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Original Research

Lack of Effect of Murine Norovirus Infection on the CD4$^+$CD45RB^{high} T-cell Adoptive Transfer Mouse Model of Inflammatory Bowel Disease

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Murine norovirus (MNV) infection is highly prevalent in laboratory mice. Although MNV infection does not typically induce clinical disease in most laboratory mice, infection may nonetheless affect mouse models of disease by altering immune responses. We previously reported that MNV altered the bacterial-induced mouse model of inflammatory bowel disease (IBD) using *Helicobacter*-infected *Mdr1a*$^{-/-}$ mice. Therefore, we hypothesized that MNV infection would exacerbate another mouse model of IBD, the T-cell adoptive transfer (AT) model. In this model, *Helicobacter* infection is used to accelerate the progression of IBD induced by AT of naïve CD4$^+$CD45RB^{high} T cells into B6.129S7-*Rag1*^{tm1Mom}/J (*Rag1*$^{-/-}$) mice. We evaluated the effects of MNV infection in both *Helicobacter*-accelerated as well as *Helicobacter*-free AT models. In our studies, *Helicobacter*-infected *Rag1*$^{-/-}$ mice that received CD4$^+$CD45RB^{high} T cells through AT rapidly developed weight loss and typhlocolitis; MNV infection had no effect on disease severity or rate of progression. In the absence of *Helicobacter* infection, progression of IBD caused by AT of CD4$^+$CD45RB^{high} T cells was slower and typhlocolitis was less severe; this inflammation likewise was unaltered by MNV infection. These results indicate that MNV infection does not alter IBD progression and severity in the CD4$^+$CD45RB^{high} T-cell AT model in *Rag1*$^{-/-}$ mice.

Abbreviations: AT, AT; CFSE, carboxyfluorescein diacetate succinimidyl ester; IBD, inflammatory bowel disease; MNV, murine norovirus

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Inflammatory bowel disease (IBD) is a progressive and multifactorial disease that affects millions of people.[38] The pathogenesis of the disease is still not understood, but a combination of poorly regulated immune responses, genetics, and host microbiota are involved.[1] Murine models of IBD have been instrumental tools to further our understanding of factors that drive disease development and progression as well as to investigate potential preventatives and therapeutics. The adoptive transfer (AT) model, induced by transferring naïve CD4$^+$CD45RB^{high} T cells into a lymphopenic host, is a well-characterized mouse model of IBD in which typhlocolitis is the result of disrupted T-cell homeostasis.[10,24,39,44,49] Therefore, this model has been used extensively to help determine factors that influence the onset, severity, and penetrance of bowel inflammation triggered by aberrant T-cell responses.

Murine norovirus (MNV) is a highly prevalent enteric RNA virus of laboratory mice that does not induce clinical disease in many stocks and strains of mice.[4,20,43] It has been shown to alter some mouse models of disease but produces only minimal changes in others.[5,14,16,18,46] For example, our laboratory previously reported that MNV infection exacerbates IBD progression in *Helicobacter bilis*-infected *Mdr1a*$^{-/-}$ mice (FVB.129P2-*Abcb1a*^{tm1Bor}) but does not cause changes in IBD scores or colon tumor incidence in *Smad3*$^{-/-}$ mice (129-*Smad3*^{tm1Par}/J).[28,29] The effect of MNV infection on IBD has also been studied in *Il10*$^{-/-}$ mice with mixed results depending on the background strain of the mouse: mice on a C57BL/6J background (B6.129P2-*Il10*^{tm1Cgn}/J or JZtm) had no changes in cecal and colonic inflammation,[17] whereas mice on a C3H/HeJBir background (C3Bir.129P2-*Il10*^{tm1Cgn}/JZtm) showed increased colonic inflammation at 4 wk after MNV infection.[5] These mixed results highlight the importance of evaluating the effects of MNV on mouse models of IBD to determine its potential to confound research findings.

