The AIM2 inflammasome is a central regulator of intestinal homeostasis through the IL-18/IL-22/STAT3 pathway

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Inflammasomes are important for maintaining intestinal homeostasis, and dysbiosis contributes to the pathology of inflammatory bowel disease (IBD) and increases the risk for colorectal cancer. Inflammasome defects contribute to chronic intestinal inflammation and increase the susceptibility to colitis in mice. However, the inflammasome sensor absent in melanoma 2 (AIM2) protects against colorectal cancer in an inflammasome-independent manner through DNA-dependent protein kinase and Akt pathways. Yet, the roles of the AIM2 inflammasome in IBD and the early phases of colorectal cancer remain ill-defined. Here we show that the AIM2 inflammasome has a protective role in the intestine. During steady state, Aim2 deletion results in the loss of IL-18 secretion, suppression of the IL-22 binding protein (IL-22BP) in intestinal epithelial cells and consequent loss of the STAT3-dependent antimicrobial peptides (AMPs) Reg3β and Reg3γ, which promotes dysbiosis-linked colitis. During dextran sulfate sodium-induced colitis, a dysfunctional IL-18/IL-22BP pathway in Aim2−/− mice promotes excessive IL-22 production and elevated STAT3 activation. Aim2−/− mice further exhibit sustained STAT3 and Akt activation during the resolution of colitis fueled by enhanced Reg3b and Reg3g expression. This self-perpetuating mechanism promotes proliferation of intestinal crypt cells and likely contributes to the recently described increase in susceptibility of Aim2−/− mice to colorectal cancer. Collectively, our results demonstrate a central role for the AIM2 inflammasome in preventing dysbiosis and intestinal inflammation through regulation of the IL-18/IL-22BP/IL-22 and STAT3 pathway and expression of select AMPs.

Keywords: antimicrobial peptides; IL-18; IL-22; IL-22BP; inflammasome; inflammatory bowel disease; microbiome; Reg3β

INTRODUCTION

Inflammatory bowel disease (IBD) manifests most commonly as ulcerative colitis (UC) and Crohn’s disease (CD), both of which are major health issues that severely impact quality of life and predispose patients to the development of colorectal cancer. CD and UC are chronic remittent inflammatory disorders that share several clinical features, including abdominal pain, diarrhea, rectal bleeding, malnutrition and fever. UC typically begins in the rectum, extends throughout the colon, with inflammation restricted to the mucosal surfaces, and is characterized by the presence of crypt abscesses. However, CD occurs predominantly in the small intestine or the proximal colon, with transmural inflammation characterized by granulomas, fissures and fistulas. Another distinction is the atypical Th2 or Th1/Th17 cytokine profile characteristic of UC or CD patients, respectively. The pathology of IBD is still incompletely understood, but environmental and genetic risk factors contribute to IBD. The immune system has a key role in restricting and shaping intestinal microbial communities, and altered interactions between mucosal immune cells, intestinal epithelial cells (IECs) and the gut microbiome, due to a breached IEC barrier, contribute to the pathology of IBD.

Inflammasomes are multi-protein platforms linking recognition of danger-associated molecular patterns and pathogen-associated molecular patterns to the activation of inflammatory caspase-1. Inflammasome sensors are pattern recognition
receptors belonging to the Nod-like receptor (NLR) or AIM2-like receptor (ALR) families.\(^5\)\(^,\)\(^6\) NLRs and ALRs contain either a PYRIN domain (PYD) or a caspase recruitment domain and cause the polymerization of the adaptor ASC and subsequent activation of caspase-1 by induced proximity. Active caspase-1 is then responsible for the maturation and release of the cytokine interleukin (IL)-1β, IL-18 and a number of leaderless proteins as well as for the induction of pyroptotic cell death.

Several inflammasomes shape the mucosal immune response in a cell type-specific manner within the gastrointestinal tract. In particular, NLRP3 and NLRC4 sense a variety of enteric pathogens and pathobionts and initiate immune responses against these pathogens.\(^7\)\(^,\)\(^8\) In addition, several ASC-containing inflammasomes are involved in the maintenance of intestinal homeostasis and the prevention of dysbiosis; consequently, they are also involved in the pathology of IBD and colitis-linked colorectal cancer.\(^3\)\(^,\)\(^4\)\(^,\)\(^10\) Initial studies suggested that inflammasome activation and the production of downstream effectors is detrimental for colitis. The induction of colitis in mice caused elevated IL-18 in hematopoietic and epithelial tissues, and IL-18 transgenic mice displayed exacerbated disease, while neutralizing IL-18 ameliorated dextran sulfate sodium (DSS)-induced colitis.\(^11\)\(^,\)\(^12\)\(^,\)\(^13\) However, other studies have shown that Il18\(^-/-\) and Il18r\(^-/-\) mice develop more severe colitis.\(^14\)\(^,\)\(^15\) Similarly, Casp1/11\(^-/-\) mice, or mice treated with caspase-1 inhibitor, show reduced IL-1β, IL-18 and IFN-γ and reduced colitis severity,\(^16\)\(^,\)\(^17\) while other studies revealed the opposite result caused by impaired tissue repair.\(^18\) In addition, deletion of the inflammasome sensor Nlrp3 or pharmacological inhibition of caspase-1 resulted in ameliorated dextran sulfate sodium (DSS)-induced colitis.\(^19\)

In contrast, several recent studies have provided more detailed insights into inflammasome signaling in colitis, revealing that it is detrimental in the hematopoietic compartment, while it is protective in IECs, where IL-18 has a role in local tissue regeneration in response to injury. Nlrp3\(^-/-\), Asc\(^-/-\) and Casp1/11\(^-/-\) mice are all hyper-susceptible to DSS-induced colitis and are required for epithelial cell integrity.\(^20\)\(^-\)\(^22\) Loss of NLRP3 inflammasome activity and the consequent loss of IL-1β and IL-18 results in dissemination of commensal bacteria into the lamina propria, leukocyte infiltration and elevated chemokine production in the colon as well as increased incidence of colorectal cancer.\(^20\)\(^-\)\(^23\) Nlrp3\(^-/-\) mice also develop dysbiosis and display reduced production of β-defensins, IL-10 and TGF-β.\(^22\)

In addition to NLRP3, the NLRP6 inflammasome in the hematopoietic and non-hematopoietic compartments also participates in colonic homeostasis. Nlrp6\(^-/-\) mice show transferrable dysbiosis and increased susceptibility to DSS and colorectal cancer, suggesting that intestinal homeostasis requires the activation of multiple inflammasomes.\(^15\)\(^,\)\(^24\)\(^,\)\(^25\) Furthermore, NLRP12 may assemble an inflammasome as well as regulate NF-κB, and Nlrp12\(^-/-\) mice are more susceptible to colitis and colorectal cancer.\(^26\)\(^,\)\(^27\) Furthermore, Nlrc4\(^-/-\) mice develop an increased colorectal tumor burden in a DSS model, although they do not show altered colitis.\(^28\) However, another study showed no effect on tumorigenesis.\(^21\)

Recently, loss of Aim2 has been implicated in the development of DSS-induced colorectal cancer through an inflammasome-independent mechanism, with no apparent effect on DSS-induced colitis.\(^29\)\(^,\)\(^30\) Non-hematopoietic AIM2 interacts with and inhibits the DNA-dependent protein kinase (DNA-PK), a PI3K-related kinase, which promotes Akt phosphorylation.\(^30\)

In addition, AIM2 expression in intestinal stem cells prevents Wnt signaling, uncontrolled proliferation and dysbiosis.\(^29\)

Here we show that Aim2\(^-/-\) mice are more susceptible to DSS-induced colitis. This phenotype is mediated by dysbiosis due to impaired IL-18 production by IECs, colitis-driven imbalanced ratios of IL-22/IL-22BP, excessive STAT3 signaling and dysregulation of the IL-18- and IL-22-dependent anti-microbial and pro-proliferative peptides of the Reg3 family.

