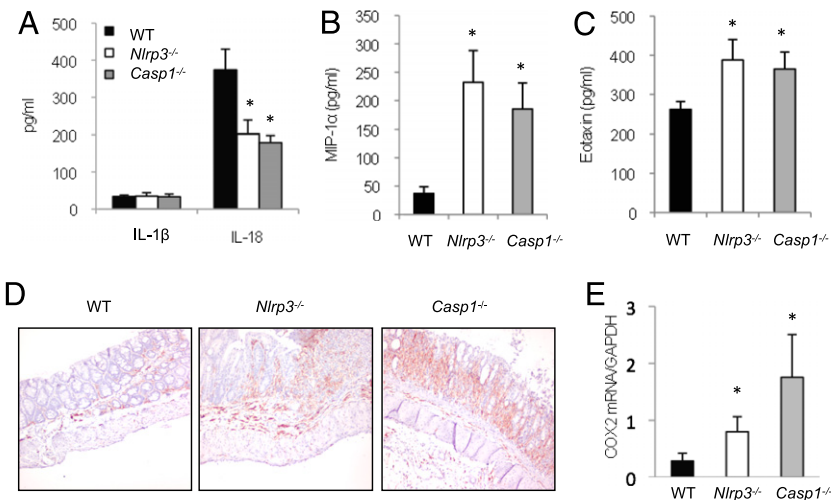


FIGURE 3. Decreased production of IL-18 in *Nlrp3*^{-/-} and *Casp1*^{-/-} colons is associated with increased inflammation and induction of tumorigenic factors. WT, *Nlrp3*^{-/-}, and *Casp1*^{-/-} mice were injected with AOM followed by 3% DSS treatment. **A–C**, Distal colons were collected at day 15 after AOM injection, and homogenates were used to determine IL-1β and IL-18 levels (**A**) and the concentrations of the chemotactic factors MIP-1α (**B**) and eotaxin (**C**) by ELISA. **D**, Colon sections were simultaneously immunostained for the macrophage marker F4/80. Original magnification ×10. **E**, Real-time PCR analysis was performed on distal colon homogenates collected at day 15 after AOM injection to measure COX-2 expression. Data represent means ± SE (*n* = 5/group). **p* < 0.05. WT, wild-type.

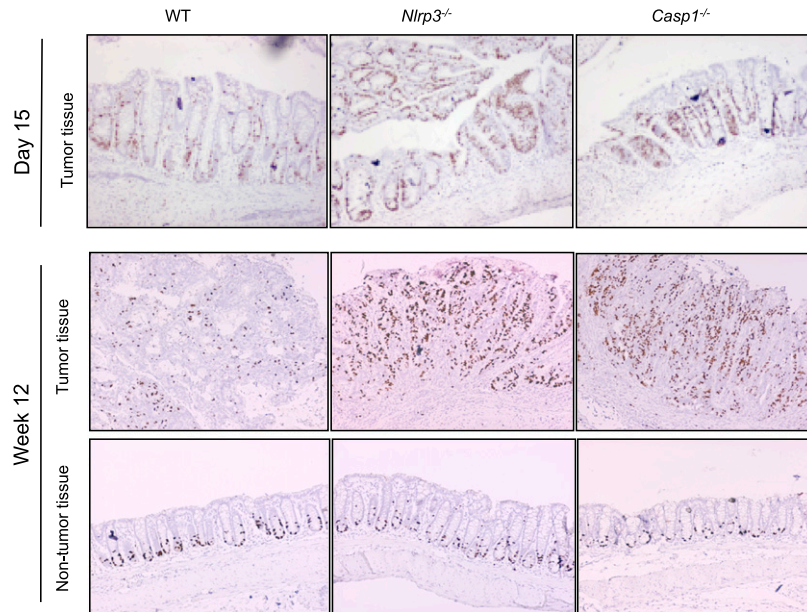


FIGURE 4. Increased hyperplastic cell proliferation in colons of AOM/DSS-treated *Nlrp3*^{-/-} and *Casp1*^{-/-} mice. WT, *Nlrp3*^{-/-}, and *Casp1*^{-/-} mice were injected with AOM followed by administration of a 3% DSS solution as described in *Materials and Methods*. Mice were injected i.p. with BrdU either at day 15 or 12 wk after AOM injection. Colon sections were immunostained to determine BrdU-positive cells. Original magnification $\times 10$. WT, wild-type.