This study investigated whether MNV affected colitis development in the T-cell AT model of IBD. Enteric organisms are known to influence the severity and progression of disease in the AT model. In this model, naïve T cells adoptively transferred into lymphopenic recipient mice proliferate extensively in response to the lymphopenic conditions, and gut microbes appear to play a significant role in disease development by eliciting an uncontrolled inflammatory response.[10,24,26,42,49] Adoptive transfer of CD4$^+$CD45RB^{high} T cells into germ-free mice, monocolonized or reduced-enteric flora mice, or mice treated with antibiotics results in absent or significantly reduced disease,[3,21,33,37,45] whereas the presence of *Helicobacter* spp. can accelerate disease

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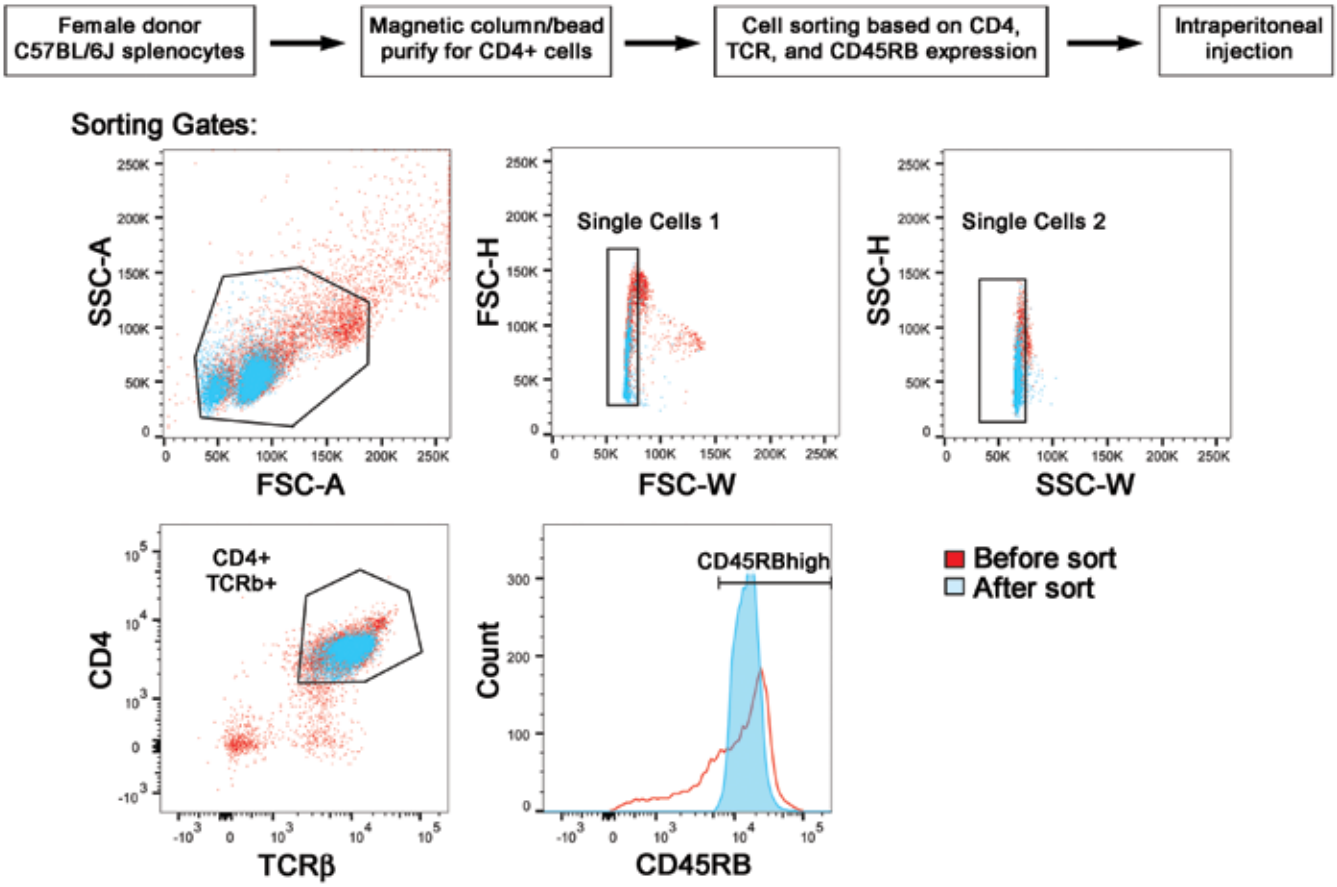

Figure 1. Collection of cells and sorting gate strategy to isolate $CD4^{+}CD45RB^{high}$ T cells for AT.

development in mice with normal enteric flora.[7,30] Because *Helicobacter* is a common enteric bacteria in laboratory mice, we evaluated the effect of MNV infection on the AT model of IBD using *H. bilis* to accelerate disease development and on this model of IBD without *H. bilis*. We determined that MNV infection does not significantly alter the AT mouse model of IBD.

