Comparative Medicine
Copyright 2017
by the American Association for Laboratory Animal Science

Vol 67, No 5
October 2017
Pages 400–406

Original Research

Lack of Effect of Murine Astrovirus Infection on Dextran Sulfate–induced Colitis in NLRP3-deficient Mice

Susan R Compton,[1,*] Carmen J Booth,[1] and James D Macy[1,2]

Murine astrovirus (MuAstV) is a recently identified, widespread infection among laboratory mice. MuAstV is found predominantly in the gastrointestinal tract of mice. Human and turkey astroviruses have been shown to disrupt tight junctions in the intestinal epithelium. The potential of MuAstV to alter research results was tested in a well-established dextran sodium sulfate (DSS)-induced colitis model in Nod-like receptor 3 (NLRP3)-deficient mice. This model offers a direct approach to determine whether MuAstV, as a component of the mouse microbiome, contributes to the issue of poor reproducibility in murine inflammatory bowel disease research. In this model, defective inflammasome activation causes loss of epithelial integrity, resulting in leakage of intestinal bacteria and colitis. Our goal was to determine whether MuAstV, which also may affect intestinal permeability, altered the onset or severity of colitis. Male and female mice (age, 8 to 12 wk) homozygous or heterozygous for an NLRP3 mutation were inoculated orally with MuAstV or mock-inoculated with media 3 or 20 d prior to being exposed to 2% DSS in their drinking water for 9 d. MuAstV infection alone did not cause clinical signs or histopathologic changes in NLRP3$^{-/-}$ or NLRP3$^{+/-}$ mice. No significant difference was seen in weight loss, clinical disease, intestinal inflammation, edema, hyperplasia, or mucosal ulceration between MuAstV- infected and mock-infected mice that received 2% DSS for 9 d. Therefore, MuAstV does not appear to be a confounding variable in the DSS colitis model in NLRP3 mice.

Abbreviations: dpi, days postinoculation; DSS, dextran sodium sulfate; MuAstV, murine astrovirus; NLRP3, Nod-like receptor 3

Although murine astrovirus (MuAstV) is common in commercial and noncommercial mouse colonies, the consequences of MuAstV infection in murine-based research and biosecurity are largely unknown.[11,26,35] Astroviruses were first observed in 1975 by electron microscopy in the stool of a child with diarrhea.[6] Astroviruses are the second most common cause of nonbacterial gastroenteritis in children, the elderly, and the immunocompromised.[6] Patients infected with human astroviruses present with diarrhea, vomiting, fever, anorexia, and abdominal pain lasting 2 to 4 d.[6] Astroviruses infect a wide range of mammalian species, including cows, sheep, pigs, turkeys, cats, dogs, and rats, causing self-limiting diarrhea and enteritis or no clinical signs.[9] Astroviruses cause very little inflammation and cellular damage, and diarrhea in turkeys has been shown to be caused by disruption of the cellular tight junction complex in the intestinal epithelium by the astrovirus capsid protein.[23-25]

Murine astrovirus was first observed in 1985 in the gut contents of NMRI and nude mice during an outbreak of diarrhea in nude mice.[18] The first complete genomic sequence of a MuAstV was elucidated in 2011, as part of a metagenomics study of feces collected from a wild *Mus musculus*.[28] Portions of other strains of MuAstV from the feces or liver of asymptomatic immunocompetent (BALB/c, C57BL/6, C3H, ICR, CD1) and immunodeficient (NOD-scid, NSG, uPA-NOG, Rag1, Rag2) adult mice have been sequenced.[26,35] To understand the role of the immune system in controlling MuAstV infection, C57BL/6 and Stat1 mice were infected with MuAstV by cohousing them with infected Rag1 mice.[35] MuAstV RNA was detected in the feces of all 3 mouse strains for 2 wk, with Rag1 mice having the highest level of MuAstV RNA. At day 14, MuAstV RNA was detected in the intestines and mesenteric lymph nodes of C57BL/6 mice; in the intestines, mesenteric lymph nodes, and spleen of Stat1 mice; and in the intestines, mesenteric lymph nodes, spleen, liver, and kidney of Rag1 mice; the authors thus concluded the humoral, cell-mediated and innate immunity are involved in controlling MuAstV infection.[35] Although no clinical signs or pathology were seen in the C57BL/6, Stat1, and Rag mice, the effect of MuAstV on research models is unknown, and MuAstV might contribute to differences in reproducibility between groups of control and experimental mice if they are housed in facilities or obtained from sources with different MuAstV infection statuses. Interferon α was shown to be important in the clearance of MuAstV, because persistently infected mice deficient in IFNα receptor (IFNαR) that were inoculated with MuAstV-STL4 shed high levels of MuAstV RNA for at least 7 wk, whereas naïve C57Bl/6 mice inoculated with MuAstV-STL4 shed lower levels of MuAstV RNA only during the first 2 wk of infection.[23] At 3 d after inoculation (dpi) with MuAstV, both C57Bl/6 and IFNαR-deficient mice had increased intestinal permeability as compared with uninfected control mice, with the IFNαR-deficient mice having a greater increase in permeability.[23] The mechanism of increased intestinal permeability associated with MuAstV is probably due to disruption of intestinal epithelial cells, given that human astrovirus has been shown

Received: 21 Apr 2017. Revision requested: 12 May 2017. Accepted: 08 Jun 2017.
[1]Section of Comparative Medicine and [2]Animal Resources Center, Yale University, New Haven, Connecticut.
[*]Corresponding author. Email: susan.compton@yale.edu.

to disrupt the tight junction protein occludin and to decrease the number of actin stress fibers in Caco2 cells.[25] Furthermore, turkey astrovirus causes redistribution of sodium hydrogen exchanger 3 from the membrane to the cytoplasm of intestinal epithelial cells, resulting in diarrhea in turkey poults.[23,24]

