Caspase-11 attenuates gastrointestinal inflammation and experimental colitis pathogenesis

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INFLAMMASOME FORMATION plays an essential role in modulating immune system homeostasis in the gut and attenuates inflammatory bowel disease (IBD) pathogenesis (4, 17, 56). Inflammasomes are cytosolic macromolecular scaffolds that recognize pathogen-associated molecular patterns and damage-associated molecular patterns. These macromolecular scaffolds are comprised of a nucleotide-binding domain and leucine-rich repeat containing protein (NLR), the adaptor apoptosis-associated speck-like protein containing a caspase-recruitment domain (CARD) (ASC; Pycard), and caspase-1 (35). Inflammasome formation results in the proteolytic activation of caspase-1 and the subsequent maturation of pro-IL-1β and pro-IL-18 into bioactive cytokines. In addition to IL-1β and IL-18, caspase-1 activation is also associated with a unique form of proinflammatory cell death, which is defined as pyroptosis (13, 19–21, 50, 53). Caspase-1 is essential for canonical inflammasome formation and function. However, a noncanonical inflammasome has recently been characterized and is associated with caspase-11 (30). The noncanonical caspase-11 inflammasome is responsible for sensing cytosolic lipopolysaccharide (LPS) from Gram-negative bacteria. Caspase-11 has also been found to be an essential regulator of the host immune response against Escherichia coli, Citrobacter rodentium, and Vibrio cholera (23, 30). In early studies, caspase-11 was shown to be an essential mediator of endotoxic shock through modulating caspase-1 activation (51). Subsequent findings revealed that caspase-11 functions as a critical initiator of other caspases, including caspase-1, under specific acute pathological conditions (29). These early studies of caspase-11 have recently gained greater significance following the revelation that most Casp1−/− mice used in previous inflammasome and IL-1β/IL-18 studies were actually Casp1−/− and Casp1−/− double mutants (30). Subsequent studies utilizing Casp1−/− mice revealed that caspase-11 modulates many biological functions that were previously considered to be solely caspase-1 dependent and has also been shown to have caspase-1-independent functions (1, 9, 29, 30, 42).

The role of the canonical NLR inflammasome and caspase-1 in IBD has been well characterized. However, very little is known regarding the contribution of caspase-11. Murine caspase-11 is the paralog of caspase-4 and caspase-5 in humans. Neither of these caspases has been closely evaluated in biological functions that were previously considered to be chronic inflammation and tumorigenesis.

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through a mechanism associated with both IL-1β and IL-18 production.

MATERIALS AND METHODS

Animals. The generation and characterization of Casp11/−/− mice have been previously described (30). All experiments were conducted with 8–10-wk-old male mice housed under specific pathogen-free conditions that were backcrossed for at least 10 generations onto the C57Bl/6 background. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were conducted under the approval of the IACUC for Virginia Tech and the Virginia Maryland Regional College of Veterinary Medicine.

Induction and assessments of experimental colitis and colitis-associated tumorigenesis. Mice were exposed to models of either acute experimental colitis, relapsing/remitting experimental colitis, or a model of inflammation-driven colon tumorigenesis. In the acute experimental colitis model, mice were exposed to a single cycle of 4% DSS (MP Biomedicals) for 4 days and evaluated 3–5 days following the removal of the DSS (54). For the cytokine reconstitution and inhibition studies, mice were given daily intraperitoneal injections with either 0.25 μg/g of recombinant mouse IL-18 (Life Technologies), 10 μg/g of IL-18-binding protein (Sino Biological), 10 μg/g of recombinant mouse IL-1β (Sigma), or 10 mg/kg of the human IL-1 receptor antagonist anakinra (Kineret) (Amgen) (17, 41, 49). The induction of relapsing/remitting experimental colitis utilized three cycles of 2.5% DSS for 5 days, with a 14-day interval between each cycle (54). Mice were evaluated 10–14 days following the last cycle of DSS. To induce inflammation-driven tumorigenesis in the colitis-associated cancer model, mice were given a single intraperitoneal injection of (10 mg/kg body wt) of azoxymethane (AOM) (Sigma Aldrich), which functions as the mutagenic agent (38). The injection of AOM was followed by the initiation of the relapsing/remitting experimental colitis model described above utilizing 2.5% DSS (38). For all models, mice were euthanized and evaluated at specific time points throughout the course of the DSS and AOM/DSS exposure or when moribund.