Materials and Methods

Animals. Female recombination-activating gene 1 knockout (*Rag1*$^{-/-}$, B6.129S7-*Rag1*^{tm1Mom}/J) and C57BL/6J mice (age, 6 to 8 wk) were purchased from The Jackson Laboratory (Bar Harbor, ME) for experiments evaluating IBD. To evaluate T-cell proliferation, male Thy1.1 C57BL/6J mice (B6.PL-*Thy1*^{a}/CyJ, The Jackson Laboratory) and male *Rag1*$^{-/-}$ mice (age, 10 to 12 wk) were bred inhouse and used. Mice were housed in autoclaved IVC (Thoren, Hazleton, PA) with autoclaved corncob bedding (The Andersons, Maumee, OH) in an SPF facility and were fed standard irradiated chow (PicoLab Rodent Diet 20, 5053, PMI Nutrition, St Louis, MO) without restriction. Autoclaved, reverse-osmosis–purified, acidified water was provided in bottles without restriction. Mice were certified by the vendor to be free of specific rodent pathogens including ectoparasites, endoparasites, *Pneumocystis murina*, *Helicobacter* spp., known enteric and respiratory bacterial pathogens and antibodies to MNV, mouse hepatitis virus, Sendai virus, pneumonia virus of mice, reovirus 3, Theiler murine encephalomyelitis virus, ectromelia virus, polyoma virus, lymphocytic choriomeningitis virus, mouse adenovirus, minute virus of mice, mouse parvovirus, mouse rotavirus, mouse cytomegalovirus, mouse thymic virus, Hantaan virus, K virus, *Encephalitozoon cuniculi*, cilia-associated respiratory bacillus, *Mycoplasma pulmonis*, and *Clostridium piliforme*. Mice were maintained under SPF conditions and group-housed. During experimental infections with MNV and *H. bilis*, mice were handled according to infection status to prevent cross-contamination. All manipulations were performed in a vertical flow animal transfer station (AniGard II, Baker, Sanford, ME) disinfected with chlorine dioxide (dilution, 1:18:1; Clidox S, Pharmacal Research Laboratories, Naugatuck, CT). All animal procedures were approved by the University of Washington's IACUC.

Experimental infection and AT. *H. bilis* and MNV4 (passage 7) were grown and propagated as previously described.[17] Prior to experimental manipulations, mice were acclimated for at least 1 wk and confirmed to be negative for MNV and *Helicobacter* spp. through RT-PCR and PCR analyses, respectively. At the end of each study, MNV and *H. bilis* infection status was confirmed through fecal RT-PCR and PCR analyses, respectively, as previously described.[17]

To evaluate MNV infection in the AT model of IBD accelerated by *H. bilis* infection, 8-wk-old female *Rag1*$^{-/-}$ mice (n = 33) were given 2×10^{7} cfu of *H. bilis* in 0.2 mL of *Brucella* broth by oral gavage at 5 d prior to AT. For the AT, all mice (n = 33) were intraperitoneally injected with 1.3×10^{5} $CD4^{+}CD45RB^{high}$ cells, obtained through FACS of cells from syngeneic female C57BL/6J donor mice. At 3 d after the AT, the mice were split into 2 groups—one group (n = 17) was infected with 1×10^{6} pfu of MNV4 through oral gavage, whereas the other group (n = 16) was sham-inoculated with 0.2 mL of uninfected clarified RAW 264.7 cell lysate. Mice were weighed at least twice each week. Animals that lost 20% or more of their baseline weight were euthanized by using an inhaled overdose of carbon dioxide gas