**MATERIALS AND METHODS**

**Mice**

Acute colitis was induced with 2–3% (w/v) DSS (molecular mass 36–40 kDa; MP Biologicals, Santa Ana, CA, USA) dissolved in sterile, distilled water provided ad libitum on experimental days 1–5, followed by normal drinking water until the end of the experiment on days 5, 7 or 14, as previously described.\(^37\) DSS was freshly dissolved every 2 days.

** Antibiotic treatment and fecal microbiota transplant **

For the antibiotics-only treatment, mice were equally treated with a combination of antibiotics for 14 days as previously described.\(^15\) Briefly, mice were given a combination of vancomycin (1 g/l), ampicillin (1 g/l), kanamycin (1 g/l) and metronidazole (1 g/l) (all from Sigma-Aldrich) for 3 weeks in their drinking water before the administration of DSS as described above. For fecal microbiota transplant (FMT), mice were treated with antibiotics as described above and then received fecal content from either WT or Aim2\(^-/-\) mice. Briefly, donor animals were weight-, gender- and age-matched single-housed WT or Aim2\(^-/-\) mice. Mice were killed, and the fecal content was immediately diluted in sterile phosphate-buffered saline, filtered through a 500-μm mesh and administered equally to each group of mice. Recipient mice were given 48 h to recover before DSS administration as described above.
**Determination of clinical colitis scores**

Weight loss, stool consistency and any presence of occult or macroscopic blood were determined daily until mice were killed at days 0, 5, 7 or 14. Stool consistency and rectal bleeding were analyzed as previously described. Normal stools (0), soft but still formed stool (1), loose stool (2), mostly liquid stool (3), diarrhea; and 0, negative hemoccult; 2, positive hemoccult; 4, blood traces visible in stool/rectal bleeding.

**Colon tissue analysis**

Mice were killed, and entire colons were isolated, measured and opened along the mesenteric border. Mesenteric fat and fecal material were removed, and colons were aseptically flushed several times with Hank’s balanced salt solution (HBBS). The entire colon was then weighed, and samples were isolated in identical order for each mouse, starting from the distal portion of the colons.

**Histology.** One centimeter of colon was fixed in 10% formalin overnight, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) and Ki-67 at the Northwestern University Mouse Histology and Phenotyping Laboratory. The severity of colitis was scored histologically using two different parameters. An inflammation score: 0, no inflammation; 1, increased inflammatory cells noted above the muscularis mucosa only; 2, increased inflammatory cells involving the submucosa and above; 3, increased inflammatory cells involving the muscularis and/or serosa. The percentage of ulceration was determined by assessing the relative extent of ulceration along the muscularis mucosa (expressed as percentage ulcerated mucosa).

**Colon tissue explants.** Biological triplicates of 0.5-cm colon pieces per mouse were isolated; pre-incubated 30 min in HBBS supplemented with 100 μg/ml gentamicin; rinsed and cultured in complete RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 μg/ml gentamicin, 1% penicillin–streptomycin and 10 mM HEPES. Supernatants were collected after 17 h, cleared by centrifugation at 12,000 g for 10 min at 4 °C and stored at −80 °C before determination of cytokine concentrations by enzyme-linked immunosorbent assay (ELISA). For cytokine measurements of select samples by ELISA, whole colon tissue was lysed in RIPA buffer containing protease and phosphatase inhibitors and results were normalized to protein levels by BCA assay.

**MPO activity.** Myeloid peroxidase (MPO) was extracted from colon homogenates (10% w/v) with 0.5% hexadecyltrimethylammonium, and biological activity was measured according to the manufacturer’s instructions (Enzo Life Sciences, Farmingdale, NY, USA).

**Cytokine measurement**

IL-1β, TNF-α, IL-6, IFN-γ (all BD Biosciences, San Jose, CA, USA), IL-18 (R&D Systems, Minneapolis, MN, USA), IL-22 (Affymetrix, Santa Clara, CA, USA) and IL-22BP (MyBiosource, San Diego, USA) were determined by ELISA from clarified culture supernatants, colon tissue explants or colon lysates. ELISA results were colony-stimulated-adjusted.

**Immunoblot**

Cells or whole colon tissues were lysed in RIPA buffer containing protease and phosphatase inhibitors. Lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes and analyzed by immunoblot with the appropriate primary antibodies and horseradish peroxidase-conjugated secondary antibodies (whole donkey antibody to rabbit IgG (NA934V) and whole sheep antibody to mouse IgG (NXA931), both from GE Healthcare (Pittsburgh, PA, USA); ECL detection (ThermoFisher, Waltham, MA, USA) and digital image acquisition (Ultralum, Claremont, CA, USA). The following antibodies were used: rabbit monoclonal antibody to phospho-STAT3 (Tyr705; clone D3A7), rabbit monoclonal antibody to total-STAT3 (clone 79D7), rabbit monoclonal antibody to phospho-Akt (Ser473; clone D9E) and rabbit monoclonal antibody to total Akt (clone C67E7) (Cell Signaling Technology, Danvers, MA, USA).

**Isolation of fecal DNA**

Immediately after euthanasia, fecal samples were collected from colons, frozen in liquid nitrogen and stored at −80 °C until further processing. DNA in colonic fecal samples was extracted using the QiaAmp DNA Stool Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions and quantified on a Synergy HT Multimode plate reader using the Take3 Micro-Volume Plate attachment (BioTek, Winooski, VT, USA).

**Quantitative real-time PCR**

Total RNA was isolated and analyzed as described. Total RNA was isolated using TRIzol reagent (Invitrogen, Thermo Fisher), treated with DNase I, reverse transcribed with iScript (Bio-Rad, Hercules, CA, USA) and analyzed by either predesigned FAM-labeled TaqMan real-time gene expression primer/probes or the iTaq Universal SYBR Green real-time PCR assays (Bio-Rad) on an ABI 7300 Real Time PCR machine (Applied Biosystems, Carlsbad, CA, USA) and displayed as relative expression compared with Actb. The following SYBR Green primer pairs for cytokines, HDPs and microbial 16S rDNA genes were used: Defcr5: 5′-AGGCTGATCCTATCCACAAAAACAG-3′, 5′-TGAAGAGCACCCCTTCTTGCG-3′;4,40 Bd4: 5′-GATTCCTCTCCTGTTCGG-3′, 5′-AAGTACAGCACACCGGCCAC-3′;4,33 Reg3b: 5′-CAGGCTGGAGGACACCTCGT-3′, 5′-TCCGTTTGTCCATGATGCTCTTC-3′;32 Reg2: 5′-GCTCCTATTGCTATGCTTGT-3′, 5′-CATGAGGAGCAGAGAGGAGACG-3′;4,40 Ile: 5′-TCCAGTGTCTTCAACAGTAA-3′, 5′-CAAGAATGTTGAGGATGCTTG-3′;4,44 Il22: 5′-TCCGGAGGCTCAGTGCTAA-3′, 5′-AGAAGCTCTTCCAGGGTGAA-3′;4,44 Il22bp: 5′-TCACGGCAGCAAGCAAGAAC-3′, 5′-GTGTCTCCAGCACAACCTCTCA-3′;4,45 Gapdh: 5′-CTCACGGGAGGGTTG-3′, 5′-TATGGGGTTGCTGGATG-3′;4,46 Mouse Intestinal Bacteroides (MB): 5′-CCACACCCCGGTTGTAA-3′, 5′-CCATGCCTTGCACTTCTCA-3′;4,46 Clostridium leptum (cluster IV) 5′-CCTCCTTGCGAGGCCTG-3′;4,47