The Nod-like receptor 3 (NLRP3; also known as NALP3, cryopyrin, cold-induced autoimmunity syndrome 1 protein [CIAS1], and PYRIN-containing Apaf-1 like protein [PYPAF1])[32] can be activated by a wide range of stimuli including bacterial peptidoglycans and muramyl dipeptide, Toll-like receptor ligands, monosodium urate and calcium pyrophosphate dehydrate crystals associated with gout, and pore-forming agents.[31] Several viruses, including adenovirus, Coxsackie B virus, encephalomyocarditis virus, influenza virus, Newcastle disease virus, Sendai virus, and vesicular stomatitis virus, are recognized by the NLRP3 inflammasome.[17,29,34] Activated NLRP3 oligomerizes and associates with the adaptor protein ASC and procaspase 1 to form the NLRP3 inflammasome.[32] The activated caspase 1 in the NLRP3 inflammasome cleaves proIL1β and proIL18 to form the mature secreted forms of these proinflammatory cytokines, which recruit innate immune cells and induce IFNγ.[32]

In humans, mutations in the *NLRP3* gene, which leads to constitutive activation of the inflammasome, cause increased production of IL1β and lead to chronic autoinflammatory diseases (hereditary fever syndromes).[1] In contrast, mice deficient in NLRP3 have decreased production of IL1β and IL18 and are resistant to the proinflammatory effects of these cytokines and are used to understand the role of the NLRP3 imflammasome in the inflammation induced by obesity or aging, type 2 diabetes, atherosclerosis, ischemia, Alzheimer disease, gout, and colitis and colitis-associated cancer.[13,14,20,22,33]

Inflammatory bowel disease is a complex multifactorial disease characterized by chronic intestinal inflammation caused by the dysregulation of the mucosal immune system in the intestine. Levels of proinflammatory cytokines IL1β, IL6, IL18, and TNFα are increased during inflammatory bowel disease,[5] and IL1β and IL18 have been shown to alter intestinal tight junctions and intestinal permeability.[5] When epithelial barrier integrity is compromised, microorganisms and toxins gain access to the underlying tissues. One well-established model of inflammatory bowel disease in mice is dextran sulfate sodium (DSS)-induced colitis. DSS is believed to cause colitis by interfering with intestinal barrier functions and stimulating inflammation in the colon.[5,36] Results from this DSS model are inconsistent, with several studies reporting that colitis was worse in NLRP3^{-} mice than in C57BL/6 mice,[2,15,36] whereas another study reported that colitis was less severe in NLRP3^{-} mice than in C57BL/6 mice.[5] The differences in colitis outcomes may be related to differences in the experimental design, including differences in the concentration of DSS used (2% to 5%), the duration of DSS exposure (5 to 9 d), and the age of the mice (8 to 16 wk). In addition, the sex of mice used was not disclosed in some of the studies. In addition, the intestinal microbiome may contribute to this disparity, given that cohoused wild-type and NLRP3^{-} mice showed similar severity of DSS- induced colitis.[4] We postulated that the MuAstV infection status of the mice used in the DSS-models of colitis might also affect the severity of DSS-induced colitis.

Materials and Methods

Mice. Homozygous C57BL/6N-Nlrp3^{tm1Vmd} (NLRP3; age, 10 wk) mice[21] were obtained from Dr Vishwa Deep Dixit (Yale University, New Haven, CT). C57BL/6J mice (age, 8 wk) were obtained from Jackson Laboratory (Bar Harbor, ME). Prior to breeding, fresh feces were collected from the anus of each mouse for MuAstV and murine norovirus RT-PCR testing and for *Tritrichomonas muris* and *Helicobacter* ssp. PCR analysis, to rule out infection with these endemic agents which are prevalent in many laboratory mouse colonies. Soiled-bedding sentinels in the room from which the NLRP3 mice were obtained were seronegative for ectromelia virus, murine rotavirus, lymphocytic choriomeningitis virus, mouse hepatitis virus, mouse parvovirus, minute virus of mice, *Mycoplasma pulmonis*, pneumonia virus of mice, Sendai virus, and Theiler encephalomyelitis virus at their most recent screening. Feces and fur plucks from the NLRP3 mice were negative for pinworms and fur mites. Twelve breeding pairs yielded 14 homozygous NLRP3$^{-/-}$ females, 30 homozygous NLRP3$^{-/-}$ males, 11 heterozygous NLRP3$^{+/-}$ females, and 9 heterozygous NLRP3$^{+/-}$ males. The second litters yielded 11 homozygous NLRP3$^{-/-}$ females, 18 homozygous NLRP3$^{-/-}$ males, 5 heterozygous NLRP3$^{+/-}$ females, and 7 heterozygous NLRP3$^{+/-}$ males. Mice were identified by using ear punches. Mice initially were housed in IVC (MicroVent, ACE, Allentown, NJ) under positive pressure. Cages containing corncob bedding (Harlan Teklad, Indianapolis, IN), rodent chow (Global 2018S, Harlan Teklad), and nesting material (Nestlets, Ancare, Bellmore, NY) were preassembled and autoclaved. Mice drank hyperchlorinated (4 to 6 ppm) water delivered by water bottles (ACE) without restriction. Because water containing 2% DSS (MW 36,000 to 50,000; MP Biomedicals, Solon, OH) leaked from the bottles that lacked silicone seals (ACE), for the second half of each experiment, mice were in housed in static cages (ACE) containing corncob bedding (Harlan Teklad), rodent chow (Global 2018S, Harlan Teklad), and nesting material. For the second half of each experiment, mice drank hyperchlorinated water containing 2% DSS or water without DSS without restriction from bottles that had silicone seals in the metal caps (Tecniplast, West Chester, PA). The mice were housed and husbanded according to standard biocontainment procedures. The animal room had a negative pressure differential relative to the corridor, a 12:12-h light: dark cycle, 10 to 15 air changes hourly, temperature of 22.2 ± 1.1 °C, and relative humidity of 50% ± 10%. All animal care and experimental procedures were performed in an AAALAC-accredited animal facility, were approved by the Yale IACUC, and were in compliance with the *Guide for the Care and Use of Laboratory Animals*[16] and in accordance with federal policies and guidelines governing the use of vertebrate animals. Mice were euthanized by CO_2 overdose followed by cardiocentesis.