Morbidity and mortality were evaluated throughout each of the experimental colitis models and included assessments of body weight, the presence of blood around the rectum or in the stool, and stool consistency. Each of these parameters were scored (scale of 0–4) and averaged to generate a cumulative semiquantitative clinical score, as previously described (4, 5). To evaluate the presence of blood in the stool, we utilized visual inspection and/or a Hemoccult Immunochemical Fecal Occult Blood Test (Beckman Coulter). Mice approaching 15% weight loss were monitored every 4 h (weight and clinical score). Animals were considered moribund and harvested on the day that weight loss reached 19% of the starting weight. The moribund data were used to generate survival curves.

Macroscopic polypl analysis and histopathology evaluation. The colon was removed from the cecum to the rectum and flushed with 1× PBS containing penicillin/streptomycin, and the length was recorded and opened longitudinally. Macroscopic polypl were identified by a trained investigator (I.C.A.) using a dissecting microscope (×10 magnification), and the size was determined by taking multiple measurements across the maximum surface of each macroscopic polypl (4, 5). To evaluate inflammation and tumor progression, the colons were Swiss rolled and fixed in 10% buffered formalin. The paraffin-embedded tissues were sectioned at 5 μm and prepared for hematoxylin and eosin (H and E) staining. H and E-stained sections were evaluated by an experienced investigator (I.C.A.) or board-certified veterinary pathologist (S.L.C.), who was blinded to genotype and treatment. Sections were evaluated and scored for inflammation, epithelial defects, crypt atrophy, hyperplasia, dysplasia/neoplasia, and area affected by disease, as previously detailed (4, 5, 37).

In addition to the evaluation of inflammation from H and E-stained sections, goblet cell hyperplasia and mucus in the colon were evaluated utilizing Alcian-blue/Periodic Acid-Schiff reaction (AB/PAS) staining (3). Briefly, a 2-mm section located ~1 cm proximal from the termination of the rectum was identified and digitally imaged in an effort to consistently observe similar regions across all colon samples and experiments. The length and area of the AB/PAS-stained regions of the epithelium were assessed utilizing ImageJ software (NIH), and data are expressed as the mean volume density (14).

Colon organ culture and cytokine assessments. To evaluate cytokine levels in the colon, we utilized an organ culture system as previously described (4, 5, 22). The bisected colon was cut into 1-cm² sections, which were washed with 1× PBS containing penicillin/streptomycin, and the weight of each section was recorded. The isolated colon sections were incubated overnight in RPMI media that contained penicillin/streptomycin but no additional supplements. Cell-free supernatants were collected, and cytokine levels were evaluated by ELISA.

Generation of chimeric mice by bone marrow reconstitution. Bone marrow transplantation was utilized to generate chimeric mice following standard protocols (26). Recipient mice were lethally irradiated with 1,100 rad (equivalent) utilizing two equal doses of X-ray irradiations 6 h apart (Rad Source Technologies), and 12 h later the mice received 5 × 10⁵ bone marrow cells from the femurs of WT and Casp11/−/− mice. The bone marrow was collected and prepared following standard protocols (26). The reciprocal bone marrow reconstitution was preformed, which resulted in the generation of the following four groups of experimental mice: WT → WT, Casp11/−/− → Casp11/−/−, Casp11/−/− → WT, and WT → Casp11/−/−. The surviving mice were subjected to the acute experimental colitis model, 6 wk after transplantation.

Human metadata analysis. We evaluated CASP4, CASP5, and Casp11 expression using a publically accessible microarray meta-analysis search engine (http://www.nextbio.com/b/search/ba nb), as described (33). The following array data sets were analyzed: GSE37283, GSE6731, GSE10714, GSE13367, GSE9452, GSE16879, GSE36807, GSE38713, GSE11223, GSE10191, GSE9686, and GSE37283.

Statistical analysis. We utilized GraphPad Prism 5 statistical software to conduct ANOVA followed by either Tukey-Kramer honestly significant different test or Newman-Keuls posttest to evaluate statistical significance for multiple comparisons. Single data point comparisons were evaluated by the Student’s two-tailed t-test. Group survival was assessed utilizing the Kaplan-Meier test. All data are presented as the means ± SE, and in all cases a P value of <0.05 was considered statistically significant.