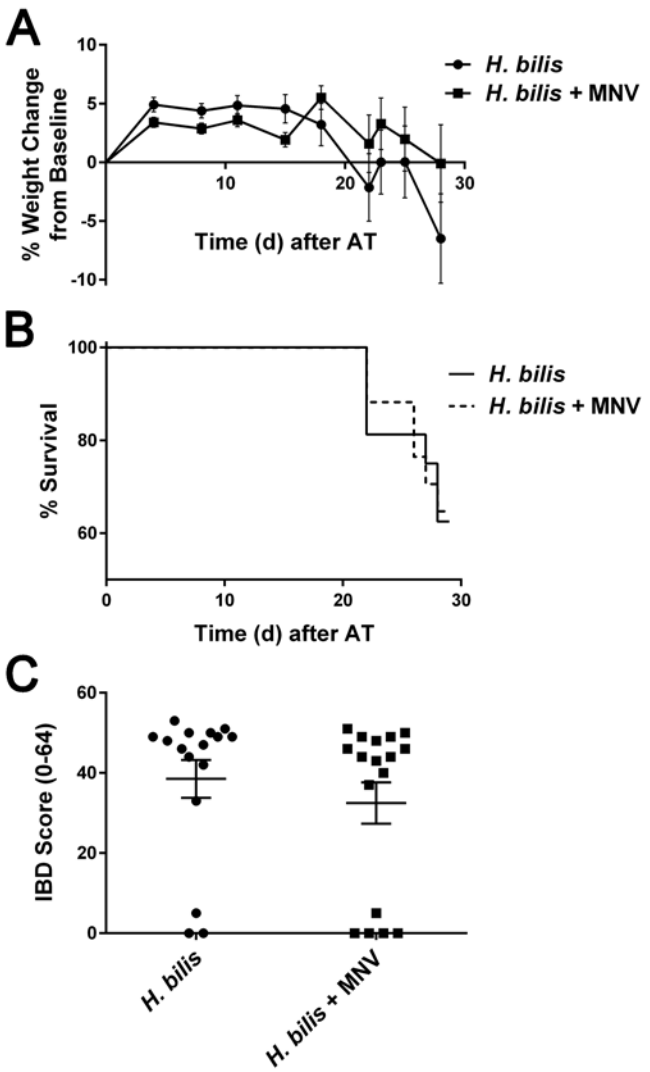

Figure 2. MNV4 does not alter (A) weight loss, (B) survival, or (C) IBD score in the $CD4^+CD45RB^{high}$ T-cell AT mouse model of IBD using a bacterial accelerator of inflammation, *H. bilis*. All *Rag1*$^{-/-}$ mice were inoculated with *H. bilis* 5 d before AT of $CD4^+CD45RB^{high}$ T cells and then infected with MNV4 (n = 17) or mock infected (n = 16) at 3 d after AT. Data are given as mean ± SEM (error bars). AT, adoptive transfer.

and necropsied; the entire study was terminated when 50% of the mice within an experimental group reached this weight loss criterion. This study termination criterion was used to attempt to balance our ability to observe altered time to disease development as well as to discern differences in histologic disease severity between groups at a specific time point.

To evaluate MNV infection in the AT model of IBD without *H. bilis*, 8-wk-old female *Rag1*$^{-/-}$ mice were intraperitoneally injected with 1.3×10^5 $CD4^+CD45RB^{high}$ cells and infected with MNV4 either prior to or after AT. Mice infected prior to AT received 1×10^6 pfu of MNV4 by oral gavage at 7 d before AT (MNV–AT group), whereas mice infected after AT received 1×10^6 pfu of MNV4 by oral gavage at 3 d after AT (AT–MNV group). Sham inoculations were performed by oral gavage of uninfected clarified RAW 264.7 cell lysates. Within the experiment, all mice received the same number (that is, 2) of oral gavages; when one group (for example, MNV–AT group) received MNV4 by gavage, all remaining mice in the other 2 groups (for example, control and AT–MNV groups) received sham inoculations. This experiment was performed 3 times, with 12 to 17 mice per group. Mice were weighed at least once weekly. Animals that lost 20% or more of their baseline weight were euthanized with an inhaled overdose of carbon dioxide gas and necropsied. Additional euthanasia criteria in these experiments included animals that developed marked clinical signs, such as severe dermatitis, blepharitis, and respiratory distress. The entire study was terminated when 50% of the mice within an experimental group reached these euthanasia criteria.