IL-18 signaling downstream of the Nlrp3 inflammasome confers protection against colitis-associated tumorigenesis

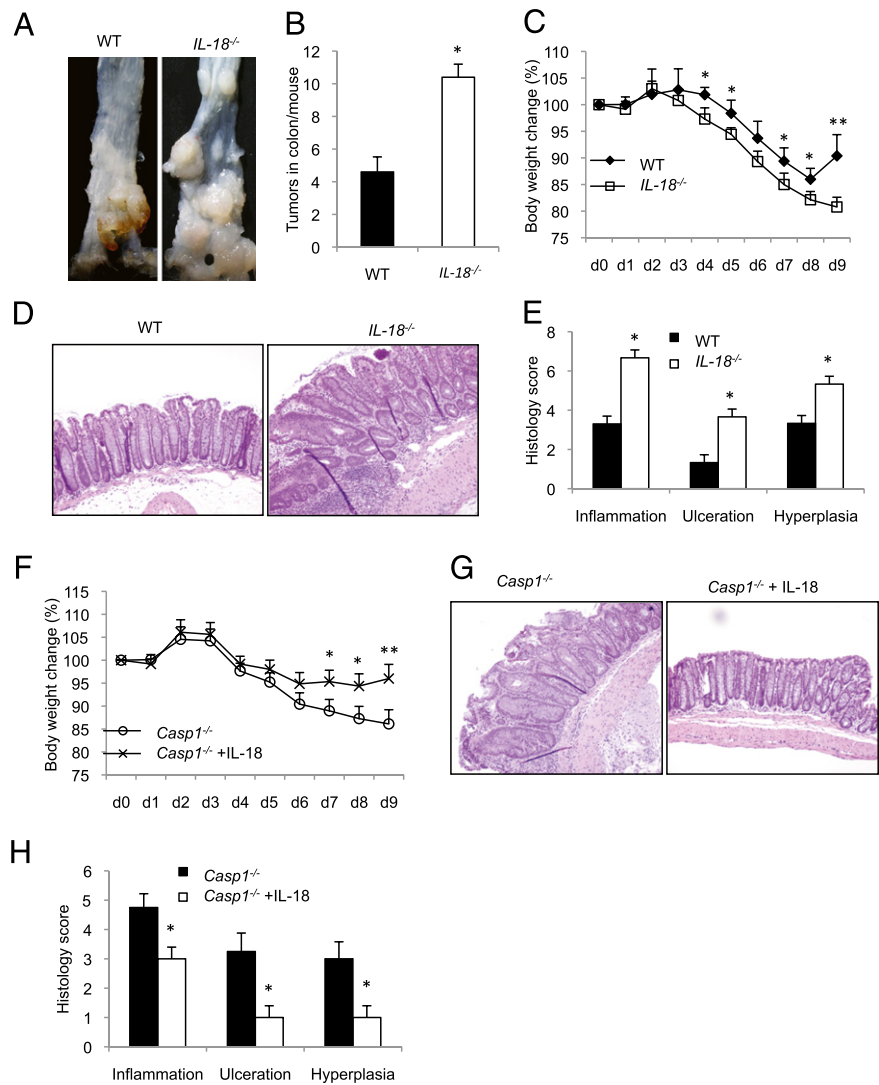
To further examine the role of IL-18 in protection against colitis-associated dysplasia and tumor development, we characterized colon inflammation and tumor development in *il-18*^{-/-} mice that were subjected to the AOM/DSS regimen described in Fig. 1A. In agreement with an important role for IL-18 in protection against colitis-associated tumorigenesis, colons of *il-18*^{-/-} mice contained significantly more tumors than did those of treated wild-type mice (Fig. 5A, 5B). To determine whether increased tumor formation in *il-18*^{-/-} mice could be linked to increased epithelial cell damage and colon inflammation during the early stages of disease, we examined phenotypic and histological signs of colitis and hyperplasia during acute colitis. To this end, wild-type and *il-18*^{-/-} mice were administered AOM followed by a 3% DSS solution during 5 d before parameters of colitis development and tumorigenesis were analyzed. The *il-18*^{-/-} mice presented with aggravated colitis, as evidenced by higher body weight loss (Fig. 5C), severe inflammation, hyperplasia, and more dysplastic cells (Fig. 5D). Semiquantitative scoring of histological colon sections for inflammation, ulceration, affected area, and hyperplasia was consistent with markedly increased disease development in *il-18*^{-/-} mice relative to the group of wild-type mice (Fig. 5E).

As a complementary approach to the use of *il-18*^{-/-} mice, we studied whether recombinant IL-18 could reverse disease progression in AOM/DSS-treated *Casp1*^{-/-} mice. Importantly, the group of *Casp1*^{-/-} mice that received recombinant IL-18 lost significantly less weight compared with *Casp1*^{-/-} mice that were refused the recombinant cytokine (Fig. 5F). Moreover, IL-18 administration provided protection against histological signs of inflammation and dysplasia (Fig. 5G). Consistently, semiquantitative scoring of inflammation, ulceration, affected area, and hyperplasia on histological colon sections was indicative of milder disease in IL-18-treated *Casp1*^{-/-} mice (Fig. 5H). These results demonstrate that IL-18 signaling downstream of the Nlrp3 inflammasome confers protection against colitis-associated colorectal tumorigenesis.