RESULTS

Caspase-11 attenuates experimental colitis. The role of the canonical inflammasome and caspase-1 in IBD has been well characterized. However, significantly less is known regarding caspase-11 in either human or mouse disease pathogenesis. Murine caspase-11 is the paralog of caspase-4 and caspase-5 in humans. An evaluation of publically available gene expression metadata compiled from 11 independent studies revealed that CASP4 and CASP5 are significantly upregulated in colon biopsies from patients with UC with active disease compared with those currently in remission (Fig. 1A). Further evaluation of these data revealed that CASP4 expression is significantly upregulated in the colonic mucosa of patients with Crohn’s disease and UC that do not respond well to the common therapeutic infliximab (Remicade) (Fig. 1B). CASP5 expression was also found to be upregulated in this study, but the difference did not reach significance (P = 0.147). In addition to IBD, we also evaluated gene expression metadata in colon biopsies from patients with colitis-associated cancer and found...
that both CASP4 and CASP5 were significantly upregulated in biopsies from patients with colitis-associated neoplasia (Fig. 1C). These findings suggest that CASP4 and CASP5 are induced directly, or as a feedback response, by intestinal inflammation and tumorigenesis and underscore the importance of additional studies to better elucidate their functions during IBD in human patients.

To better characterize the role of Caspase-11 in UC, we utilized Casp11−/− mice in a common model of DSS-mediated acute experimental colitis. This model is commonly employed in IBD research and studies evaluating the contribution of acute experimental colitis. This model is commonly employed for 4 days, and morbidity and mortality were evaluated. The clinical score is a composite of scores associated with weight loss, stool consistency, and bleeding. *P < 0.001; #P < 0.02. For all data shown, the fold changes in expression values for each gene transcript were averaged between all studies, and groups were evaluated.

![Fig. 1. Expression of human CASP4 and CASP5. A: CASP4 and CASP5 expression in colonic biopsies from patients with ulcerative colitis (UC). Data are presented as the fold change values of affected tissue from patients with UC compared with healthy controls. *P = 0.001; B: CASP4 and CASP5 expression in colonic biopsies from patients with UC before treatment with infliximab compared with healthy controls (GSE16879). *P = 0.0248. C: CASP4 and CASP5 expression in colon biopsies from patients with UC and neoplasia compared with healthy controls (GSE37283). *P < 0.001; #P < 0.02. For all data shown, the fold changes in expression values for each gene transcript were averaged between all studies, and groups were evaluated.](http://ajpgi.physiology.org/)

![Fig. 2. Casp11−/− mice exhibit increased susceptibility to experimental colitis. Wild-type (WT) and Casp11−/− mice received 4% dextran sulfate sodium (DSS) for 4 days, and morbidity and mortality were evaluated. A: Kaplan-Meier plot of WT and Casp11−/− mouse survival. Mice were considered moribund when weight loss reached −19% from baseline. WT DSS/Casp11−/− DSS, *P = 0.0002. B: Casp11−/− mice demonstrate significant weight loss following DSS exposure compared with WT animals. *P = 0.0018; #P < 0.0001; +P = 0.0001. C: Casp11−/− mice demonstrate increased clinical parameters associated with disease progression compared with WT mice. The clinical score is a composite of scores associated with weight loss, stool consistency, and bleeding. *P = 0.0316; #P = 0.0400; +P < 0.0001. Data shown are representative of 4 independent experiments and depict the means ± SE. WT mock, n = 5; WT DSS, n = 5; Casp11−/− mock, n = 5; Casp11−/− DSS, n = 5.](http://ajpgi.physiology.org/)
Further characterization of the individual parameters that comprise the HAI score revealed a significant increase in the parameters associated with distal- and mid-colon inflammation and defects in the epithelial barrier (Fig. 3, C–J). These data are consistent with another recent publication that evaluated Casp11−/−Casp11−/− and Casp11−/− mice in a similar model of experimental colitis (16). In the work recently published by Demon et al. (16), Casp11−/− animals were also found to be hypersensitive to DSS compared with WT animals (16). Together, these studies strongly support a protective role for caspase-11 in the colon during gastrointestinal inflammation.

Casp11−/− hypersensitivity is associated with reduced levels of IL-1β and IL-18. Mice lacking functional canonical caspase-1 inflammasomes have ablated IL-1β and IL-18 levels in the colon during experimental colitis and colitis-associated tumorigenesis (4, 17, 18, 56). Both IL-1β and IL-18 are important regulators of immune system homeostasis, epithelial cell regeneration and repair, and stromal cell stimulation in the gut (10, 18, 40, 44). Thus we sought to evaluate these cytokines in the Casp11−/− colon following DSS exposure. Colon organ cultures were established from DSS and mock-treated animals to evaluate local cytokine levels. Following overnight incuba-