To evaluate MNV infection on T-cell proliferation after AT into a lymphopenic host, isoflurane-anesthetized 10- to 12-wk-old male *Rag1*$^{-/-}$ mice received through retroorbital injection 1×10^6 $CD4^+$ T cells that had been labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE). The $CD4^+$ T cells were obtained from the spleens of donor male Thy1.1 C57BL/6J mice by using negative selection ($CD4^+$ T Cell Isolation Kit II, Miltenyi Biotec, Auburn, CA) and then stained with 5 µM CFSE (CellTrace CFSE Cell Proliferation Kit, ThermoFisher Scientific, Waltham, MA). One group of mice (n = 3) was infected with 1×10^6 pfu of MNV4 through oral gavage at 2 d before AT of CFSE-labeled $CD4^+$ T cells, whereas another group (n = 3) was infected 3 d after AT. Control animals (n = 4) received CFSE-labeled $CD4^+$ T cells but were sham-gavaged with uninfected RAW 264.7 cell lysate. Mice were euthanized by an inhaled overdose of carbon dioxide gas at 5 d after AT. Splenocytes from these mice were stained with antibodies specific for CD4 (RM4-5; eBioscience, San Diego, CA) and CD90.1/Thy1.1 (OX7; BD Biosciences, San

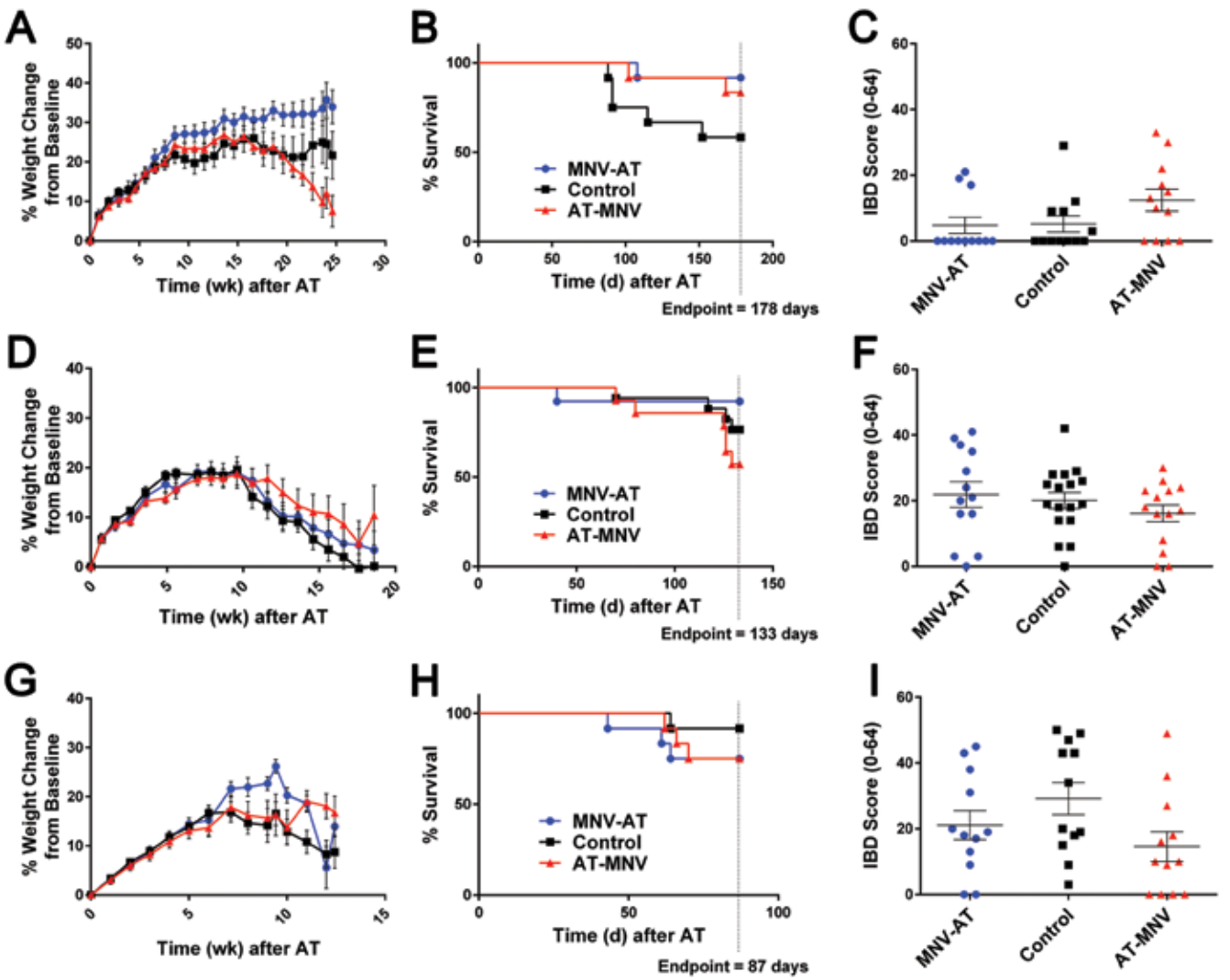

Figure 3. Effects of MNV4 on (A, D, and G) weight loss, (B, E, and H) survival, and (C, F and I) IBD score in the $CD4^+CD45RB^{high}$ T-cell AT mouse model of IBD in the absence of an accelerator of inflammation, *H. bilis*. *Rag1*$^{-/-}$ mice were infected with MNV4 at 7 d before AT of $CD4^+CD45RB^{high}$ T cells (MNV–AT), infected 3 d after AT (AT–MNV), or mock-infected (control). Results of 3 independent experiments are shown: (A through C) study 1 (n = 12 mice per group); (D through F) study 2 (n = 13 to 17 mice per group), and (G through I) study 3 (n = 12 mice per group). Statistical differences between groups in percent weight change from baseline: (A) MNV–AT compared with control: weeks 18 through 22 and 24 until endpoint; control compared with AT–MNV: weeks 22 to endpoint; MNV–AT compared with AT–MNV: week 18 to endpoint. (D) Control compared with AT–MNV: week 15 and endpoint. (G) MNV–AT compared with control: weeks 8 through 11; control compared with AT–MNV, week 11 to endpoint; MNV–AT compared with AT–MNV, weeks 9 and 12. Data are given as mean ± SEM (error bars). AT, adoptive transfer.

Jose, CA) and evaluated by FACS. Single-cell lymphocytes were gated according to forward and side light scatter and then antibody surface markers to obtain $CD4^+Thy1.1^+$ cells. T-cell proliferation in the $CD4^+Thy1.1^+$ cell population was evaluated through CFSE expression, similar to methods previously described,[11] to differentiate cells that were undergoing spontaneous proliferation or homeostatic proliferation or that remained undivided.