The Nlrp3 inflammasome activates the tumor suppressor STAT1 in the colon via IL-18-mediated IFN- γ production

Our results showed that impaired production of IL-18 downstream of the Nlrp3 inflammasome contributes to aggravated colitis-associated tumorigenesis (Figs. 3, 5). IL-18 was initially described as the cytokine responsible for induction of IFN- γ production (22), and IFN- γ was attributed potent antitumor activity in a variety of experimental tumorigenesis models (34–38). In agreement with the defective IL-18 production in colons of AOM/DSS-treated *Nlrp3*^{-/-} and *Casp1*^{-/-} mice (Fig. 3A), we found that IFN- γ mRNA levels were dramatically lower in colon homogenates of the latter genotypes relative to those of AOM/DSS-treated wild-type mice (Fig. 6A). Diminished IFN- γ production was confirmed at the protein level by IFN- γ -specific ELISA (Fig. 6B). IFN- γ -mediated antitumor signaling involves activation of the transcription factor STAT1 (36). Notably, *stat1*^{-/-} mice are highly susceptible to tumorigenesis, classifying STAT1 as a tumor suppressor (39, 40). To determine whether decreased production of IL-18 and IFN- γ in colons of AOM/DSS-treated *Casp1*^{-/-} mice affected STAT1 activation levels, phospho-specific STAT1 Abs were used to examine STAT1 activation by Western blotting. STAT1 activation was a consequence of AOM/DSS treatment, because basal STAT1 activation levels in wild-type colons were significantly upregulated following AOM/DSS treatment (Fig. 6C). However, AOM/DSS-induced STAT1 activation was dramatically reduced in colons of *Casp1*^{-/-} mice during early stages of tumorigenesis (day 15 after AOM treatment) (Fig. 6C). In contrast, phosphorylation of the NF- κ B inhibitor I κ B was not affected (Fig. 6D), demonstrating the specificity of these results. Immunohistochemical analysis of wild-type colons indicated increased phospho-STAT1 activation in epithelial cells and infiltrating immune cells upon AOM/DSS treatment (Fig. 6E). Colons of AOM/DSS-treated *Casp1*^{-/-} mice contained significantly less cells staining positive for phospho-STAT1 (Fig. 6E), suggesting that STAT1 signaling in epithelial and immune cells may both contribute to protection against tumorigenesis.

FIGURE 5. IL-18 signaling downstream of caspase-1 is critical for protection against colitis-associated tumorigenesis. WT and *IL-18*^{-/-} mice were administered AOM on day 0 and were then given a 3% DSS solution during three 5-d cycles as described in *Materials and Methods*. **A**, Eleven weeks after AOM injection, mice were sacrificed to determine tumor development in the colon. **B**, Tumors observed in the whole colon were counted. **C**, Body weight change of WT and *IL-18*^{-/-} mice was monitored for 9 d after DSS administration. **D**, Representative images of inflamed and hyperplastic areas in the distal colon at day 10 after DSS administration. H&E staining; original magnification $\times 10$. **E**, Semiquantitative histological scoring of inflammation, ulceration, and hyperplasia in whole colons of WT and *IL-18*^{-/-} mice at day 10 after DSS administration. **F**, *Casp1*^{-/-} mice were treated with recombinant IL-18 on days 0, 2, 4, 6, and 8 after DSS administration. Body weight change was monitored for 9 d. **G**, Representative images of inflamed and hyperplastic areas in the distal colon at day 10 after DSS administration. H&E staining; original magnification $\times 10$. **H**, Semiquantitative scoring of inflammation, ulceration, and hyperplasia in the whole colon at day 10 after DSS treatment. Data represent means \pm SE ($n = 5$ /group). * $p < 0.05$. WT, wild-type.



In agreement with an important role for IFN- γ in inducing STAT1 activation, STAT1 phosphorylation was restored by treating *Casp1*^{-/-} mice with 200 IU recombinant IFN- γ at days 5, 7, 9, and 11 after AOM treatment (Fig. 6F). Similarly, administration of recombinant IL-18 restored phospho-STAT1 levels in AOM/DSS-treated *Casp1*^{-/-} mice to those observed in colons of AOM/DSS-treated wild-type mice (Fig. 6G). Thus, IL-18- and IFN- γ -mediated activation of the tumor suppressor STAT1 may play a critical role in protection against colitis-associated tumorigenesis upon activation of the Nlrp3 inflammasome.

Discussion

Chronic inflammation is increasingly recognized as a critical risk factor for the development of colorectal cancer (4). Members of the NLR protein family are expressed on epithelial and professional APCs residing in the colonic mucosa and lamina propria and play key roles in regulating the immune response against commensal microorganisms in the gut. Notably, defective activation of the NLR member NOD1 has been reported to enhance inflammatory cytokine production against commensal bacteria in the gut and prime the colorectal mucosa for increased cell proliferation and tumor formation during colitis in mice (25). Moreover, mutations in the NLR protein NOD2 are linked with the development of Crohn's disease in humans (41, 42). It has been established that Crohn's disease patients are at increased risk of developing