RT-PCR and PCR analyses. Fresh feces were collected from the anus of each mouse and were frozen at –70 °C until processing. RNA was extracted from mouse feces by using RNeasy kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. RT- PCR analysis was performed by using previously described primers for MuAstV (MuAstV-BF and MuAstV-BR[26]) or murine norovirus (MNV5033 and MNV 5542[12]), iScript One-Step RT-PCR kit with SYBR Green (Biorad, Hercules, CA) and a thermocycler (CFX Connect, Biorad). The reaction conditions were 10 min at 50 °C; 5 min at 95 °C; and 40 cycles of 10 s at 95 °C, 20 s at 59 °C, and 30 s at 72 °C. All assays contained negative and positive controls.

Fresh feces were collected from the anus of each mouse and were frozen at –70 °C until processing. DNA was extracted from mouse feces by using DNeasy kits (Qiagen) according to the manufacturer's instructions. PCR analysis for *Helicobacter* ssp. was performed by using previously described primers,[12] iTaq Universal SYBR Green kit (Biorad), and a thermocycler (CFX Connect, Biorad). *T. muris* PCR analysis was performed by using the primers TM689F (5′ GGC GAA CGG TGG AAT GTT

TT 3′) and TM1201R (5′ ACC CTC TTC CTC CTG CTT GA 3′), iTaqUniversal SYBR Green kit (Biorad), and a thermocycler (CFX Connect, Biorad). The reaction conditions were 3 min at 95 °C followed by 40 cycles of 30 s at 95 °C, 30 s at 54.8 °C, and 60 s at 72 °C. All assays contained negative and positive controls.

DNA was extracted from ear punch tissue by using DNeasy kits (Qiagen) according to the manufacturer's instructions. Genotyping PCR was performed by using the primers cryopyrin-F (5′ CCA GCC AGA GTG GAG TGA GTA AAC 3′) and cryopyrin-R (5′ CAG TCA CCT GCG AGT GAG TGA AGA C 3′) or cryopyrin-M (5′ CCG GTG GAT GTG GAA TGT GTG C 3′) and cryopyrin-R. PCR analysis was performed by using *Taq* polymerase (Roche Diagnostics, Mannheim, Germany) and a thermocycler (PTC100, MJ Research, Watertown, MA). The reaction conditions were 2 min at 95 °C; 10 cycles of 45 s at 95 °C and 90 s at 60 °C; and 25 cycles of 45 s at 95 °C, 30 s at 62 °C, and 45 s at 68 °C. Mice homozygous for the mutant *NLRP3* gene produced a 318-bp fragment, and heterozygous mice produced 318- and 665-bp fragments.

Infection with MuAstV. Fecal homogenates from 6 MuAstV RNA-positive CD1 mice were pooled and clarified by centrifugation at 10 × *g* for 2 min at room temperature.[8] Three female SW mice (CRL:CFW[SW]; age, 6 wk) were obtained from Charles River (Kingston NY), and each mouse was inoculated orally with 20 µL of clarified fecal homogenate. Mice were euthanized by CO_2 5 d after inoculation, and sections of the intestine were collected from each mouse. These tissue samples were processed as 10% homogenates in DMEM; RT-PCR analysis indicated that the colonic homogenates had the highest level of MuAstV RNA.[8] Experimental mice in the current study were inoculated with pooled colonic homogenates that had been clarified by centrifugation and passed through a 0.22-µm filter. This MuAstV-Y stock was negative for nucleic acids from *Helicobacter* spp., mouse hepatitis virus, mouse parvovirus, murine norovirus, murine rotavirus, reovirus, Theiler encephalomyelitis virus, and *Tritrichomonas* ssp., according to PCR analysis.[8] A portion of ORF1b and the complete ORF2 region of MuAstV strain Y were sequenced (GenBank accession no. KX683863).[8] Mice were inoculated orally with 20 µL of a 10% colonic homogenate containing MuAstV-Y[8] or 20 µL of Dulbecco minimal essential media.

Histopathologic analysis. After euthanasia by CO_2 overdose, mice were examined for gross lesions. The colon was removed at the ileocecocolic junction and rectum, and fecal material was washed from the colon by using PBS. Colons were immersed in Bouin fixative (Ricca, Arlington TX), trimmed, processed, and embedded lengthwise in paraffin. Sections (5 µm) were cut and stained with hematoxylin and eosin (ThermoFisher, Kalamazoo MI). Each section was evaluated for changes in the mucosa, submucosa, muscularis externa and serosa. Severity of colitis was scored by using an established semiquantitative colitis scoring system that combines scores for edema, mucosal ulceration, inflammation, and hyperplasia (0, absent; 1, minimal [<10% of tissue affected]; 2, mild [10% to 25%]; 3, moderate [25% to 50%]; 4, marked [50% to 75%]; and 5, severe [75% to 100%]);[27] the scorer was blinded to experimental groups.

Statistical analysis. Two-tailed *t* tests were performed by using Excel (Microsoft, Redmond, WA). A *P* value of less than 0.05 denotes statistical significance.