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TGGCGSAGTTA-3', 5'-GAATTAACCATGATCAGTCCGCTT-3';30,46 Clostridium cocoides (cluster XI) 5'-ACTCTCTACGGGAGGACG-3', 5'-GCTCTTAGTTCGATTACGTCAT-3';40,46 Lactobacillus: 5'-AGCATAGGGAGCTTCCTCA-3', 5'-CACCGCTACACATGGAGG-3';40,46 Bifidobacterium: 5'-CGGTTGAAATATGAAAG-3', 5'-CGGTAACTGAGCAATGAAA-3';48 Prevotella: 5'-CCTWCGATGATGGG-3', 5'-CAACCTAGCTTGGCT-3';48 TM7: 5'-GAGAGATGACGGCCAG-3', 5'-GACCTGACATATCCTCCTTCG-3';49 all bacteria: 5'-CGGGTGACC-3', 5'-GCGTGACC-3', 5'-TGAATACGTTCCCGG-3'.

For isolation of intestinal epithelial cells

For qPCR. Cleaned colons were cut into 1-cm pieces and cleared of debris by three additional washes in ice-cold HBSS. IECs were then isolated following treatment of colon pieces with 5 mM EDTA and 2 mM DTT in HBSS at 37°C for 30 min. IECs fractions were then treated with TRIzol (Invitrogen, ThermoFisher) and stored at −80°C until further processing. The purity of IEC fractions was assessed by flow cytometry (BD LSR II), and cells were >85% EpCAM+ (rat anti-mouse EpCAM antibody, clone G8.8, eBioscience, San Diego, CA, USA) (data not shown).

For tissue culture. Organoids were obtained as previously described.50 Briefly, after cleaning, whole colons were cut into pieces, washed and incubated with 1 mg/ml collagenase D and 100 μg/ml dispase II for 1 h, then organoids were purified by successive sedimentation steps in 2% (w/v) sorbitol-HBSS. Organoids were then pelleted; re-suspended and cultured in DMEM medium supplemented with 2.5% FBS, 1% sodium pyruvate, 0.25 U/ml insulin, 100 U/ml penicillin, 30 μg/ml streptomycin and 100 μg/ml gentamicin. IECs were treated with recombinant IL-18 (500-1000 ng/ml; R&D Systems), recombinant IL-22 (25-50 ng/ml; eBiosciences), recombinant Reg3β (2 μg/ml; R&D Systems), recombinant Reg3γ (1 μg/ml; R&D Systems) and the neutralizing rat monoclonal anti-IL-18 antibody (10 μg/ml, clone 92-10C; R&D Systems).

Immortalization of bone marrow-derived macrophages

AM12-C11 alveolar macrophages infected with the J2 virus (ATCC CRL-2456) were used as the source for J2 virus production as previously described.31 Briefly, AM12-C11 cells were grown in DMEM, supplemented with 4 mM l-glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 5 mM HEPES and 10% FBS. Cleared culture supernatants containing virus were collected from confluent cells after 3 days by centrifugation and 0.45 μm filtration. These were then used to infect BMDMs that had been differentiated for 7 days in L929-conditioned medium 1:1 (v/v) in 10-cm Petri dishes in the presence of 3 μg/ml Polybrene (Sigma, St Louis, MO, USA) at 32°C for 16 h, followed by culture in L929-conditioned medium. A homozygous immortalization of bone marrow-derived macrophages (iBMDM) population was obtained after 4-6 weeks.

DNA transfection of iBMDM and IECs

Lipofectamine 2000 transfection reagent (Invitrogen, ThermoFisher) was used for the transfection of iBMDM or IECs with fectal DNA or poly(dA:dT) as indicated, and IL-1β or IL-18 secretion was measured after 17 h by ELISA. Alternatively, cells were lysed in TRIzol (Invitrogen, ThermoFisher), and total RNA or protein was extracted following the manufacturer's instructions.

Statistical analysis

Graphs represent the mean ± s.e.m. A standard two-tailed unpaired t-test and unpaired, non-parametric Mann-Whitney U-test were used for statistical analysis of groups. Values of P<0.05 were considered significant and are listed in the figure legends (Prism 5, GraphPad, La Jolla, CA, USA). The investigators were blinded to the genotype of the mice/cells, but they were blinded to all sample and tissue analyses. Sample sizes were selected on the basis of preliminary results to ensure a power of 80% with 95% confidence between populations. All samples were analyzed in triplicate and were repeated at least three times, showing a representative result.

RESULTS

AIM2 regulates intestinal homeostasis

Several inflammasomes maintain intestinal homeostasis, and their loss causes intestinal dysbiosis.3,4,10,15,20,27 We therefore investigated if AIM2 expression also contributes to a balanced intestinal microbiota and whether deletion of Aim2 would alter the composition of gut bacterial communities. We determined the relative presence of several bacterial strains from fecal DNA extracted from the colons of SPF WT and Aim2−/− mice by quantitative PCR utilizing specific bacterial 16S rDNA primers.40,46–49 In Aim2−/− mice, we detected increased population of Prevotella, Bacteroides and mouse intestinal Bacteroides (MIB) within the phylum of Bacteroidetes compared with WT mice (Figure 1a). In particular, the increased abundance of Prevotella is reminiscent of Nlrp6−/− mice.13 However, contrary to Nlrp6−/− and Asc−/− mice, which display an increased number of members of the phylum TM7,13 Aim2−/− mice showed reduced TM7 (Figure 1b). Hence, the various inflammasomes appear to involve distinct mechanisms of regulating the composition of the intestinal microbiota. We did not detect significant differences in the abundance of key Gram-positive or Gram-negative groups, including Lactobacillus, Bacillus, Eubacteria and Clostridium from the phylum Firmicutes, Bifidobacterium from the phylum Actinobacteria or Escherichia from the phylum Proteobacteria (Figure 1c). Overall, we found that AIM2 contributed to the homeostasis of the intestinal microbiota, and its loss caused the dysbiosis of select intestinal microbiota instructions.
AIM2 protects from acute DSS-induced colitis and prevents intestinal damage