Fig. 3. Caspase-11 attenuates gastrointestinal inflammation during experimental colitis. A: increased inflammation and epithelial cell damage was observed in hematoxylin and eosin (H and E)-stained colon sections from DSS-treated Casp11−/− mice evaluated on day 9. Scale bar = 250 μm. B: significant increases in colon histopathology were observed in DSS-treated Casp11−/− animals. The severity of inflammation, epithelial defects, crypt atrophy, and area affected in H and E-stained sections through the mid and distal colon were scored on a scale of 0–4 and summed to generate a Histologic Activity Index (HAI). *P < 0.004; #P < 0.0001; +P = 0.017. C–J: individual parameters assessed in the HAI score revealed significant disease-associated histopathology in both the distal and mid colon from Casp11−/− mice. C–F: DSS induced a significant increase in distal colon inflammation (*P < 0.0446; #P = 0.0011; +P = 0.042), epithelial defects (*P = 0.0312; #P < 0.0001; +P = 0.050), crypt atrophy (*P < 0.0417; #P < 0.0001; +P = 0.0265), and area affected (*P < 0.0265; #P < 0.0001; +P = 0.0265) in Casp11−/− mice. G–J: DSS exposure induces mild histopathology in the mid colon of WT animals. However, significant increases in inflammation (*P < 0.0256; #P < 0.0001), epithelial defects (*P < 0.0159; #P < 0.0001), crypt atrophy (*P < 0.0060; #P < 0.0001), and area affected (*P < 0.0012; #P < 0.0001) were observed in the Casp11−/− mid colon. Data shown are representative of 4 independent experiments and depict the means ± SE. WT mock, n = 5; WT DSS, n = 5; Casp11−/− mock, n = 5; Casp11−/− DSS, n = 5.
tion, the organ culture supernatants were harvested, and IL-1β, IL-18, and IL-6 levels were determined by cytokine-specific ELISA. Following DSS administration, all three cytokines were significantly increased in colon sections harvested from WT mice (Fig. 4, A–C). However, the IL-1β levels were significantly attenuated, and IL-18 levels were significantly ablated in the Casp11−/− mice (Fig. 4, A and B). IL-6 levels were not significantly altered in the Casp11−/− colons (Fig. 4C). In addition to the evaluation of cytokines by ELISA, we also assessed Casp1 gene expression in colons freshly harvested during necropsy (Fig. 4D). Casp1 gene expression in WT mice was not influenced by DSS exposure. However, in the absence of caspase-11, we observed a significant increase in Casp1 gene expression (Fig. 4D). These data may partially explain the attenuated, rather than ablated, levels of IL-1β observed in the Casp11−/− mice and may reflect a compensatory change in caspase gene expression in these animals.

IL-1β and IL-18 reconstitution attenuates experimental colitis pathogenesis in Casp11−/− mice. Despite extensive studies, the role of IL-1β and IL-18 in the modulation of IBD and experimental colitis remains unclear and in many cases paradoxical. In the context of the inflammasome, studies have shown that the increased hypersensitivity to DSS in caspase-1 inflammasome-deficient animals is associated with decreased levels of IL-18 in the colon (10, 18). To directly evaluate this potential mechanism in the Casp11−/− mice, animals were treated daily with either recombinant murine IL-18- or IL-18-binding protein during the DSS administration. WT mice that received IL-18bp (αIL-1β) demonstrated a significant increase in weight loss, clinical score, and histopathological features associated with disease pathogenesis compared with mice that received IL-18 or saline (Fig. 5, A–C). Conversely, Casp11−/− mice that received IL-18 injections during DSS exposure demonstrated a significant attenuation in disease pathogenesis. Although IL-18 reconstitution did not fully rescue the Casp11−/− mice, we did observe a significant 23% improvement in the weight loss between the mice that received IL-18 compared with the Casp11−/− mice injected with saline or IL-18bp (Fig. 5D). Likewise, the IL-18-treated animals demonstrated significant improvements in clinical and histopathological features associated with disease progression (Fig. 5, E and F). Together, these data indicate that the Casp11−/− hypersensitivity to DSS is at least partially associated with IL-18 attenuation.

In addition to IL-18, we also evaluated IL-1β utilizing recombinant mouse IL-1β and the human IL-1 receptor antagonist anakinra (Kinera). WT mice treated with anakinra (αIL-1β) during the DSS exposure demonstrated a significant increase in weight loss compared with the saline-treated WT mice (Fig. 5G). However, we also observed a significant, albeit more moderate, increase in weight loss in animals treated with recombinant IL-1β (Fig. 5G). In addition to weight loss, WT animals treated with anakinra also demonstrated significantly increased clinical and histopathological features associated with experimental colitis (Figs. 5, H and I). Together, these data are consistent with prior studies that have found both protective and exacerbating effects of altered IL-1β levels in the colon (2, 7, 11, 12, 36, 40, 43, 45, 48). In the Casp11−/− mice, IL-1β reconstitution significantly attenuated weight loss, clinical parameters associated with disease, and histopathology compared with the saline- and anakinra-treated animals (Fig. 5, J–L). Together, our data suggest that loss of both IL-1β and IL-18 contribute to the increased DSS hypersensitivity observed in the Casp11−/− mice in the experimental colitis model.