Experiment 1. The initial design of the experiment used equal numbers of male and female mice and of mice homozygous and heterozygous for the mutant *NLRP3* gene. Mice heterozygous for *NLRP3*, bred at Yale, were used as controls because their microbiome is likely more similar to that of the mice homozygous for *NLRP3* than that of C57BL/6 mice purchased from a commercial vendor. Breeding yielded more male than female mice and more NLRP3$^{-/-}$ than NLRP3$^{+/-}$ mice. The design of the first experiment was altered to use 40 NLRP3$^{-/-}$ homozygous and 20 NLRP3$^{+/-}$ heterozygous mice (age, 8 to 12 wk). Mice were allocated into the following 4 groups: (A) MuAstV only: 7 homozygous males, 3 homozygous females, and 2 heterozygous males; (B) MuAstV and DSS: 4 homozygous males, 6 homozygous females, 5 heterozygous males, and 4 heterozygous females; (C) untreated controls (no MuAstV or DSS): 10 homozygous males; and (D) DSS only: 5 homozygous males, 5 homozygous females, 5 heterozygous males, and 4 heterozygous females. Mice were housed 3 to 5 per cage, segregated by sex; homozygous and heterozygous mice were cohoused, when possible, to reduce the potential effect of microbiome differences between the 2 genotypes of mice in this model. All groups B and D cages housed at least 2 NLRP3$^{-/-}$ and 2 NLRP3$^{+/-}$ mice. Two of the 3 group A cages and both group C cages housed only male NLRP3$^{-/-}$ mice. At 5 d prior to the initiation of the experiment, feces were collected from all experimental mice for MuAstV and murine norovirus RT-PCR testing and *T. muris* and *Helicobacter* ssp. PCR analysis. On experimental day 0, all 60 mice were weighed, the 31 mice in groups A and B were inoculated with MuAstV, and the 29 mice in groups C and D with inoculated with media. All mice were weighed and observed daily for 14 d for clinical signs of colitis, such as dehydration, hunching, ruffled fur, inactivity, diarrhea, hematochezia, and rectal prolapse. At 3 dpi, fecal pools were collected from each cage for MuAstV RT-PCR analysis. At 5dpi, mice in group A and C were moved to static cages and were given water bottles containing hyperchlorinated water and mice in group B and D were moved to static cages and were given water bottles containing hyperchlorinated water containing 2% DSS. Water intake was monitored in all cages. On day 7 and 11, individual feces were collected from mice for MuAstV RT-PCR. Mice were euthanized if they had a weight loss greater than 15%. On day 14, all mice were euthanized by CO_2 overdose, ear punches were performed for confirmatory genotyping, and colons were harvested for histopathologic evaluation. After euthanasia, a clinical score was determined based on fecal consistency (0, formed; 1, semiformed or unformed; 2, no feces) and presence of blood in the feces (0, no blood; 1, dark feces; 2, gross rectal bleeding). The scorer was blinded to mouse genotype but not treatment.

Experiment 2. Subjective clinical scoring during the first experiment suggested that the perceived clinical disease was slightly more severe in mice that received both MuAstV and DSS as compared with those that received only DSS; therefore a follow-up experiment was performed. The second experiment used 26 NLRP3$^{-/-}$ homozygous and 12 NLRP3$^{+/-}$ heterozygous mice (age, 8 to 12 wk). Mice were allocated into 2 groups: group B (6 homozygous males; 7 homozygous females, 3 heterozygous males, and 3 heterozygous females) received MuAstV and DSS, and group D (6 homozygous males, 7 homozygous females, 4 heterozygous males, and 2 heterozygous females) received DSS only. Mice were housed at 4 or 5 per cage, segregated by sex, for 20 d prior to DSS treatment in an attempt to increase the similarity of the microbiomes between the homozygous and heterozygous mice. On experimental day 0, all 38 mice were weighed, the 19 group B mice were inoculated with MuAstV, and the 19 group D mice were inoculated with media. All mice were weighed and observed daily for 29 d for clinical signs, such as dehydration, hunching, ruffled fur, inactivity, diarrhea, hematochezia, and rectal prolapse,. At 20 dpi, mice were moved to static cages and were given water bottles containing 2% DSS in hyperchlorinated water. At 20 dpi, group B mice would be

Table 1. Average clinical and histopathologic data in mice that received DSS during experiment 1

group: inoculum	Sex	Genotype	*n*	Weight loss (%)	Water intake (mL)	Clinical score	Histopathology score
B: MuAstV	male	homozygous	4	14	3.6	2.0	14.9
B: MuAstV	male	heterozygous	5	12		2.4	14.9
B: MuAstV	female	homozygous	6	13	2.9	1.8	15.0
B: MuAstV	female	heterozygous	4	12		2.5	15.0
D: media	male	homozygous	5	16	3.2	1.6	15.4
D: media	male	heterozygous	5	15		1.9	14.4
D: media	female	homozygous	5	12	3.0	1.0	14.5
D: media	female	heterozygous	4	13		1.3	15.5
Overall			38	13	3.2	1.8	14.9

chronically infected with MuAstV and any antiMuAstV immune response would be well-established.[8,35] For all cages, water intake was monitored for 9 d. At 6 d after DSS treatment (26 dpi), feces were collected from mice for MuAstV RT-PCR analysis. Mice were euthanized if they had a weight loss greater than 15%. On day 29, mice were euthanized by CO_2, ear punches were performed for confirmatory genotyping, and colons were harvested for histopathologic evaluation. A clinical score was assigned according to fecal consistency and presence of blood in the feces. The scorer was blinded to the genotype of the mouse but not the treatment.

Results

Experiment 1. At the start of the experiment, all were mice were negative for MuAstV, murine norovirus, *Helicobacter* ssp., and *T. muris* nucleic acids. MuAstV RT-PCR analysis of pooled feces from each cage at 3 dpi confirmed that all group A (treated with MuAstV only) and B (MuAstV and DSS) cages contained mice that were shedding MuAstV and that mice in the group C (untreated controls) and D (DSS only) cages were free of MuAstV. MuAstV RT-PCR analysis of feces from individual mice on 7 and 11 dpi confirmed that all group A mice were still shedding MuAstV and that all group C and D mice were still free of MuAstV. In contrast, MuAstV RT-PCR analysis of feces from individual group B mice on 7 and 11 dpi, (2 and 6 d after DSS treatment) showed that MuAstV, was no longer being shed from any of the group B mice.