Compelling evidence supports the notion that alterations in the intestinal microbiota are linked to the development of IBD and exacerbate IBD pathology, which subsequently changes the intestinal milieu and promotes altered mucosal immunity in genetically susceptible individuals. Consequently, the intestinal microbiota of IBD patients differ from that of healthy people and show a 30–50% reduced commensal biodiversity.\(^5\) In addition, several inflammamasomes have been linked to the immune response during experimental colitis. We therefore investigated if \(\text{Aim2}^{-/-}\) mice were more susceptible to colitis. Oral administration of DSS in the drinking water causes apoptosis of IECs within basal crypts and affects the integrity of the mucosal barrier, which promotes the subsequent mucosal influx of commensal microbes, intestinal inflammation and eventually colitis.\(^3\) We orally administered the commonly used dose of 3% DSS in drinking water to age- and gender-matched WT and \(\text{Aim2}^{-/-}\) mice for 5 days and assessed the early stage of the disease starting at day 5, the peak of the disease starting at day 7 and the resolution phase of acute colitis through day 14 (Figure 2a). Starting at day 7, \(\text{Aim2}^{-/-}\) mice suffered more severe weight loss compared with WT mice. While WT mice lost up to 11.5% body weight, peaking at day 8, \(\text{Aim2}^{-/-}\) mice lost up to 21.9% body weight, and their recovery began a day later than WT mice (Figure 2b). However, the recovery curve of \(\text{Aim2}^{-/-}\) mice was steeper than that of WT mice, indicating that a loss of AIM2 may accelerate the resolution of inflammation and promote tissue repair. The increased susceptibility of \(\text{Aim2}^{-/-}\) mice is reminiscent of other inflammamasome component-deficient mice, including \(\text{Nlrp3}^{-/-}\) and \(\text{Asc}^{-/-}\), to 2% DSS-induced colitis (Figure 2c). \(\text{Aim2}^{-/-}\) mice also displayed severe diarrhea compared with WT mice, as indicated by worse stool consistency (Figure 2d) and rectal bleeding scores (Figure 2e). Colon-length reduction is a gross assessment of the severity of DSS-induced colitis.\(^5\) In agreement with the more severe colitis symptoms, \(\text{Aim2}^{-/-}\) mice also displayed a 9% shorter colon than WT mice at the peak of the disease on day 7 (Figures 2f and g); however, colon length (Figure 2g) and thickness (Figure 2h) were not significantly different upon resolution of colitis at day 14. We verified these clinical parameters by histological examination of paraffin-embedded colon sections. DSS administration resulted in notable histopathological changes in H&E-stained sections of colon tissue at the peak of the disease 7 days after DSS treatment, whereas the changes were less pronounced during recovery at day 14 (Figure 2i). DSS treatment caused extensive loss of crypts and more severe ulceration in the colons of \(\text{Aim2}^{-/-}\) mice compared with WT mice. \(\text{Aim2}^{-/-}\) mice also showed highly reduced goblet cells and increased severity of inflammation, as determined by the presence of infiltrating leukocytes, which often reached the transmural levels in \(\text{Aim2}^{-/-}\) mice during acute colitis. Semi-quantitative scoring of these histological parameters confirmed that the severity of colitis was significantly higher in \(\text{Aim2}^{-/-}\) mice compared with WT mice during acute colitis (Figure 2j). In addition, \(\text{Aim2}^{-/-}\) mice also displayed a greater extent of ulceration and necrotic lesions along the muscularis mucosa compared with WT mice during acute colitis, but not after colitis recovery, providing additional evidence for accelerated intestinal repair in \(\text{Aim2}^{-/-}\) mice (Figure 2k). Increased leukocytic infiltration in the colons of \(\text{Aim2}^{-/-}\) mice in response to DSS administration was further evident by higher MPO activity in the colons of \(\text{Aim2}^{-/-}\) mice at the acute phase (Figure 2l).
Overall, these results indicate that AIM2 is an important component that contributes to the protection from acute DSS-induced colitis and that AIM2 deficiency promotes an inflammatory intestinal environment. Imbalanced cytokine responses have a central role in the pathology of IBD. We therefore analyzed cytokines secreted from colon tissue explants ex vivo during the acute phase of disease at days 5 and 7 after DSS administration. In agreement with more severe colitis, we detected increased TNF-α, IL-1β and IL-22 levels in Aim2−/− mice (Figure 2m), and these results were verified by direct analysis of colon tissue lysates (data not shown). However, we observed reduced IL-6 in Aim2−/− mice during the peak of the disease at day 7, which is in agreement with a protective role for IL-6 in IECs during colitis (Figure 2m). In contrast, IL-18 was reduced at all time points (Figure 2m), in agreement with impaired inflammasome activation reminiscent of Asc−/− mice.

Figure 2  Aim2−/− mice are more susceptible to DSS-induced colitis. (a) Timeline of DSS administration and analysis of colitis. WT and Aim2−/− mice were fed 3% DSS in drinking water for 5 days, followed by regular drinking water until euthanasia. Because of the increased susceptibility to colitis, Nlrp3−/−, Asc−/− and WT mice were given 2% DSS. All samples were harvested at day 0 (controls), day 5 (onset of colitis), day 7 (peak of colitis) and day 14 (resolution of colitis) following DSS administration. (b, c) Body weight loss was monitored during the progression of colitis induced with (b) 3% and (c) 2% DSS (b: n=10, c: n=5). (d) Diarrhea score and (e) gross/occult rectal bleeding were evaluated daily (n=10). (f, g) Colon lengths were measured at necropsy (f) representative pictures, (g) length at day 0 (n=6–7), day 5 (n=8), day 7 (n=10) and day 14 (n=15–16) after DSS administration. (h) Colon thickness was measured at day 14 as a gross assessment of the resolution of colitis (n=15–16). (i) Colon tissues harvested at day 0, day 7 and day 14 were stained with H&E to assess the severity of colitis at different phases, with representative images shown. (j, k) Semi-quantitative scoring of histopathology (n=6–7) for (j) inflammation and (k) ulceration, (l) Myeloperoxidase (MPO) activity was determined and normalized per mg colon tissue at day 0 and day 5 of colitis (n=6–7). (m) Washed colon tissue explants were weighed and cultured ex vivo for 17 h, and cleared colon culture supernatants from colon biopsies normalized to weight at day 0 (n=2–6), day 5 (n=6–8) and day 7 (n=9–11) were analyzed by ELISA for TNF-α, IL-1β, IL-6, IL-18 and IL-22. Error bars represent ±s.e.m.; *P<0.05.
Dysbiosis in Aim2−/− mice is responsible for the heightened susceptibility to DSS-induced colitis

To investigate a causal connection between dysbiosis and the increased susceptibility to colitis in Aim2−/− mice, we treated mice with an antibiotic cocktail (ABx) to eliminate the microbiota for 2 weeks, followed by induction of DSS-induced colitis (Figure 3a). After treatment with ABx, both WT and Aim2−/− mice failed to develop significant DSS-induced colitis, as indicated by equal but modest body weight loss, even at day 9 after DSS administration, which is past the peak of colitis (Figure 3b). Consequently, there were no differences in colon length at day 9 (Figures 3c and d). To directly demonstrate that the altered microbiota in Aim2−/− mice was responsible for the susceptibility to colitis, we randomly assigned WT mice to two groups that were subjected to ABx treatment, after which these mice did not show any differences in body weight (Figure 3e).

We next isolated and pooled fecal material from the colons of three WT or Aim2−/− donor mice that had not been treated with ABx and performed a FMT by gavage at days 1 and 3 into ABx-treated WT recipient mice (Figure 3a). Fecal material from Aim2−/− mice caused more severe colitis than fecal material from WT mice as determined by body weight (Figure 3f) and significantly shortened colon length on day 7 after DSS administration (Figures 3g and h). These results indicate that dysbiosis is responsible for the susceptibility to DSS-induced colitis in Aim2−/− mice, as fecal material of Aim2−/− mice alone was sufficient to recapitulate the colitis prone phenotype observed in Aim2−/− mice.