The pathogenesis in Casp11−/− mice is associated with both hematopoietic and nonhematopoietic sources. Both epithelial cells and macrophages can significantly influence gastrointestinal inflammation, and specific NLR inflammasomes have been associated with modulating experimental colitis through both of these cellular compartments (4, 18, 56). However, there appears to be compartment specificity for each individual inflammasome forming NLR, such as NLRP3 that functions through the hematopoietic compartment and NLRP6 that functions through epithelial cells (4, 18). Casp11 is expressed
Fig. 5. IL-1β and IL-18 reconstitution attenuates experimental colitis pathogenesis in Casp11−/− mice. A–C: WT mice demonstrated significantly increased weight loss, clinical features associated with disease progression, and HAI scores following daily injections of recombinant mouse IL-18-binding protein (DSS + αIL-18) during DSS administration. No significant differences were observed between WT mice receiving vehicle injections (DSS) and mice receiving recombinant mouse IL-18 (DSS + IL-18). A: *P = 0.0338; #P = 0.0499. B: *P = 0.0269; #P = 0.0024. C: *P = 0.0004; #P < 0.0001. D–F: Casp11−/− mice demonstrated significantly attenuated weight loss, clinical scores, and HAI following daily injections with recombinant IL-18 (DSS + IL-18) during DSS exposure compared with vehicle-treated animals (DSS). IL-18bp administration (αIL-18) did not affect weight loss in the Casp11−/− animals. D: *P = 0.0479; #P = 0.0365. E: *P = 0.0233; #P = 0.0031. F: *P = 0.0233. G–I: WT mice receiving daily injections of the IL-1 receptor antagonist anakinra (DSS + αIL-1β) or recombinant mouse IL-1β (DSS) significantly increased weight loss, clinical scores, and histopathology compared with vehicle-treated animals (DSS) (G) *P = 0.0238; #P = 0.0456. H: *P = 0.0087. I: *P = 0.0005; #P = 0.0484. J–L: IL-1β administration during DSS exposure (DSS + IL-1β) significantly attenuated weight loss, clinical score, and colon histopathology in Casp11−/− mice compared with anakinra-treated mice (DSS + αIL-1β) and vehicle-treated animals (DSS). J: *P = 0.0396; #P = 0.0042. K: *P = 0.050; #P = 0.0157. L: *P = 0.0005; #P = 0.0022. Data shown are representative of at least 2 independent experiments and depict the means ± SE. For all studies, n = 7 for all mouse groups shown.

Throughout the colon, including high levels of expression in epithelial cells and macrophages (Fig. 6A). Thus, to evaluate caspase-11 function in these cellular compartments, we generated WT and Casp11−/− chimeric mice using adoptive bone marrow transplantation (Fig. 6B). The mice were lethally irradiated and received reciprocal bone marrow (4, 5). Near-complete chimerism was achieved after a 6-wk reconstitution phase. The bone marrow chimera procedure enhanced the severity of colitis in all of the Casp11−/− mice (donors and recipients), requiring animals to be harvested on day 7, rather...
than day 9 (Fig. 6C). Following DSS exposure, the Casp11−/− → Casp11−/− mice recapitulated the general phenotype observed for the nonchimeric Casp11−/− mice and were significantly more hypersensitive in the experimental colitis model compared with the WT → WT animals (Fig. 6C). Likewise, both sets of chimeric mice were significantly more hypersensitive compared with the WT → WT animals (Fig. 6C). The Casp11−/− → WT and WT → Casp11−/− animals demonstrated weight loss and colon length reductions that were equivalent to the Casp11−/− → Casp11−/− mice and significantly altered compared with the WT → WT animals (Fig. 6, C and D). Histopathological evaluation of colons isolated from the chimeric mice revealed that all of the Casp11−/− animals (both donor and recipient) had significantly increased HAI scores compared with the WT → WT mice (Fig. 6E). Interestingly, semiquantitative scoring of the individual parameters
that compose the HAI score revealed that the WT → Casp11−/− mice demonstrated greater epithelial cell defects characterized by enhanced epithelial tattering, large areas of erosion, and extensive areas of ulceration, whereas the Casp11−/− → WT mice demonstrated enhanced inflammatory cell infiltration and increased crypt atrophy (Fig. 6, F–K). Representative histopathology images illustrate the enhanced inflammation commonly observed in the Casp11−/− → WT mice and the enhanced epithelial damage and atrophy in the WT → Casp11−/− and Casp11−/− → Casp11−/− mice (Fig. 6, L–O). Together, these data suggest that caspase-11 functions through both hematopoietic and nonhematopoietic compartments. These data are consistent with findings previously reported for various NLRs, caspase-1, and ASC, which indicate that canonical inflammasomes significantly modulate experimental colitis through both monocytes and epithelial cells (4, 17, 18, 39, 56).