sporadic colorectal cancer (43). In agreement, polymorphisms in the gene encoding NOD2 have been associated with increased susceptibility to gastrointestinal tumorigenesis (44). More recently, mutations in the gene encoding Nlrp3 were linked with increased susceptibility to Crohn's disease in humans (26). Recent reports from our and other groups demonstrated that DSS-induced colitis in Nlrp3-deficient mice is associated with an increased destruction of the epithelial barrier in the gut, inducing systemic dispersion of colonic microflora and an exaggerated inflammatory response (27, 29). Nlrp3 plays a central role in activation of caspase-1 and secretion of the proinflammatory cytokines IL-1 β and IL-18 (30). Caspase-1-deficient mice also were hypersensitive to DSS- and 2,4,6-trinitrobenzene sulfonic acid-induced colitis (27, 29), indicating that Nlrp3 protects against colitis through the production of caspase-1-dependent cytokines. Indeed, the phenotype of *Casp1*^{-/-} mice was rescued by administration of recombinant IL-18 (27, 29). Moreover, mice lacking the inflammasome inhibitor caspase-12 were resistant to acute colitis, although (paradoxically) they were more susceptible to AOM/DSS-induced colorectal tumorigenesis (27).

In this study, we showed that increased inflammatory responses and destruction of the epithelial barrier led to enhanced dysplasia and tumorigenesis in colons of AOM/DSS-treated *Nlrp3*^{-/-} and *Casp1*^{-/-} mice. Our observations are in agreement with a recent report showing that mice lacking Nlrp3, ASC, or caspase-1 were

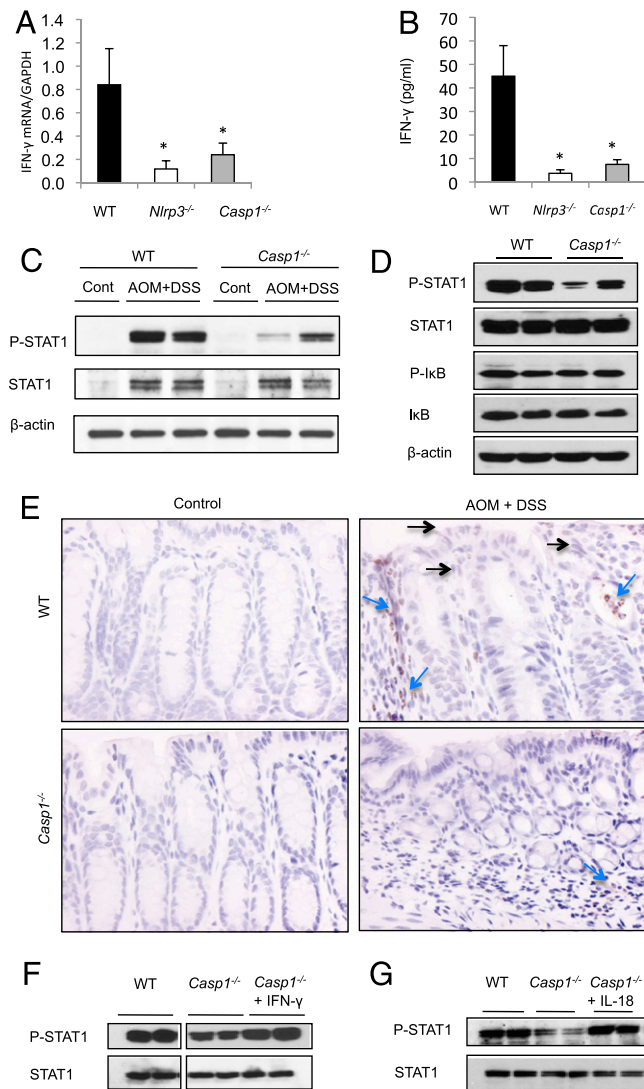


FIGURE 6. The Nlrp3 inflammasome activates the tumor suppressor STAT1 via IL-18-mediated IFN- γ production. *A* and *B*, WT, *Nlrp3*^{-/-}, and *Casp1*^{-/-} mice were administered AOM on day 0 and were then given a 3% DSS solution for 5 d as described in *Materials and Methods*. IFN- γ production in colon tissue at day 15 after AOM injection was analyzed by real-time PCR (*A*) and ELISA (*B*). Data represent means \pm SE ($n = 5$ /group). * $p < 0.05$. *C* and *D*, Homogenates of the distal colon were prepared 15 d after AOM injection and analyzed for total STAT1, phospho-STAT1, total I κ B, and phospho-I κ B by Western blotting. β -actin was used as a loading control. *E*, Colon tissue sections collected from control mice and AOM/DSS-treated WT and *Casp1*^{-/-} mice (at day 15 after AOM) were immunostained for phospho-STAT1. Black arrows indicate phospho-STAT1-positive epithelial cells; blue arrows indicate phospho-STAT1-positive inflammatory cells. Original magnification $\times 40$. *F*, *Casp1*^{-/-} mice were treated with IFN- γ at 5, 7, 9, and 11 d after AOM administration. On day 15 after AOM treatment, STAT1 activation in colon homogenates was compared with that of AOM/DSS-treated WT and *Casp1*^{-/-} mice by Western blotting. *G*, *Casp1*^{-/-} mice were treated with IL-18 at 5, 7, 9, and 11 d after AOM treatment. On day 15 after AOM treatment, STAT1 activation in colon homogenates was compared with that of AOM/DSS-treated WT and *Casp1*^{-/-} mice by Western blotting. WT, wild-type.