Weight loss was not detected in the 2 groups of mice (A and C) that did not receive DSS; instead these mice showed an average weight gain of 5% over the final 9 d of the experiment. Mice in these 2 groups showed no clinical signs, drank an average of 4.3 mL of water daily and, after euthanasia, they had clinical scores of 0 (formed feces with no fecal blood observed). Histopathology scores ranged from 0 to 2 (possible maximum, 20), with an average score of 0.8 due to mild colonic inflammation or edema. No significant changes were seen between untreated control mice and those that received only MuAstV, regardless of the genotype or sex of the mice.

All mice in groups B (MuAstV and DSS) and D (DSS only) lost weight (6% to 18%, Table 1) during the 9-d period that they drank 2% DSS in their water. A total of 12 mice from these 2 groups were euthanized due to excessive weight loss (greater than 15%) prior to the conclusion of the experiment on day 14 (4 mice on day 12, and 8 on day 13; 7 male and 5 female; 6 NLRP3$^{-/-}$ and 6 NLRP3$^{+/-}$; 6 from group B and 6 from group D). Mice in groups B and D drank an average of 3.2 mL of water during the 9 d that they were receiving DSS, which might have resulted in dehydration and contributed to the weight loss observed (Table 1). After euthanasia, a clinical score was assigned according to fecal consistency and the presence of fecal blood. A few mice had normal, formed feces with no evidence of bleeding, but most mice had semiformed dark-red feces, and a few mice had no evidence of feces but gross rectal bleeding. The average clinical score for the 38 mice was 1.8 (Table 1). There were no genotype- or sex-associated significant differences in the clinical scores. However, female NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice infected with MuAstV that received DSS treatment (group B) had higher average clinical scores (score, 2.1) than female NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice that received only DSS treatment (group D; score, 1.1; $P = 0.02$; Table 1).

Mild to moderate colonic hyperplasia was present in the colons of all but 2 mice in groups B and D (average score, 2.3). All group B and D mice had mild to marked edema (average score, 3.4), mild to marked inflammation (average score, 3.3), mild to marked crypt loss (average score, 3.4), and mild to marked ulceration in the epithelium of the colon (average score, 2.5). The combined histopathology scores for the mice in groups B and D ranged from 11.0 to 18.5 (Table 1). Despite the slight difference in clinical scores between group B and D mice, no significant differences in histopathology scores were seen between male and female mice or between NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice or between the mice in groups B and D (Figure 1).

Experiment 2. A follow-up experiment was performed, in which mice were infected with MuAstV for 20 d prior to treatment with DSS to investigate the effect of established MuAstV infection on the DSS model of colitis. At the start of the experiment, all were mice were negative for MuAstV, murine norovirus, *T .muris*, and *Helicobacter* ssp. nucleic acids. MuAstV RT-PCR analysis of fecal pools collected from each cage at 4 dpi and of feces collected from individual mice at 6, 13 and 20 dpi confirmed that all group B mice (MuAstV and DSS) were shedding MuAstV and that all group D mice (DSS only) were free of MuAstV. MuAstV RNA levels in the feces of group B mice were 7 to 8 times higher at 6 dpi as compared with the levels at 13 and 20 dpi. In contrast, MuAstV RT-PCR analysis of feces from individual group B mice on 26 dpi (that is, 6 d after DSS treatment) showed that none of them shed MuAstV any longer.

All mice in groups B (MuAstV and DSS) and D (DSS only) lost weight (2% to 20%, Table 2) during the 9-d period that they drank water containing 2% DSS. In addition, 5 mice (three male and 2 female; 4 NLRP3$^{-/-}$and 1 NLRP3$^{+/-}$; 1 from group B and 4 from group D) were euthanized prior to the conclusion of the experiment (1 on day 7 and 4 on day 8 after DSS) due to weight loss greater than 15%. Mice in groups B and D drank an average of 3.4 mL during the 9 d they were receiving DSS (Table 2). After euthanasia, a clinical score was determined according to fecal consistency and the presence of fecal blood. A few mice had normally formed feces with no evidence of bleeding, but most mice had semiformed, dark-red feces, and a few mice had no evidence of feces but gross rectal bleeding. The average clinical