The AIM2 inflammasome is functional in IECs and impacts colonic IL-22BP production

AIM2 assembles a DNA-sensing inflammasome in macrophages, which is essential for host defense against viral and bacterial pathogens.33,35,61,62 We therefore investigated whether genomic DNA from the commensal microbiota also induces AIM2 inflammasome activation in IECs. As gut bacteria make up the majority of feces, we isolated fecal genomic DNA (fDNA) from intestinal fecal samples. As expected, transfection of fDNA into immortalized WT iBMDM caused the release of IL-1β and IL-18, which was completely abrogated in Aim2−/− iBMDM and matched the loss of response observed in Asc−/− iBMDM and Casp1/11−/− iBMDM (Figures 4a and b). We next examined, whether IECs respond to fDNA with IL-18 release, as epithelial cells are not a major source of IL-1β. Transfection of fDNA into WT IECs resulted in IL-18 release, although this was less than that observed in macrophages. Importantly, IL-18 release was significantly reduced in IECs isolated from Aim2−/− mice (Figure 4c), indicating that IECs assemble an AIM2 inflammasome that senses fDNA.

![Figure 3](image-url)

**Figure 3** Transferrable dysbiosis in Aim2−/− mice is responsible for the susceptibility to DSS-induced colitis. (a) Timeline of ABx, FMT and DSS administration and analysis of colitis. Mice were fed 3% DSS in drinking water for 5 days, followed by regular drinking water until euthanasia at day 7 or 9. (b-d) Mice were treated for 3 weeks with an antibiotics cocktail, followed by administration of 3% DSS. (b) Body weight loss was monitored daily, and (c) colon length was determined at necropsy on day 9 after DSS administration. (d) Representative images are shown. (e-h) WT mice were treated with antibiotics as above, then (e) body weight was determined before re-colonization with either WT or Aim2−/− fecal microbiota by oral gavage before colitis induction with 3% DSS. (f) Body weight was determined daily, and (g, h) colon length was determined at necropsy on day 7 after DSS administration. (h) Representative images are shown. The results represent individual mice (n=7–10). Error bars represent ± s.e.m.; *P<0.05.
IL-18 has a key role in maintaining intestinal homeostasis. Specifically, inflammasome-mediated IL-18 suppresses the transcription of Il22bp in CD11c+ dendritic cells (DCs). Il22bp encodes the soluble IL-22 receptor A2, also known as IL-22BP, which is produced by myeloid cells as well as IECs. IL-22BP lacks a transmembrane and intracellular domain and therefore antagonizes IL-22 signaling, which also has a key role in intestinal inflammation and repair. IL-22 is produced by a heterogeneous population of lymphocytes, including subsets of Th17, Th22 and ILC3 cells, but not by IECs. Because IECs responded to fecal microbiota DNA with IL-18 production, we investigated if this response could directly affect IL-22BP. We therefore isolated IECs from naive mice and determined the expression of Il22bp. Similar to macrophages, we found that Il18 was not regulated at the transcriptional level in IECs of naive mice (Figure 4d). However, IL-18 production from colon tissue explants was significantly reduced in naive Aim2−/− mice (Figure 4d), indicating that it is regulated by AIM2 inflammasome-mediated proteolytic maturation and secretion. In accordance with the impaired IL-18 production, IECs from Aim2−/− mice expressed significantly more Il22bp than IECs isolated from WT mice (Figure 4f). In agreement with our qPCR results in IECs, colon tissue from Aim2−/− mice also produced significantly elevated levels of IL-22BP (Figure 4g).

Additional support for the involvement of the AIM2 inflammasome in regulating Il22bp came from the observation that Casp1/11−/− IECs also showed elevated Il22bp expression (Figure 4h). To directly verify that Il22bp expression was dependent on IL-18, we treated IECs with recombinant IL-18 for 4 and 8 h, which resulted in a 60% reduction in Il22bp expression (Figure 4i). Conversely, incubating IECs for 8 h in the presence of an anti-IL-18 neutralizing antibody significantly increased Il22bp expression, while an isotype control antibody had no effect (Figure 4j). Our results indicate that AIM2 is necessary to promote IL-18 secretion from IECs, which in turn directly regulates the expression of Il22bp, and the loss of AIM2 likely contributes to an imbalanced IL-22BP/IL-22 ratio.

**AIM2 controls anti-microbial peptide production in IECs**

A key function of IECs is the production of AMPs to shape and maintain a healthy luminal microbiota, and IL-18 and IL-22 have a key role in this process. Because we observed an altered intestinal microbiota in Aim2−/− mice, and because AMP expression and the antimicrobial barrier are impaired in murine colitis and human IBD, we determined the expression of several key AMPs in IECs isolated from naive mice. Expression of the α-defensin cryptdin-5 (Defcr5), the β-defensin Bd14 and the C-type lectin regenerating islet-derived (Reg) 2 were
significantly elevated in Aim2−/− IECs (Figure 5a). In contrast, the expression of Reg3β and Reg3g was completely impaired in Aim2−/− IECs (Figure 5b). In addition, the expression of several other AMPs, including Reg1, Bd3 and the cathelicidin Cramp, was unchanged in Aim2−/− IECs (Figure 5c). Our results indicate that AIM2 is necessary for the appropriate expression of select AMPs, specifically members of the Reg3 family. Defective AMP expression in Aim2−/− IECs was likely responsible for the observed dysbiosis in naive Aim2−/− mice and consequently the heightened susceptibility of Aim2−/− mice to colitis. Therefore, AIM2 has a key role in maintaining intestinal homeostasis.

AIM2 controls anti-microbial peptide production and resolution of intestinal inflammation during DSS-induced colitis

Because AMP expression is altered during acute colitis and the loss of Aim2 affects AMP expression in naive mice, we investigated AMP expression during DSS-induced colitis. We isolated IECs from WT and Aim2−/− mice at the acute and repair phases of colitis 5 and 14 days post DSS administration, respectively, and investigated the production of epithelial AMPs that were altered in naive Aim2−/− IECs. Contrary to naive Aim2−/− IECs, where the expression of Reg3b and Reg3g was impaired, we observed that both AMPs were significantly elevated in Aim2−/− IECs at both time points during DSS-induced colitis, whereas WT IECs were not significantly altered (Figure 6a). In contrast, expression of Defc5 was strongly induced during colitis. However, Aim2−/− IECs showed reduced Defc5 expression compared with WT IECs 5 days post DSS administration, but they normalized during repair at day 14 (Figure 6b). Bd14 expression was elevated during acute colitis and was further elevated in Aim2−/− IECs, but it was downregulated during repair 14 days after DSS administration (Figure 6c). Reg3b and Reg3g were the only AMPs that were consistently upregulated in Aim2−/− IECs during acute colitis and repair. Because their expression has been linked to IL-22, 71 we examined the expression of IL-22 (Figure 6d). The induced Il22bp expression on day 5 compared with day 0 correlated with elevated IL-22 (Figure 2m). Consistent with the increased Il22bp expression during the resolution phase at day 14 after DSS administration, IL-22 was reduced in Aim2−/− mice (Figure 6e). In addition, TNF-α, IL-1β and IFN-γ levels were also all reduced in Aim2−/− mice 14 days after DSS administration (Figure 6e), suggesting a more rapid resolution of intestinal inflammation in Aim2−/− mice.