hypersusceptible to AOM/DSS-induced colorectal tumor formation (28). However, the mechanism by which the Nlrp3 inflammasome confers protection against colitis-associated tumorigenesis remained obscure (45). We demonstrated that IL-18 production was significantly reduced in colons of AOM/DSS-treated *Nlrp3*^{-/-} and

Casp1^{-/-} mice, and, more importantly, that colons of AOM/DSS-treated *il-18*^{-/-} mice recapitulated the increased tumor burdens seen in mice lacking Nlrp3 or caspase-1. These results suggested a critical role for IL-18 production downstream of the Nlrp3 inflammasome in protection against colitis-associated neoplasia. In agreement, administration of recombinant IL-18 markedly reduced disease progression in AOM/DSS-treated *Casp1*^{-/-} mice.

IL-18 was previously assigned an antitumor function in a variety of experimental tumor models (46–49). It was reported to inhibit tumor growth and angiogenesis (50–52) and was associated with repair and restitution of ulcerated epithelium (53). We and others showed that IL-18 is involved in repair of the epithelial layer of the gut by maintaining proper levels of epithelial cell proliferation during the acute stage of DSS-induced colitis (27, 29). DSS-induced damage and erosion of the epithelial layer is repaired by rapid proliferation of stem cells residing at the base of crypts (54). Intriguingly, while IL-18 promotes enterocyte proliferation to repair chemically induced injury of colonic epithelium, we showed in this study that it also inhibits hyperplasia during chronic stages of colitis. This apparent discrepancy may be explained by differential roles of IL-18 during the acute and chronic stages of colitis (53). Moreover, we only observed higher proliferation rates in dysplastic regions of the colon epithelium of AOM/DSS-treated *Nlrp3*^{-/-} and *Casp1*^{-/-} mice, but not in non-tumor regions. These observations suggest that IL-18 exerts its protective effect in two stages. First, during acute DSS-induced colitis, it contributes to restoring epithelial barrier integrity by induced controlled proliferation of stem cells at the crypt base and turnover of damaged epithelial cells. This prevents systemic dispersion of commensal microflora and the induction of exaggerated inflammatory responses. However, during remission and chronic stages of colitis, IL-18 inhibits epithelial cell proliferation in neoplastic regions of the colon epithelium. This may be achieved, at least in part, through the induction of IFN- γ production. Indeed, IL-18 was originally identified as the “IFN- γ -inducing factor” (22), and IFN- γ has been described as a pleiotropic cytokine with potent antitumor activity (34, 37). In this regard, we demonstrated a markedly diminished production of IFN- γ in colons of AOM/DSS-treated *Nlrp3*^{-/-} and *Casp1*^{-/-} mice. Notably, IFN- γ signaling was previously shown to confer protection against experimental colitis (55). In agreement with a biphasic role for Nlrp3-mediated IL-18 production in colitis-associated tumorigenesis, a recent report described a biphasic role for IFN- γ during DSS-induced colitis with promotion of intestinal epithelial cell proliferation at early stages and induction of anti-proliferative responses at later stages (56). IFN- γ mediates its effect through IFN- γ R, which is expressed on both normal and malignant cells (57). Its biological effects are mediated by a number of intracellular signaling pathways, the best characterized of which is the JAK-STAT pathway. Once IFN- γ R is activated, it phosphorylates JAK1 and JAK2, which further induces phosphorylation and nuclear translocation of STAT1. We showed that phosphorylated STAT1 levels were markedly reduced in colons of AOM/DSS-treated *Casp1*^{-/-} mice, but they were restored upon stimulation with either IFN- γ or IL-18. These results indicate that STAT1 signaling is affected in the absence of a functional Nlrp3 inflammasome. Once in the nucleus, STAT1 binds with γ -activated sequences in IFN- γ -responsive genes to induce transcription of genes involved in cell proliferation, differentiation and cell death (58, 59). Thus, IFN- γ -mediated STAT1 activation downstream of IL-18 may play an important role in maintaining gut homeostasis and inhibiting tumor development during colitis.

In conclusion, we characterized the role and the mechanism by which activation of the Nlrp3 inflammasome confers protection

against the development of inflammation-associated colorectal tumorigenesis. **We showed that Nlrp3 inflammasome-dependent IL-18 production prevents neoplastic events, possibly through the induction of IFN- γ production and STAT1 signaling.** These results suggest that strategies aimed at producing or delivering mature IL-18 in the colon may prove beneficial in preventing colorectal tumor development in the context of chronic inflammation.

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Disclosures

The authors have no financial conflicts of interest.