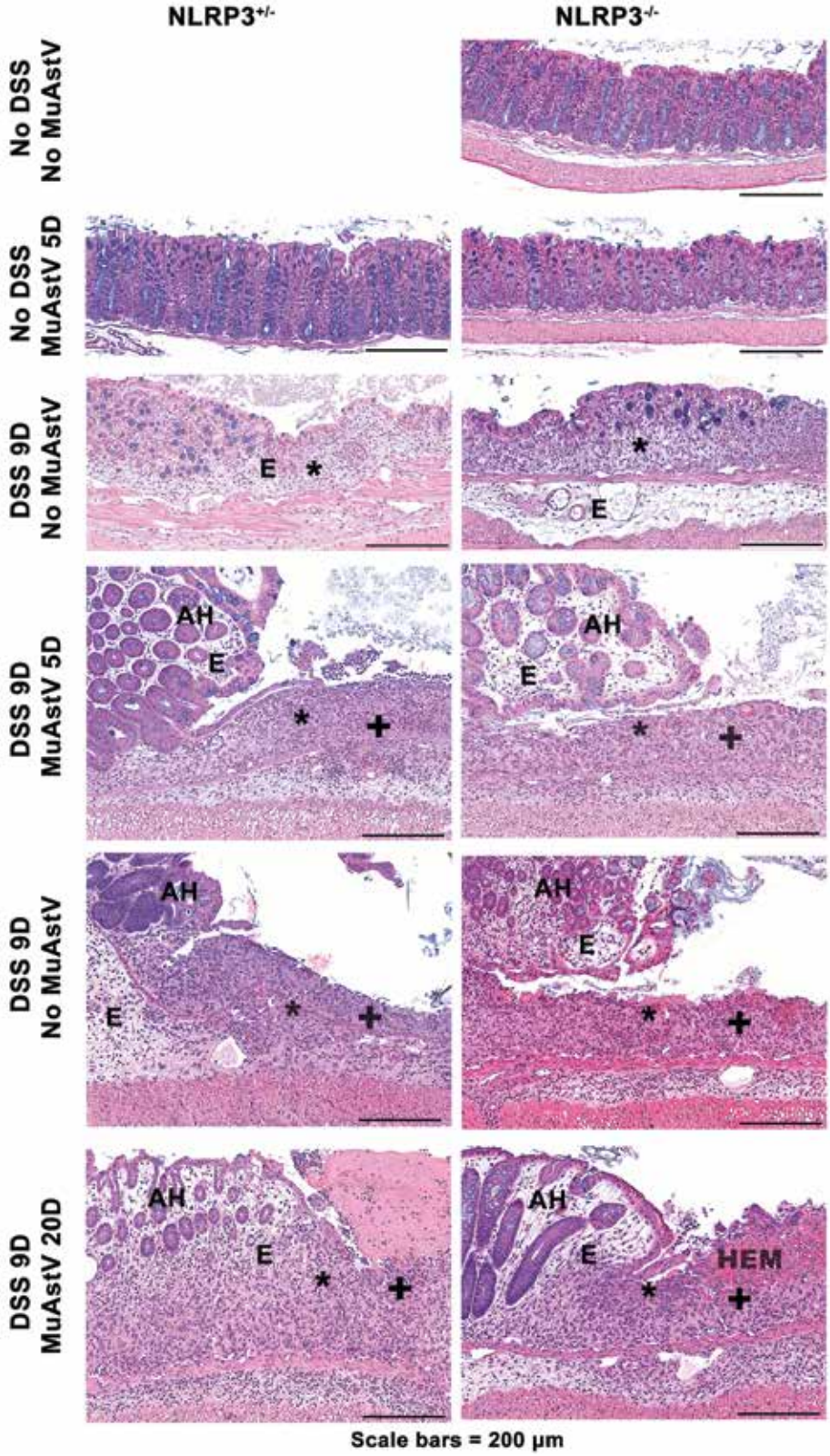

Figure 1. Representative sections of mouse colon. Left panels, heterozygous NLRP3$^{+/-}$ mice; right panels, homozygous NLRP3 $^{-/-}$ mice. Row 1, left panel: Blank; histopathologic analysis of uninfected, untreated NLRP3$^{+/-}$ mice was not performed. Row 1, right panel: experiment 1 group C NLRP3 $^{-/-}$ mice that did not receive DSS or MuAstV have an unremarkable, normal colon. Row 2: experiment 1 group A mice that received MuAstV have an unremarkable, normal colon. Row 3: experiment 1 group D mice that received DSS for 9 d. Row 4: experiment 1 group B mice that were infected with MuAstV for 5 d prior to receiving DSS for 9 d. Row 5: experiment 2 group D mice that received DSS for 9 d. Row 6: experiment 2 group D mice that were infected for 20 d with MuAstV prior to receiving DSS for 9 d. There were no differences in crypt loss (*), edema (E), ulceration (+), inflammation severity, or adenomatous hyperplasia (AH) between NLRP3 $^{-/-}$ and NLRP3$^{+/-}$ mice that received DSS for 9 d in both experiments regardless of whether the mice were also infected with MuAstV for 5 or 20 d (rows 3 through 6). Other changes included ulceration and hemorrhage (HEM).

Table 2. Average clinical data and histopathologic scores in mice that received DSS during experiment 2

Group:inoculum	Sex	Genotype	*n*	Weight loss (%)	Water intake (mL)	Clinical score	Histopathology score
B: MuAstV	male	homozygous	6	15	3.6	2.7	15.2
B: MuAstV	male	heterozygous	3	14		2.0	13.5
B: MuAstV	female	homozygous	7	11	3.1	1.7	14.8
B: MuAstV	female	heterozygous	3	11		1.7	14.2
D: media	male	homozygous	6	15	3.7	2.1	14.6
D: media	male	heterozygous	4	16		1.9	13.7
D: media	female	homozygous	7	14	3.2	2.5	15.9
D: media	female	heterozygous	2	8		2.0	13.3
Overall			38	13	3.4	2.1	14.7

score for the 38 mice was 2.1 (Table 2). There were no significant differences in the average clinical scores observed between mice that were or were not infected with MuAstV, between female and male mice, or between NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice. The combined histopathology scores for the mice in groups B and D mice ranged from 11.0 to 17.5 (Table 2). All mice had mild to moderate colonic hyperplasia (average score, 2.7) mild to marked edema (average score, 3.5), mild to marked inflammation (average score, 3.4), mild to marked crypt loss (average score, 3.1) and mild to marked ulceration in the epithelium of the colon (average score, 2.1). No significant differences in individual or combined histopathology scores were present between male and female mice or between mice in groups B and D. Although the combined histopathology scores differed statistically between homozygous and heterozygous mice (15.1 compared with 13.7; $P = 0.02$), the difference is probably not clinically significant.

Average clinical scores for the group B and D mice did not differ between the 2 experiments (1.8 compared with 2.1; Tables 1 and 2). In addition, average histopathology scores for the group B and D mice did not differ between the experiments (14.9 compared with 14.7; Tables 1 and 2).

Discussion

We noted no clinical signs or histopathologic changes in the colons of NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice infected with MuAstV, similar to what has previously been reported in MuAstV- infected Rag, Stat1, C57BL/6, and SW mice.[8,35] In addition, we saw no effect of MuAstV on DSS-induced colitis in NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice. Clinical scores, histopathology scores, and percentage weight loss were consistent between the 2 experiments, regardless of duration of MuAstV infection. Therefore, our hypothesis that MuAstV would alter the severity of DSS-induced colitis in NLRP3 mice was disproven.