AIM2 regulates tissue repair through the IL-18/IL-22/STAT3/Reg3 axis

IL-22 can have opposing roles during colitis by exacerbating acute inflammation and antimicrobial immunity but also enhancing epithelial cell proliferation and tissue repair, thus contributing to the development of tumors. 64 However, the role of IL-22, which signals primarily through STAT3, has not yet been investigated in the context of intestinal inflammation in Aim2−/− mice. We therefore isolated IECs from WT and Aim2−/− mice at the acute and repair phases of colitis at days 5 and 14 after DSS administration, respectively. Aim2−/− colons revealed enhanced phosphorylation of STAT3 on Tyr705 both at days 5 and 14 after DSS treatment, while total STAT3 was not different between WT and Aim2−/− colons (Figures 7a and b). These results suggest that IL-22 may be central to the elevated STAT3 signaling in Aim2−/− IECs during the acute phase of colitis. However, because IL-22 was reduced in Aim2−/− colons at day 14, additional factors may contribute to elevated STAT3 activation during intestinal repair. To investigate the cellular mechanisms associated with the differential regulation of STAT3 in IECs, we isolated WT IECs and treated organoids with recombinant IL-18 or IL-22. While IL-18 at any concentration failed to induce phosphorylation of STAT3, recombinant IL-22 induced phosphorylation of STAT3 in IECs (Figures 7c and d and data not shown), confirming that IL-22 alone elicits a direct effect on STAT3 signaling in IECs. In addition, an anti-IL-18 blocking antibody did not affect IL-22-mediated STAT3 phosphorylation (Figure 7d). To detect STAT3 phosphorylation in vitro, we treated cells for only 45 min. During this short

Figure 5 Aim2 deficiency impairs antimicrobial peptide production in IECs. (a–c) IECs from naive WT and Aim2−/− mice were analyzed by real-time PCR for the expression of (a) Defc5, Bd14 and Reg2, (b) Reg3b and Reg3g and (c) Reg1, Bd3 and Cramp (n = 14–16). The data are presented as fold induction compared with a randomly chosen WT mouse. Error bars represent ± s.e.m.; *P<0.05.
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Figure 6 Loss of Aim2 promotes Reg3β/γ production and improved recovery from colitis. Colitis was induced in WT and Aim2−/− mice with 3% DSS. (a–d) IECs were isolated at days 0, 5 and 14 after DSS administration and were analyzed by real-time PCR for the expression of (a) Reg3β and Reg3γ, (b) Defcr5 and (c) Bd14 and (d) Il22bp (n = 7–16). The data are presented as fold induction compared with naive WT mice shown in Figure 5. (e) Spontaneous release of IL-22, TNF-α, IL-1β and IFN-γ was measured by ELISA from cleared culture supernatants obtained after ex vivo culture for 17 h of colonic tissue explants obtained 14 days after DSS administration. (n = 4–5), and error bars represent ± s.e.m.; *P < 0.05.

time, IL-18 could not have modulated IL-22 through the regulation of IL-22BP, as shown in Figure 6d. Reg3γ expression requires STAT3 activation. Therefore, we investigated the expression of Reg3β and Reg3γ proteins in isolated IECs, which produce IL-18 but not IL-22. Expression of Reg3 members was dependent on IL-18 because Reg3β and Reg3γ expression was diminished in the presence of an IL-18 neutralizing antibody (Figure 7e). In addition, Reg3β and Reg3γ expression was completely abrogated in Aim2−/− IECs (Figure 7e), indicating that their expression is dependent on AIM2 inflammasome-mediated IL-18 release.

Because IECs do not produce IL-22, the AIM2 inflammasome likely contributes to Reg3β and Reg3γ expression through IL-18 and IL-22 by both STAT3-dependent and -independent mechanisms. To confirm this hypothesis and to directly demonstrate the contribution of IL-18 and/or IL-22 to the regulation of Reg3β and Reg3γ expression in IECs, we stimulated WT IECs with recombinant IL-18 and IL-22 as above and determined the expression of Reg3β and Reg3γ by qPCR. Both IL-18 (Figure 7f) and IL-22 (Figure 7g) increased Reg3β and Reg3γ expression after 17 h, but they had a more potent effect on Reg3γ, indicating that both IL-18 and IL-22 can directly induce expression of Reg3β and Reg3γ in IECs.

While the elevated IL-22 during acute colitis was likely sufficient for the enhanced STAT3 activation in Aim2−/− colon tissue, it could not explain the sustained STAT3 activation upon resolution of the disease, as the IL-22 levels were significantly lower in Aim2−/− colonic tissue. Reg3 proteins are known to have both antimicrobial and proliferative properties, and Reg3β promotes the development of pancreatic tumors at least in part through effects on STAT3 signaling. We therefore investigated whether the elevated IL-18/IL-22/STAT3-driven expression of Reg3β and Reg3γ observed in Aim2−/− IECs could in turn further amplify STAT3-driven responses. Incubation of IECs with both recombinant Reg3β and Reg3γ induced phosphorylation of STAT3 on Tyr705 and Akt on Ser473 as determined by immunoblot (Figure 7h), both of which promote cell proliferation and tissue repair. Thus, elevated expression of Reg3β and Reg3γ in Aim2−/− IECs contributed to sustained STAT3 activation during the resolution of intestinal inflammation and promoted cellular proliferation via activation of the Akt pathway in IECs. Indeed, such an improved repair is evident from body weight gain during the resolution phase of colitis, which started at day 9 in WT mice and day 10 in Aim2−/− mice. A linear regression analysis revealed that the slope (b) of the recovery in Aim2−/− mice was 74% increased compared with WT mice (Figure 7i). We therefore directly analyzed cell proliferation in colonic lamina propria tissue sections by Ki-67 staining. We observed no significant difference in proliferation on day 0. However, during acute colitis on day 5, the lamina propria of Aim2−/− mice still contained a larger number of Ki-67+ proliferative cells compared with WT tissue, although this normalized during resolution at day 14 (Figures 7j and k), indicating that AIM2 controls intestinal repair through sustained cell proliferation.
AIM2 regulates Reg3β/γ production through IL-18 and IL-22 and regulates intestinal tissue repair during colitis. (a, b) Colitis was induced in WT and Aim2−/− mice with 3% DSS. Colon tissue obtained at days 5 and 14 after DSS administration was analyzed for phosphorylated STAT3 (p-STAT3) on Tyr 705 and total STAT3 (STAT3), (a) quantified by densitometry and (b) analyzed by immunoblot (n=5). (c, d) IECs from naive WT mice were left untreated or incubated (c) with recombinant IL-22 (50 ng/ml) for 0, 30 and 60 min and (d) with IL-22 (50 ng/ml) or IL-18 (1 μg/ml) for 45 min and analyzed for phosphorylated STAT3 (p-STAT3) on Tyr 705 and total STAT3 (STAT3) by immunoblot. Where indicated, a neutralizing antibody against IL-18 (10 μg/ml) was added 30 min before cytokine treatment. (e) IECs from naive WT, Aim2−/− and Asc−/− mice were left untreated or incubated with a neutralizing antibody against IL-18 (10 μg/ml) for 17 h, analyzed by real-time PCR for the expression of Reg3β and Reg3γ and presented as fold expression compared with naive WT IECs. (f, g) IECs from naive WT mice were incubated with recombinant (f) IL-18 (1 μg/ml) or (g) IL-22 (50 ng/ml) for 0 (Ctrl) and 17 h, as indicated, and analyzed for the expression of Reg3β and Reg3γ as above and presented as fold expression compared with Ctrl. (h) IECs from naive WT mice were incubated with recombinant Reg3β and Reg3γ for 0, 15, 30 and 60 min, as indicated, lysed and analyzed for phosphorylated STAT3 (p-STAT3) on Tyr 705, total STAT3 (STAT3), phosphorylated Akt (p-Akt) on Ser 473 and total Akt (Akt) by immunoblot as above. A representative experiment is shown. (i) Colitis was induced with 3% DSS in WT and Aim2−/− mice, and body weight was measured daily during the recovery phase (day 7 to day 14). The slope (b) determined by linear regression analysis is indicated and plotted for the recovery phase. (j, k) Immunohistochemical analysis for Ki-67 in colon tissue sections at days 0, 5 and 14. (j) A representative image is shown, and (k) quantification is presented as Ki-67+ cells per crypt (n=3–8). Error bars represent ± s.e.m.; *P<0.05.
In summary, we demonstrated that the AIM2 inflammasome has a key role in maintaining intestinal homeostasis by promoting adequate expression of AMPs in IECs. As a consequence, loss of Aim2 resulted in transferrable dysbiosis, which rendered Aim2−/− mice more susceptible to DSS-induced colitis and promoted excessive and sustained STAT3 activity in IECs through IL-22 signaling and Reg3 expression. This Reg3β/γ-mediated amplification mechanism contributed to the subsequent improved recovery of Aim2−/− mice by promoting proliferation and providing a mechanism for the recently observed higher susceptibility of Aim2−/− mice to colorectal cancer.29,30