References

- Choi, P. M., and M. P. Zelig. 1994. Similarity of colorectal cancer in Crohn's disease and ulcerative colitis: implications for carcinogenesis and prevention. *Gut* 35: 950–954.
- Eaden, J. A., K. R. Abrams, and J. F. Mayberry. 2001. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 48: 526–535.
- Ekbow, A., C. Helmick, M. Zack, and H. O. Adami. 1990. Ulcerative colitis and colorectal cancer: a population-based study. *N. Engl. J. Med.* 323: 1228–1233.
- Itzkowitz, S. H., and X. Yio. 2004. Inflammation and cancer, IV: colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 287: G7–G17.
- Coussens, L. M., and Z. Werb. 2002. Inflammation and cancer. *Nature* 420: 860–867.
- Grivennikov, S. I., F. R. Greten, and M. Karin. 2010. Immunity, inflammation, and cancer. *Cell* 140: 883–899.
- Krelin, Y., E. Voronov, S. Dotan, M. Elkabets, E. Reich, M. Fogel, M. Huszar, Y. Iwakura, S. Segal, C. A. Dinarello, and R. N. Apte. 2007. Interleukin-1 β -driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors. *Cancer Res.* 67: 1062–1071.
- Tamura, K., Y. Fukuda, H. Sashio, N. Takeda, H. Bamba, T. Kosaka, S. Fukui, K. Sawada, K. Tamura, M. Satomi, et al. 2002. IL18 polymorphism is associated with an increased risk of Crohn's disease. *J. Gastroenterol.* 37(Suppl. 14): 111–116.
- Greten, F. R., L. Eckmann, T. F. Greten, J. M. Park, Z. W. Li, L. J. Egan, M. F. Kagnoff, and M. Karin. 2004. IKK β links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 118: 285–296.
- Karin, M. 2006. Nuclear factor- κ B in cancer development and progression. *Nature* 441: 431–436.
- Kanneganti, T. D., M. Lamkanfi, and G. Nuñez. 2007. Intracellular NOD-like receptors in host defense and disease. *Immunity* 27: 549–559.
- Kopp, E., and R. Medzhitov. 2003. Recognition of microbial infection by Toll-like receptors. *Curr. Opin. Immunol.* 15: 396–401.
- Kawai, T., and S. Akira. 2007. Signaling to NF- κ B by Toll-like receptors. *Trends Mol. Med.* 13: 460–469.
- Rhee, S. H., E. Im, and C. Pothoulakis. 2008. Toll-like receptor 5 engagement modulates tumor development and growth in a mouse xenograft model of human colon cancer. *Gastroenterology* 135: 518–528.
- Kanneganti, T. D., N. Ozören, M. Body-Malapel, A. Amer, J. H. Park, L. Franchi, J. Whitfield, W. Barchet, M. Colonna, P. Vandenabeele, et al. 2006. Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature* 440: 233–236.
- Martinon, F., K. Burns, and J. Tschopp. 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL- β . *Mol. Cell* 10: 417–426.
- Ghayur, T., S. Banerjee, M. Hugunin, D. Butler, L. Herzog, A. Carter, L. Quintal, L. Sekut, R. Talanian, M. Paskind, et al. 1997. Caspase-1 processes IFN- γ -inducing factor and regulates LPS-induced IFN- γ production. *Nature* 386: 619–623.
- Kuida, K., J. A. Lippke, G. Ku, M. W. Harding, D. J. Livingston, M. S. Su, and R. A. Flavell. 1995. Altered cytokine export and apoptosis in mice deficient in interleukin-1 β converting enzyme. *Science* 267: 2000–2003.
- Nold-Petry, C. A., M. F. Nold, J. W. Nielsen, A. Bustamante, J. A. Zepp, K. A. Storm, J. W. Hong, S. H. Kim, and C. A. Dinarello. 2009. Increased cytokine production in interleukin-18 receptor alpha-deficient cells is associated with dysregulation of suppressors of cytokine signaling. *J. Biol. Chem.* 284: 25900–25911.
- Kalina, U., D. Kauschat, N. Koyama, H. Nuernberger, K. Ballas, S. Koschmieder, G. Bug, W. K. Hofmann, D. Hoelzer, and O. G. Ottmann. 2000. IL-18 activates STAT3 in the natural killer cell line 92, augments cytotoxic activity, and mediates IFN- γ production by the stress kinase p38 and by the extracellular regulated kinases p44^{erk-1} and p42^{erk-2}. *J. Immunol.* 165: 1307–1313.
- Takeda, K., H. Tsutsui, T. Yoshimoto, O. Adachi, N. Yoshida, T. Kishimoto, H. Okamura, K. Nakanishi, and S. Akira. 1998. Defective NK cell activity and Th1 response in IL-18-deficient mice. *Immunity* 8: 383–390.
- Okamura, H., H. Tsutsui, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Torigoe, T. Okura, Y. Nukada, K. Hattori, et al. 1995. Cloning of a new cytokine that induces IFN- γ production by T cells. *Nature* 378: 88–91.
- Horwood, N. J., N. Udagawa, J. Elliott, D. Grail, H. Okamura, M. Kurimoto, A. R. Dunn, T. Martin, and M. T. Gillespie. 1998. Interleukin 18 inhibits osteoclast formation via T cell production of granulocyte macrophage colony-stimulating factor. *J. Clin. Invest.* 101: 595–603.
- Maxwell, J. R., R. Yadav, R. J. Rossi, C. E. Ruby, A. D. Weinberg, H. L. Aguila, and A. T. Vella. 2006. IL-18 bridges innate and adaptive immunity through IFN- γ and the CD134 pathway. *J. Immunol.* 177: 234–245.