In experiment 1, female mice that were infected with MuAstV and received DSS treatment (group B) had higher average clinical scores than female mice that received only DSS treatment (group D). Although the difference was statistically significant ($P = 0.02$), this difference was not mirrored in the histopathology scores or by differences in weight loss, and the percentage weight loss did not differ between the females in groups B and D in experiment 1. In addition this difference in clinical scores did not occur in the second experiment. In general, the clinical score did not correlate well with histopathology scores or percentage weight loss. Therefore, conclusions based on subjective clinical scores may not be corroborated by more objective data, such histopathology scores and weight loss.

In light of reported differences in the severity of DSS-induced colitis in female and male C57Bl/6 mice[3] and the recent NIH recommendation that both males and females be used in animal studies, our experimental populations included both female and male mice. We did not observe any difference in the severity of DSS-induced colitis between male and female mice. Previous studies of DSS-induced colitis in NLRP3$^{-/-}$ used male mice only[2,36] or did not report the sex of mice evaluated.[4,5,15] To our knowledge, this report is the first to compare the severity of DSS-induced colitis between female and male NLRP3$^{-/-}$ mice.

In the second experiment, a statistically significant ($P = 0.02$)—but probably clinically irrelevant—difference in the combined histopathology scores, but not in clinical scores or weight loss percentage, emerged between NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice. This difference did not occur in the first experiment. Previous DSS-induced models of colitis in NLRP3$^{-/-}$ mice have used C57Bl/6 mice as the control mice rather than NLRP3$^{+/-}$ mice.[2,4,5,15,35] In all 5 previous studies, the severity of colitis differed between the NLRP3$^{-/-}$ and C57Bl/6 mice. In most of the studies, the source of the C57Bl/6 mice and how the mice were housed were unclear. In one study,[4] cohousing of the NLRP3$^{-/-}$ and C57Bl/6 mice abrogated the difference in colitis severity that emerged when the 2 genotypes of mice were housed separately. In our study, 8- to 10-wk-old NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice were cohoused for 5 d (first experiment) or 20 d (second experiment) prior to administration of DSS. Apparently the *NLRP3* gene has less effect on DSS-induced colitis than previously reported and that the previously observed difference in colitis severity likely was due to differences between the microbiomes of the NLRP3$^{-/-}$ and C57Bl/6 mice. The role of intestinal microbiome in the severity DSS-induced colitis has been reported in several other strains of genetically engineered mice, including ASC$^{-/-}$, Casp1$^{-/-}$, Casp3/11$^{-/-}$, and NLRP6$^{-/-}$ mice, where the severity of the colitis in the wildtype mice increased after they were cohoused with the genetically engineered mice.[7,10] Prior to infection, both the NLRP3$^{-/-}$ and C57Bl/6 mice would have contributed to the microbiome of NLRP3$^{+/-}$ mice during the breeding and neonatal periods, whereas only NLRP3$^{-/-}$ mice would have contributed to the microbiome of the NLRP3$^{-/-}$ mice. By cohousing NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice prior to DSS administration, we attempted to normalize the microbiomes between the 2 genotypes of mice. Ideally, NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice would have been littermates and thus exposed to the same microbiome from birth.[19] DSS treatment has been shown to transiently alter the microbiome in the mouse intestine, skewing the microbiome toward an inflammation-associated microbiota characterized by increases in *Bacteroidetes*, *Deferribacteraceae*, and *Enterobacteriaceae*.[30] Whether a DSS-induced change in the microbiome played a role in the clearance of MuAstV from the intestine of DSS-treated mice, whether the cell type(s) susceptible to MuAstV infection were lost due to mucosal ulceration, or whether the DSS-induced inflammatory response resulted in clearance of the virus remains unknown currently.

In conclusion, MuAstV did not influence the severity of DSS-induced colitis in NLRP3$^{-/-}$ and NLRP3$^{+/-}$ mice, and careful experimental design to account for microbiome effects is essential in murine intestinal studies where inflammation is a component of the disease.

Acknowledgments

This work was funded by grants from the American College of Laboratory Animal Medicine and Grants for Laboratory Science (GLAS) from the American Association for Laboratory Animal Science. We thank Alison Faruolo for technical assistance.