Caspase-11 does not attenuate disease pathogenesis during chronic relapsing/remitting experimental colitis and colitis-associated tumorigenesis. In addition to their protective roles in experimental colitis, canonical inflammasomes have also been shown to significantly protect the host from tumorigenesis in models of colitis-associated cancer (4, 17, 27, 28, 56). Thus we hypothesized that the increased inflammation and ablated levels of IL-18 in the Casp11−/− mice would promote tumorigenesis over the course of the relapsing remitting experimental colitis model. To evaluate inflammation-driven tumorigenesis, we utilized the well-characterized AOM/DSS model (38). The AOM/DSS-treated WT and Casp11−/− mice showed a significant increase in morbidity and weight loss compared with the mock-treated animals (Fig. 7A). However, unlike the findings from the acute model, no significant differences were observed in morbidity or mortality in the Casp11−/− mice compared with similarly treated WT animals (Fig. 7A). Upon necropsy, the number of macroscopic polyps in each colon was evaluated. Polyps were observed in the majority of WT and Casp11−/− animals treated with AOM/DSS (Fig. 7B). However, no significant differences were observed in either the number or size of polyps between the Casp11−/− and WT mice (Fig. 7B). Histopathology evaluation revealed a significant increase in colon inflammation in the animals exposed to DSS and AOM/DSS (Fig. 7C). Likewise, we also observed increased epithelial barrier defects, hyperplasia, and dysplasia in the AOM/DSS-treated animals (Fig. 7C). However, we did not observe any substantial pathological differences between the Casp11−/− and WT mice. Consistent with these observations, additional semiquantitative HAI assessments revealed a significant increase in disease pathogenesis in all of the AOM/DSS-treated animals compared with the mock-treated mice and animals treated with only AOM (Fig. 7D). Likewise, DSS alone significantly increased colon inflammation in both WT and Casp11−/− mice (Fig. 7D). However, in all cases, no significant differences were observed between the Casp11−/− and WT animals (Fig. 7D). Together, these data reveal that caspase-11 plays a robust role in attenuating acute experimental colitis but has a diminished role in the chronic relapsing remitting colitis model and in the context of inflammation-driven colon tumorigenesis.

Recent studies evaluating the mechanism associated with canonical inflammasome attenuation of colitis and colitis-associated tumorigenesis have shown a strong correlation with defective goblet cell autophagy and mucin granule exocytosis (28, 55). For example, recent studies revealed that mice lacking a functional NLRP6 inflammasome demonstrated a significant expansion of the bacterial phyla Bacteroidetes (Prevotellaceae), which directly correlated with IBD severity (28). Additional mechanistic studies indicated that Nlrc6−/− and Asc−/− mice have defective goblet cell autophagy and subsequently reduced mucin granule exocytosis, which allows Prevotellaceae to colonize and establish persistent infections deep in the crypts of these animals (53). This persistent infection in the caspase-1 inflammasome-deficient mice in turn contributes to the enhanced inflammation observed in models of colitis-associated tumorigenesis (28). To evaluate this mechanism in the Casp11−/− mice, we evaluated mucus production in the colon using AB/PAS staining (Fig. 7E). AB/PAS staining was further evaluated using a semiquantitative scoring system that has been previously employed for similar assessments of mucus in the lungs (3). AOM/DSS-treated mice exhibited a significant increase in goblet cell hyperplasia and mucus production compared with mock-treated animals (Fig. 7F). However, no significant differences were observed between Casp11−/− and WT mice (Fig. 7F). Therefore, caspase-11 does not appear to influence mucus production in the colon during inflammation-driven tumorigenesis.