- Chen, G. Y., M. H. Shaw, G. Redondo, and G. Nuñez. 2008. The innate immune receptor Nod1 protects the intestine from inflammation-induced tumorigenesis. *Cancer Res.* 68: 10060–10067.
- Villani, A. C., M. Lemire, G. Fortin, E. Louis, M. S. Silverberg, C. Collette, N. Baba, C. Libouille, J. Belaiche, A. Bitton, et al. 2009. Common variants in the NLRP3 region contribute to Crohn's disease susceptibility. *Nat. Genet.* 41: 71–76.
- Dupauly-Chicoine, J., G. Yeretssian, K. Doiron, K. S. Bergstrom, C. R. McIntire, P. M. LeBlanc, C. Meunier, C. Turbide, P. Gros, N. Beauchemin, et al. 2010. Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* 32: 367–378.
- Allen, I. C., E. M. TeKippe, R. M. Woodford, J. M. Uronis, E. K. Holl, A. B. Rogers, H. H. Herfarth, C. Jobin, and J. P. Ting. 2010. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. *J. Exp. Med.* 207: 1045–1056.
- Zaki, M. H., K. L. Boyd, P. Vogel, M. B. Kastan, M. Lamkanfi, and T. D. Kanneganti. 2010. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* 32: 379–391.
- Ippagunta, S. K., D. D. Brand, J. Luo, K. L. Boyd, C. Calabrese, R. Stienstra, F. L. Van de Veerdonk, M. G. Netea, L. A. Joosten, M. Lamkanfi, and T. D. Kanneganti. 2010. Inflammasome-independent role of apoptosis-associated speck-like protein containing a CARD (ASC) in T cell priming is critical for collagen-induced arthritis. *J. Biol. Chem.* 285: 12454–12462.
- Okayasu, I., T. Ohkusa, K. Kajura, J. Kanno, and S. Sakamoto. 1996. Promotion of colorectal neoplasia in experimental murine ulcerative colitis. *Gut* 39: 87–92.
- Lamkanfi, M., and T. D. Kanneganti. 2010. Nlrp3: an immune sensor of cellular stress and infection. *Int. J. Biochem. Cell Biol.* 42: 792–795.
- Greenhough, A., H. J. Smart, A. E. Moore, H. R. Roberts, A. C. Williams, C. Paraskeva, and A. Kaidi. 2009. The COX-2/PGE $_2$ pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis* 30: 377–386.
- Dighe, A. S., E. Richards, L. J. Old, and R. D. Schreiber. 1994. Enhanced in vivo growth and resistance to rejection of tumor cells expressing dominant negative IFN γ receptors. *Immunity* 1: 447–456.
- Lesinski, G. B., B. Badgwell, J. Zimmerer, T. Crespin, Y. Hu, G. Abood, and W. E. Carson, III. 2004. IL-12 pretreatments enhance IFN- α -induced Janus kinase-STAT signaling and potentiate the antitumor effects of IFN- α in a murine model of malignant melanoma. *J. Immunol.* 172: 7368–7376.
- Sato, T., C. Sella, N. S. Young, and J. P. Maciejewski. 1997. Inhibition of interferon regulatory factor-1 expression results in predominance of cell growth stimulatory effects of interferon- γ due to phosphorylation of Stat1 and Stat3. *Blood* 90: 4749–4758.
- Shankaran, V., H. Ikeda, A. T. Bruce, J. M. White, P. E. Swanson, L. J. Old, and R. D. Schreiber. 2001. IFN γ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 410: 1107–1111.
- Street, S. E., J. A. Trapani, D. MacGregor, and M. J. Smyth. 2002. Suppression of lymphoma and epithelial malignancies effected by interferon γ . *J. Exp. Med.* 196: 129–134.
- Badgwell, B., G. B. Lesinski, C. Magro, G. Abood, A. Skaf, and W. Carson, III. 2004. The antitumor effects of interferon- α are maintained in mice challenged with a STAT1-deficient murine melanoma cell line. *J. Surg. Res.* 116: 129–136.
- Stephanou, A., and D. S. Latchman. 2003. STAT-1: a novel regulator of apoptosis. *Int. J. Exp. Pathol.* 84: 239–244.
- Hugot, J. P., M. Chamaillard, H. Zouali, S. Lesage, J. P. Cézard, J. Belaiche, S. Almer, C. Tysk, C. A. O'Morain, M. Gassull, et al. 2001. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411: 599–603.
- Ogura, Y., D. K. Bonen, N. Inohara, D. L. Nicolae, F. F. Chen, R. Ramos, H. Britton, T. Moran, R. Karaliuskas, R. H. Duerr, et al. 2001. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411: 603–606.
- Munkholm, P. 2003. Review article: the incidence and prevalence of colorectal cancer in inflammatory bowel disease. *Aliment. Pharmacol. Ther.* 18(Suppl. 2): 1–5.
- Roberts, R. L., R. B. Gearry, M. D. Allington, H. R. Morrin, B. A. Robinson, and F. A. Frizelle. 2006. Caspase recruitment domain-containing protein 15 mutations in patients with colorectal cancer. *Cancer Res.* 66: 2532–2535.
- Anthony, K. 2010. Immunology: the inflammasome protects? *Nat. Rev. Cancer* 10: 383.
- Micallef, M. J., T. Tanimoto, K. Kohno, M. Ikeda, and M. Kurimoto. 1997. Interleukin 18 induces the sequential activation of natural killer cells and