References

1. **Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J.** 2004. NALP3 forms an IL1β-processing inflammasome with increased activity in Muckle–Wells autoinflammatory disorder. Immunity **20:**319–325.
2. **Allen IC, TeKippe EM, Woodford RT, Uronis JM, Holl EK, Rogers AB, Herfarth HH, Jobin C, Ting JP.** 2010. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. J Exp Med **207:**1045–1056.
3. **Bábíčková J, Tothova L, Lengyelova E, Bartonova A, Hodosy J, Gardlik R, Celec P.** 2015. Sex differences in experimentally induced colitis in mice: a role of estrogens. Inflammation **38:**1996–2006.
4. **Bauer C, Duewell P, Lehr HA, Endres S, Schnurr M.** 2012. Protective and aggravating effects of NLRP3 inflammasome activation in IBD models: influence of genetic and environmental factors. Dig Dis **30 Suppl 1:**82–90.
5. **Bauer C, Duewell P, Mayer C, Lehr HA, Fitzgerald KA, Dauer M, Tschopp J, Endres S, Latz E, Schnurr M.** 2010. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. Gut **59:**1192–1199.
6. **Bosch A, Pinto RM, Guix S.** 2014. Human astroviruses. Clin Microbiol Rev **27:**1048–1074.
7. **Brinkman BM, Becker A, Ayiseh RB, Hildebrand F, Raes J, Huys G, Vandenabeele P.** 2013. Gut microbiota affects sensitivity to acute-DSS-induced colitis independently of host genotype. Inflamm Bowel Dis **19:**2560–2567.
8. **Compton SR, Booth CJ, Macy JM.** Murine astrovirus infection and transmission in neonatal CD1 mice. J Am Assoc Lab Anim Sci **56:**402–411.
9. **De Benedictis P, Schultz-Cherry S, Burnham A, Cattoli G.** 2011. Astrovirus infections of humans and animals—molecular biology, genetic diversity and interspecies transmissions. Infect Genet Evol **11:**1529–1544.
10. **Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, Flavell RA.** 2011. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell **145:**745–757.
11. **Farkas T, Fey B, Keller G, Martella V, Egyed L.** 2012. Molecular detection of novel astroviruses in wild and laboratory mice. Virus Genes **45:**518–525.
12. **Grove KA, Smith PC, Booth CJ, Compton SR.** 2012. Age-associated variability in susceptibility of Swiss Webster mice to MPV and other excluded murine pathogens. J Am Assoc Lab Anim Sci **51:**789–796.
13. **Haneklaus M, O'Neill LAJ.** 2015. NLRP3 at the interface of metabolism and inflammation. Immunol Rev **265:**53–62.
14. **Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez J, Shulman GI, Gordon JI, Hoffman HM, Flavell RA**. 2012. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature **482**:179–185.
15. **Hirota SA, Ng J, Leung A, Khajah M, Parhar K, Li Y, Lam V, Potentier MS, Ng K, Bawa M, McCafferty DM, Rioux KP, Ghosh S, Xavier RJ, Calgan SP, Tschopp J, Muruve D, McDonald JA, Beck PL.** 2011. The NLRP3 inflammasome plays a key role in the regulation of intestinal homeostasis. Inflamm Bowel Dis **17:**1359–1372.
16. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
17. **Kanneganti TD.** 2010. Central roles of NLRs and inflammasomes in viral infection. Nat Rev Immunol **10:**688–698.
18. **Kjeldsberg E, Hem A.** 1985. Detection of astroviruses in gut contents of nude and normal mice. Arch Virol **84:**135–140.
19. **Laukens D, Brinkman BM, Raes J, DeVos M, Vandenabeele P.** 2015. Heterogeneity of the gut microbiome in mice: guidelines for optimizing experimental design. FEMS Microbiol Rev **40:**117–132.
20. **Leemans JC, Cassel SL, Sutterwala FS.** 2011. Sensing damage by the NLRP3 inflammasome. Immunol Rev **243:**152–162.
21. **Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM.** 2006. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature **440:**228–232.
22. **Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J.** 2006. Gout-associated uric acid crystals activate NALP3 inflammasome. Nature **440:**237–241.
23. **Marvin SA, Huerta CT, Sharp B, Frieden P, Cline TD, Schultz-Cherry S.** 2015. Type I interferon response limits astrovirus replication and protects against increased barrier permeability in vitro and in vivo. J Virol **90:**1988–1996.
24. **Meliopoulos VA, Marvin SA, Freiden P, Moser LA, Nighot P, Ali R, Blikslager A, Reddivari M, Heath RJ, Koci MD, Schultz-Cherry S.** 2016. Oral administration of astrovirus capsid protein is sufficient to induce acute diarrhea in vivo. MBio **7:**1–6.
25. **Moser LA, Carter M, Schultz-Cherry S.** 2007. Astrovirus increases epithelial barrier permeability independently of viral replication. J Virol **81:**11937–11945.
26. **Ng TFF, Kondov NO, Hayashimoto N, Uchida R, Cha Y, Beyer AI, Wong W, Pesavento PA, Suemizu H, Meunch MO, Delwart EL.** 2013. Identification of an astrovirus commonly infecting laboratory mice in the US and Japan. PLoS Pathog **8:**1–9.
27. **O'Connor W JR, Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y, Kolls JK, Flavell RA.** 2009. A protective function for interleukin 17A in T cell-mediated intestinal inflammation. Nat Immunol **10:**603–609.
28. **Phan TG, Kapusinsky B, Wang C, Rose RK, Lipton HL, Delwart EL.** 2011. The fecal viral flora of wild rodents. PLoS Pathog **7:**1–16.
29. **Rajan JV, Rodgiguez D, Miao EA, Aderem A.** 2011. The NLRP3 inflammasome detects encephalomyocarditis virus and vesicular stomatitis virus infection. J Virol **85:**4167–4172.
30. **Schwab C, Berry D, Rauch I, Rennisch I, Ramesmayer J, Hainzl E, Heider S, Decker T, Kenner L, Muller M, Strobl B, Wagner M, Schleper C, Loy A, Urich T.** 2014. Longitudinal study of murine microbiota activity and interactions with the host during acute inflammation and recovery. ISME J **8:**1101–1114.
31. **Sutterwala FS, Haasken S, Cassel SL.** 2014. Mechanism of NLRP3 inflammasome activation. Ann N Y Acad Sci **1319:**82–95.
32. **Tschopp J, Martinon F, Burns K.** 2003. NALPs: a novel protein family involved in inflammation. Nat Rev Mol Cell Biol **4:**95–104.
33. **Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt E, Ravussin E, Stephens JM, Dixit VD.** 2011. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med **17:**179–188.
34. **Wang X, Jiang W, Yan Y, Gong T, Han J, Tian Z, Zhou R.** 2014. RNA viruses promote activation of the NLRP3 inflammasome through a RIP1–RIP3–DRP1 signaling pathway. Nat Immunol **15:**1126–1133.
35. **Yokoyama CC, Loh J, Zhao G, Stappenbeck TS, Wang D, Huang HV, Virgin HW, Thackray LB.** 2012. Adaptive immunity restricts replication of novel murine astroviruses. J Virol **86:**12262–12270.
36. **Zaki MH, Boyd KL, Vogel P, Kastan MB, Lamkanfi M, Kanneganti TD.** 2010. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity **32:**379–391.