In the acute experimental colitis model, our data indicate that the hypersensitivity associated with caspase-11 deficiency is correlated with IL-1β and IL-18 ablation. To evaluate this mechanism in the Casp11−/− mice, we evaluated IL-18 levels in the organ culture supernatants upon completion of the relapsing/remitting colitis model and the AOM/DSS inflam-
Fig. 7. Caspase-11 deficiency did not influence the progression of colitis-associated tumorigenesis. A: weight loss was evaluated throughout the azoxymethane (AOM)/DSS model. *P < 0.0001; #P < 0.0001. B: macroscopic polyps were identified in the distal colons harvested from Casp11−/− and WT mice. The number and maximal cross-sectional area of macroscopic polyps was quantified. ND, not detected. C: representative histopathology emphasizing increased inflammation in the DSS- and AOM/DSS-treated WT and Casp11−/− animals. Scale bar = 100 μm. D: composite HAI score was generated for each set of colons harvested following the completion of the colitis-associated cancer model. Histopathology analysis revealed increased colon inflammation, area associated with disease, hyperplasia, and mild dysplasia in both WT and Casp11−/− mice treated with both AOM and DSS. *P = 0.0093; #P = 0.0021; †P = 0.0021; ‡P = 0.0380. E: Alcian-blue/Periodic Acid-Schiff reaction (AB/PAS) staining was used to quantify mucus production. Data shown are representative images of AB/PAS cells. Scale bar = 100 μm. F: mucus levels were quantified utilizing AB/PAS imaging analysis along a 2-mm section of colon, located ~1 cm proximal from the termination of the rectum (represented as Vs). *P = 0.0053; #P = 0.0228. G: organ cultures were generated to determine the levels of IL-18 in the colon. *P = 0.0032; #P = 0.0203; †P = 0.00432; ‡P = 0.0321; ††P = 0.0001. Data shown are representative of 3 independent experiments and depict the means ± SE. For all experimental groups, n = 7.

G147
DISCUSSION

Canonical NLR inflammasome activation is an essential modulator of immune system homeostasis and epithelial barrier function in the gut. Indeed, several recent studies have demonstrated that dysregulated inflammasome signaling and caspase-1 activation are associated with IBD and cancer pathogenesis (4, 17, 18, 56). Although caspase-1 and canonical inflammasome signaling during enteric pathogen infection, gastrointestinal inflammation, and tumorigenesis has been well studied, the contribution of caspase-11 and the noncanonical inflammasome during disease pathogenesis has only recently been explored. The majority of prior studies characterizing caspase-11 and the human paralogs caspase-4 and caspase-5 have focused on acute enteric bacteria exposure, sepsis, and endotoxic shock models (24, 31, 32, 34, 51). These prior studies have revealed that these caspases directly bind cytosolic LPS and lipid A from Gram-negative bacteria with a high level of affinity and specificity (46). This binding is associated with the respective CARD domains and facilitates caspase oligomerization and subsequent activation (46). Here, we expand upon the prior studies associated with canonical inflammasome signaling and the human studies associated with caspase-4 and caspase-5 by demonstrating that caspase-11 plays a protective role during acute experimental colitis in mice.

Caspase-11 modulation of experimental colitis pathogenesis has been previously evaluated in the Casp11−/− mice (16). Consistent with our findings, the previous study reported significantly increased morbidity, colonic tissue damage, and leukocyte infiltration following DSS exposure in the Casp11−/− animals (16). However, the proposed mechanisms significantly differ. The Demon et al. study (16) suggests that DSS sensitivity in the Casp11−/− mice is independent of caspase-1 and the canonical inflammasome attributable to increased levels of IL-1β and IL-18 in the colon and increased release of the pyroptosis marker high-mobility group box 1 in the Casp11−/− mice. Thus the previous study indicates that inflammasome activation following DSS relies on caspase-1 but not caspase-11 and further suggests another unidentified role for caspase-11 in modulating disease pathogenesis (16). This is in contrast to our data, which show attenuated levels of IL-1β and IL-18 in the Casp11−/− mice and suggest that caspase-11 contributes to the function of the canonical inflammasome in the experimental colitis model. It should be noted that there are a few technical differences in the experimental design between the two studies that could underlie these mechanistic differences. For example, in the present study, we utilized an organ culture method to evaluate secreted cytokine levels in intact colon tissue. We have found that this technique is highly effective in quantifying IL-1β and IL-18 in the colon by ELISA (4, 5). However, in the Demon et al. study (16), colons were excised and mechanically homogenized (16).

In our experience, this technique is ideal for evaluating procytokine and cleaved cytokine levels by Western blot but can often result in an overestimation of the IL-1β and IL-18 levels in the sample attributable to measuring both procytokines and cleaved forms of the cytokines. In addition to experimental design differences, prior studies have also shown that environmental variability can also dramatically influence DSS-induced experimental colitis pathogenesis attributable in part to differences in microflora, diet, and other vivarium-specific considerations (52). Although this issue was partially addressed by Demon et al. (16) through the use of mice at two independent institutions, this may reflect that both vivariums have similar skewes in their microflora. Thus it is possible that microbiome differences between the animals used in the prior studies and here could significantly influence the activation of caspase-11 and interaction between the canonical and noncanonical inflammasomes. Despite the mechanistic differences noted between the two studies, the overall finding that caspase-11 and the noncanonical inflammasome attenuate experimental colitis pathogenesis is highly consistent. Future studies of this novel and unique signaling pathway will certainly yield further mechanistic insight that will help to clarify the current discrepancies.