DISCUSSION

IBD is a highly complex disease involving genetic and environmental factors. Several inflammasomes in IECs protect from colitis and colitis-associated colorectal cancer.15,20–25,28 In addition, AIM2 protects against colorectal cancer, but this mechanism has been shown to be independent of the AIM2 inflammasome.29,30 However, we demonstrated that the AIM2 inflammasome provides a key protective mechanism that prevents dysbiosis-mediated colitis. The AIM2 inflammasome was at least partially responsible for producing adequate levels of IL-18 to maintain well-balanced expression of intestinal AMPs. AIM2 is activated by cytosolic double-stranded DNA during viral and bacterial infection33,61,62 and has consequently been linked to self-DNA-driven autoimmunity.75–77

Here we demonstrated that AIM2 is activated in IECs in response to double-stranded DNA isolated from fecal microbiota and that this response mediates the release of IL-18 but not IL-1β in IECs, while macrophages respond with the release of both cytokines in an AIM2 inflammasome-dependent manner. Hence, IL-18 production was significantly reduced in Aim2−/− mice. Local inflammasome-mediated IL-18 production in the intestine is required for maintaining intestinal health by suppressing dysbiosis from colitogenic microbiota species.15 In agreement with the importance of IL-18 in preventing dysbiosis, we observed that AIM2 inflammasome-deficient mice were more susceptible to DSS-induced colitis, suggesting that DSS-induced injury sensitizes the gut to microbiome-derived DNA and effectively induces IL-18 production through activation of the AIM2 inflammasome in the colon, reminiscent of several other inflammasome sensors. Global deletion of inflammasome sensors and effectors has yielded conflicting results in DSS–colitis models. However, the current understanding is that inflammasome activation in infiltrating myeloid cells is detrimental during colitis, primarily through excessive IL-1β production, but inflammasome activation in IECs and the resulting release of IL-18 is necessary for the prevention of dysbiosis and colitis. In addition, the housing of mice determines the composition of the intestinal microbiota and therefore contributes to the susceptibility of mice to DSS-induced colitis and likely also to the relative contribution of a particular inflammasome. We therefore investigated the DSS response in previously analyzed inflammasome-deficient mice, including Asc−/− and Casp11/11−/− mice. These mice showed a similar heightened susceptibility to DSS as previously reported,20–22 suggesting that the higher susceptibility to DSS-induced colitis in Aim2−/− mice was not solely caused by our housing environment. As expected from the acute inflammation during early colitis, we observed elevated levels of TNF-α, IL-1β and IL-22, which were reduced during the resolution phase in Aim2−/− colons, suggesting the existence of improved repair mechanisms in Aim2−/− mice; this was further supported by enhanced Reg3β and Reg3γ expression. The increased production of IL-1β was not surprising, as the hyper-inflammatory environment in the colons of Aim2−/− mice was reflected by increased leukocyte infiltration as well as the participation of several other inflammasomes in IL-1β production during colitis.3,10 Hence, loss of only Aim2 did not prevent other inflammasomes from producing this cytokine, which is in agreement with another recent study that observed elevated IL-1β levels in colon tissue of Aim2−/− mice during the development of colorectal cancer.30

To further investigate the underlying mechanism by which the AIM2 inflammasome protected from colitis, we focused on signaling downstream of IL-18 in IECs based on the impaired IL-18 release from Aim2−/− colons. IL-22 is a key cytokine produced during colitis by subsets of CD4+ T cells and ILC3s, and its soluble inhibitory receptor, IL-22BP, is downregulated by IL-18 produced by the NLRP3 and NLRP6 inflammasomes.63 IL-22BP is mainly produced by lamina propria CD103+CD11b+ DCs and to a lesser extent by IECs.45 IL-22BP and IL-22 are reciprocally expressed during colitis, with IL-22BP being suppressed and IL-22 upregulated to enable enhanced IL-22 signaling in the inflamed intestine, especially in the IECs. The central role of IL-22 in the immunity of superficial tissues has recently emerged, and IL-22 has opposing roles during the progression of colitis. IL-22 exacerbates inflammation during the acute phase, while it promotes tissue repair during the resolution phase, which can lead to colorectal cancer.64 In agreement with impaired IL-18 production in Aim2−/− IECs, we observed enhanced production and release of IL-22BP, which prevented appropriate IL-22 signaling in naive mice. During acute colitis, both WT and Aim2−/− IECs showed significant reductions of IL22bp, yet we observed elevated IL-22 levels and increased phosphorylation of STAT3 on Tyr705 in Aim2−/− colonic tissue. Hence, derailed IL-18 and IL-22 signaling during colitis was not sufficient to dampen the increased intestinal IL-22 levels. IL-22 signals preferentially through STAT3, and we also observed increased phosphorylation of STAT3 on Tyr705 in IECs directly activated with recombinant IL-22, but not with IL-18, even when testing up to 50-fold higher IL-18 concentrations.

In addition to its pro-inflammatory and tissue repair functions, IL-22 also has a key anti-microbial role.64 Hence, the impaired IL-22 signaling likely affected intestinal homeostasis, and overwhelming evidence links dysbiosis to the pathology of IBD and colitis.52 Indeed, naïve Aim2−/− mice, even at a young age, already displayed dysbiosis of select bacterial groups. In particular, we detected increased populations of the phylum Bacteroidetes, which are mutualistic Gram-negative, obligate anaerobic, non-endospore-forming bacilli that represent the most substantial portion of the microbiota. Species within the genus Prevotella were particularly increased, and these are frequently recovered from anaerobic infections and are also...
more abundant in \textit{Nlrp6}^−/− and \textit{Asc}^−/− mice.\textsuperscript{15} However, we detected no changes in several other bacterial groups. In contrast to \textit{Nlrp6}^−/− and \textit{Asc}^−/− mice, which show increased TM7,\textsuperscript{15} we observed reduced TM7 numbers in \textit{Aim2}^−/− mice, indicating that multiple inflammasomes within IECs collaborate to prevent dysbiosis.