- cytotoxic T lymphocytes to protect syngeneic mice from transplantation with Meth A sarcoma. *Cancer Res.* 57: 4557–4563.
47. Micallef, M. J., K. Yoshida, S. Kawai, T. Hanaya, K. Kohno, S. Arai, T. Tanimoto, K. Torigoe, M. Fujii, M. Ikeda, and M. Kurimoto. 1997. In vivo antitumor effects of murine interferon- γ -inducing factor/interleukin-18 in mice bearing syngeneic Meth A sarcoma malignant ascites. *Cancer Immunol. Immunother.* 43: 361–367.
 48. Osaki, T., W. Hashimoto, A. Gambotto, H. Okamura, P. D. Robbins, M. Kurimoto, M. T. Lotze, and H. Tahara. 1999. Potent antitumor effects mediated by local expression of the mature form of the interferon- γ inducing factor, interleukin-18 (IL-18). *Gene Ther.* 6: 808–815.
 49. Osaki, T., J. M. Péron, Q. Cai, H. Okamura, P. D. Robbins, M. Kurimoto, M. T. Lotze, and H. Tahara. 1998. IFN- γ -inducing factor/IL-18 administration mediates IFN- γ - and IL-12-independent antitumor effects. *J. Immunol.* 160: 1742–1749.
 50. Cao, E., X. Zang, U. A. Ramagopal, A. Mukhopadhyaya, A. Fedorov, E. Fedorov, W. D. Zencheck, J. W. Lary, J. L. Cole, H. Deng, et al. 2007. T cell immunoglobulin mucin-3 crystal structure reveals a galectin-9-independent ligand-binding surface. *Immunity* 26: 311–321.
 51. Coughlin, C. M., K. E. Salhany, M. Wysocka, E. Aruga, H. Kurzawa, A. E. Chang, C. A. Hunter, J. C. Fox, G. Trinchieri, and W. M. Lee. 1998. Interleukin-12 and interleukin-18 synergistically induce murine tumor regression which involves inhibition of angiogenesis. *J. Clin. Invest.* 101: 1441–1452.
 52. Hegardt, P., B. Widegren, L. Li, B. Sjögren, C. Kjellman, I. Sur, and H. O. Sjögren. 2001. Nitric oxide synthase inhibitor and IL-18 enhance the anti-tumor immune response of rats carrying an intrahepatic colon carcinoma. *Cancer Immunol. Immunother.* 50: 491–501.
 53. Reuter, B. K., and T. T. Pizarro. 2004. Commentary: the role of the IL-18 system and other members of the IL-1R/TLR superfamily in innate mucosal immunity and the pathogenesis of inflammatory bowel disease: friend or foe? *Eur. J. Immunol.* 34: 2347–2355.
 54. Radtke, F., and H. Clevers. 2005. Self-renewal and cancer of the gut: two sides of a coin. *Science* 307: 1904–1909.
 55. Sheikh, S. Z., K. Matsuoka, T. Kobayashi, F. Li, T. Rubinas, and S. E. Plevy. 2010. Cutting edge: IFN- γ is a negative regulator of IL-23 in murine macrophages and experimental colitis. *J. Immunol.* 184: 4069–4073.
 56. Nava, P., S. Koch, M. G. Laukoetter, W. Y. Lee, K. Kolegraff, C. T. Capaldo, N. Beeman, C. Addis, K. Gerner-Smidt, I. Neumaier, et al. 2010. Interferon- γ regulates intestinal epithelial homeostasis through converging β -catenin signaling pathways. *Immunity* 32: 392–402.
 57. Boehm, U., T. Klamp, M. Groot, and J. C. Howard. 1997. Cellular responses to interferon- γ . *Annu. Rev. Immunol.* 15: 749–795.
 58. Darnell, J. E., Jr., I. M. Kerr, and G. R. Stark. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 264: 1415–1421.
 59. Gough, D. J., D. E. Levy, R. W. Johnstone, and C. J. Clarke. 2008. IFN γ signaling: does it mean JAK-STAT? *Cytokine Growth Factor Rev.* 19: 383–394.