Our data showing reduced IL-1β and IL-18 levels in the Casp11−/− mice and the findings from the cytokine reconstitution studies suggest that canonical inflammasome function during acute inflammation and colitis is dependent on caspase-11. These data are highly consistent with results previously reported for Il-1β−/−, Il-18−/−, Casp11−/−/Casp11−/−, Asc−/−, and various NLR-deficient mice that show significantly enhanced experimental colitis pathogenesis associated with IL-1β and IL-18 ablation (4, 8, 17, 18, 44, 56). It should be noted that both increased and attenuated levels of IL-1β and IL-18 have been shown to significantly influence IBD pathogenesis (6, 18, 44, 47). Thus it appears that these highly related cytokines have both positive and negative regulatory effects on IBD. In the context of canonical inflammasome modulation of experimental colitis, recent studies have shown that both IL-1β and IL-18 function during epithelial cell repair and barrier reconstitution during the resolution phase of colitis (8). Thus these critical processes have been found to be defective in the absence of proper inflammasomes and are associated with enhanced experimental colitis pathogenesis.

Caspase-11 is broadly expressed in both hematopoietic- and nonhematopoietic-derived cells, including macrophages and epithelial cells (Fig. 6) (16). Consistent with this expression pattern, the data generated from our adoptive transfer studies suggest that caspase-11 modulates disease pathogenesis through both compartments. This is supported by the data that show equivalent levels of disease progression between the Casp11−/− → Casp11−/−, WT → Casp11−/−, and Casp11−/− → WT groups (Fig. 6). These findings are consistent with multiple prior studies using various NLR-deficient, Asc−/−, and Casp1−/−/Il1−/− mice, which have indicated that canonical inflammasomes function in both hematopoietic-derived cells and epithelial cells to attenuate disease severity (reviewed in Ref. 15). It is currently unclear why loss of canonical or noncanonical inflammasome functions in either the hematopoietic or nonhematopoietic compartments results in an exaggerated response in the experimental colitis model. However, one possible explanation is that inflammasomes exert cell-type and temporal-specific nonredundant effects on macrophages and epithelial cells, which have similar physiological outcomes associated with disease pathogenesis. This is supported by prior NLRP6 and NLRP3 studies. Both NLRP6 and NLRP3 are inflammasome-forming NLRs that have been shown to ameliorate experimental colitis progression (4, 17, 54, 56). Prior studies evaluating NLRP6 revealed that IL-18 secretion by intestinal epithelial cells increases the expression of CCL5
and induces proliferation of these cells through the local activation of IL-6 signaling (18, 28). In these studies, epithelial cell production of IL-18 was correlated with effective barrier regeneration and repair and inhibited gut colonization by colitogenic microbiota (18, 28). Conversely, NLRP3 has been shown to ameliorate experimental colitis through macrophages and hematopoietic-derived cells (4, 25). In the absence of NLRP3, macrophages produce attenuated levels of IL-1β and IL-18, as well as reduced anti-inflammatory IL-10 and protective growth factor TGF-β (25). Thus, even with intact inflammasomes in the epithelial cells, loss of the NLRP3 inflammasome in macrophages dramatically enhanced experimental colitis progression (3).

Caspase-11 attenuates the progression of acute experimental colitis. Previous studies evaluating caspase-11 function strongly support a synergistic model where the noncanonical inflammasome can effectively modulate inflammatory responses and cytokine maturation following acute inflammation and Gram-negative bacteria exposure (30, 42, 51). Our data support this model in the context of gastrointestinal inflammation. However, as shown in the present study, caspase-11 does not seem to modulate canonical inflammasome function in the chronic relapsing/remitting DSS model or during inflammation-driven tumorsogenesis. In support of this conclusion, we show that Casp11−/− and WT mice have similar levels of mucus production and colon IL-18 levels. These two parameters are highly associated with disease severity in these models and are directly correlated to canonical caspase-1 inflammasome activity (4, 17, 18, 56). Together, these data identify caspase-11 as a critical factor protecting the host during acute DSS-induced colonic injury and inflammation but not during chronic inflammation and tumorsogenesis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


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