Appropriate antimicrobial barriers and intestinal homeostasis, which are lost during colitis, are maintained by AMPs, which are primarily produced by IECs.\textsuperscript{67,70} In agreement with altered luminal microbiota, naïve \textit{Aim2}^−/− mice displayed dysregulated expression of several key AMPs. In particular, expression of both murine \textit{Reg3} members \textit{Reg3b} and \textit{Reg3g} was downregulated in \textit{Aim2}^−/− mice. Surprisingly, not all AMPs were lost, and several were even expressed at increased levels in \textit{Aim2}^−/− mice, including \textit{Defcr5}, \textit{Bd14} and \textit{Reg2}, while others were expressed at levels equal to WT mice. Elevated expression of some AMPs may potentially constitute a compensatory mechanism to mitigate the loss of \textit{Reg3} members. Alternatively, the selective loss of \textit{Reg3} members may explain the amplification of only some bacterial groups in \textit{Aim2}^−/− mice. AIM2 was therefore required for balanced AMP expression in IECs. AIM2 also collaborates with other inflammasomes, as dysregulated AMP expression is also observed in \textit{Nlrp3}^−/− mice.\textsuperscript{22} IL-18 and IL-22 have both been linked to the expression of AMPs in IECs,\textsuperscript{68,69} and IL-22 in particular is linked to the expression of \textit{Reg3} members.\textsuperscript{71} The IL-22 downstream signaling component \textit{STAT3} has an innate antibacterial role in the lung and protects against MRSA pneumonia through the induction of \textit{Reg3g}.\textsuperscript{72} Therefore, the impaired IL-18 and IL-22 signaling in \textit{Aim2}^−/− mice could explain the loss of \textit{Reg3b} and \textit{Reg3g}, and we directly demonstrated that both cytokines were sufficient for \textit{Reg3b} and \textit{Reg3g} expression in IECs. Impaired IL-18 and IL-22 signaling and the resulting defect in AMP expression in \textit{Aim2}^−/− IECs consequently promoted dysbiosis and ultimately increased the susceptibility of \textit{Aim2}^−/− mice to colitis. Indeed, by fecal microbiota transplant and antibiotic treatment experiments, we directly demonstrated that a transferrable dysbiosis was responsible for the susceptibility to colitis in \textit{Aim2}^−/− mice. Hence, AIM2 has a key role in maintaining intestinal homeostasis. Excessive IL-22 signaling and downstream \textit{STAT3} activation during acute colitis further exacerbated inflammation in \textit{Aim2}^−/− mice. However, while \textit{STAT3} activity was markedly reduced during the resolution phase at day 14 after DSS administration, it was still significantly elevated in \textit{Aim2}^−/− colons. This was surprising, as we detected lower IL-22 levels than in WT mice, suggesting that IL-22 alone was likely not sufficient for the elevated \textit{STAT3} signaling. Therefore, \textit{Aim2} deficiency also affected other \textit{STAT3}-activating pathways or pathways that sustained IL-22-mediated activities.

While phosphorylation of \textit{STAT3} has been identified in IBD patients and in mice, it promotes acute intestinal inflammation,\textsuperscript{78,79} IEC \textit{STAT3} also has an important role in wound healing and tissue repair during the resolution of DSS-induced colitis by promoting IEC survival and proliferation, a repair mechanism that if left unchecked results in increased susceptibility to colorectal cancer.\textsuperscript{56,80,81} Furthermore, \textit{STAT3} regulates \textit{Reg3} expression, p\textit{STAT3}+ Th17-cell activation and IL-17 production; accordingly, conditional deletion of \textit{Stat3} in IECs exacerbates intestinal inflammation.\textsuperscript{73} Therefore, the increased IL-22 signaling present in \textit{Aim2}^−/− colon tissues was likely responsible for the elevated expression of \textit{Reg3b} and \textit{Reg3g} through \textit{STAT3}-mediated signaling during acute colitis. In turn, \textit{Reg3} proteins further amplified and sustained \textit{STAT3} activation in IECs after IL-22 levels had decreased during intestinal repair. Hence, our findings suggest that \textit{Reg3} proteins themselves are responsible for the sustained \textit{STAT3} activation in IECs, even after acute colitis has been resolved in a positive feed-forward loop. In addition to \textit{STAT3} activation, \textit{Reg3} proteins also promote Akt phosphorylation in IECs. While \textit{Aim2}^−/− mice were more susceptible to acute intestinal inflammation, the above changes ultimately facilitated enhanced intestinal tissue repair by promoting proliferation. Hence, AIM2 regulates proliferation of intestinal cells at the base of crypts, which contain Ki-67\textsuperscript{+} intestinal stem cells and other progenitors, including the Delta-like 1\textsuperscript{+} (Dll\textsuperscript{+}) cells, which are the common precursors of the Paneth, goblet and the enteroidendocrine cells, and the secretory label-retaining cells (LRCs), which differentiate into Paneth and enteroidendocrine cells.\textsuperscript{82-84} As a chemoattractant for macrophages, \textit{Reg3β} also promotes tissue repair\textsuperscript{42} and M2 macrophage differentiation.\textsuperscript{74} Hence, it is feasible that elevated \textit{Reg3β} may be responsible for recruiting M2 macrophages into the inflamed colon to initiate intestinal repair, a hypothesis that is supported by the reduced pro-inflammatory M1-type cytokines TNF-α, IL-1β and IFN-γ in \textit{Aim2}^−/− colon tissue 14 days after DSS administration. This finding is of importance, as two recent studies demonstrated that \textit{Aim2}^−/− mice are more susceptible to induced and spontaneous forms of colorectal cancer.\textsuperscript{29,30} Although an inflammasome-independent mechanism has been suggested based on unchanged IL-1β release, \textit{Aim2}^−/− mice show increased proliferation of enterocytes and intestinal stem cells through aberrant Akt activation and expression of proliferative genes, but not \textit{Reg3b} or \textit{Reg3g}.\textsuperscript{29,30} AIM2 also interacts and inhibits the PI3K family member DNA-PK upstream of Akt.\textsuperscript{30} \textit{Aim2}^−/− mice display dysbiosis, although it involves different bacterial species than those detected in our study, such as reduced \textit{Prevotella}, indicating housing differences likely have a role in the composition of the microbiota in \textit{Aim2}^−/− mice.\textsuperscript{29,30} During the preparation of this manuscript, Zaki \textit{et al.} also demonstrated AIM2 inflammasome-mediated control of dysbiosis, AMPs and colitis, but they also identified different bacteria amplified in \textit{Aim2}^−/− mice, such as \textit{Escherichia coli}, which were not affected in our study.\textsuperscript{45}

In summary, our findings provide important insights into the mechanisms by which AIM2 maintains intestinal homeostasis and prevents colitis and colorectal cancer by demonstrating that AIM2-mediated IL-18 production is a key step in preventing intestinal inflammation. Consequently, loss of \textit{Aim2} results in dysregulated IL-18-dependent IL-22BP/IL-22 equilibrium, which is required for balanced AMP expression by IECs and results in the loss of \textit{Reg3b} and \textit{Reg3g} expression and dysbiosis in naïve mice. Dysbiosis increases the susceptibility of \textit{Aim2}^−/− mice to experimental colitis in response to damage by DSS, which
Further dysregulates IL-22 secretion and consequently induces Reg3β and Reg3γ expression in Aim2−/− mice. Reg3β and Reg3γ perpetuate STAT3 and Akt activation in a forward amplification loop to sustain IEC proliferation and tissue repair, which may contribute to the previously reported heightened susceptibility to colorectal cancer (Figure 8). 29,30,86

CONCLUSIONS

In the present study, we demonstrate that the AIM2 inflammasome is activated in intestinal epithelial cells by fecal microbiota DNA as a protective mechanism to prevent dysbiosis and colitis, and to regulate intestinal tissue repair through the IL-18/IL-22/STAT3/Reg3 pathway.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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