Histopathology and inflammation scoring. The cecum and colon were collected, processed, and scored for IBD through histologic exam, as previously described.[17] Additional tissues including lung, esophagus, stomach, liver, and small intestine were immersion-fixed in 10% phosphate-buffered formalin and prepared for routine histologic exam.

FACS. To isolate $CD4^+CD45RB^{high}$ cells, single-cell suspensions were generated from the spleens of 8-wk-old, female, syngeneic C57BL/6J mice. RBC were lysed, and $CD4^+$ cells were isolated through negative selection ($CD4^+$ T Cell Isolation Kit II, Miltenyi Biotec). $CD4^+$-enriched cells were blocked with antiCD16/CD32 antibody (2.4G2, BD Biosciences) and then stained with antiCD4 (RM4-5; eBioscience), antiTCRβ (H57-597; BD Biosciences), and antiCD45RB (16A; BD Biosciences) antibodies. FACS was performed (FACSAria II, BD Biosciences) to obtain $TCR\beta^+CD4^+CD45RB^{high}$ cells (Figure 1) The purities of sorted cells exceeded 98% for all AT experiments.

Statistical analysis. Statistical analyses were performed by using Prism version 5 or 6 (GraphPad Software, San Diego, CA). The unpaired Student t test or Mann–Whitney test was used to compare 2 groups. For more than 2 groups, one-way ANOVA with the Tukey multiple comparisons test or the Kruskal–Wallis test with the Dunn multiple-comparisons test was used to compare IBD scores and T-cell proliferation, whereas 2-way ANOVA with the Tukey multiple-comparisons test was used to compare body weight at each time point. Survival curves were evaluated by using the Mantel–Cox log-rank test. Average weights were calculated from surviving mice within each group at each time point. P values less than 0.05 were considered statistically significant.

Results

Lack of MNV4-induced effect on typhlocolitis in the *Helicobacter*-accelerated T-cell AT model of IBD. We first sought to determine whether MNV infection altered typhlocolitis in the *H. bilis*-accelerated T-cell AT model of IBD. *Rag1*$^{-/-}$ mice all received CD4^{+}CD45RB^{high} T cells to induce colitis and *H. bilis* to accelerate disease, and then mice were split into 2 groups to compare MNV4-infected mice with sham-infected controls. As expected, mice developed clinical signs consistent with IBD, including diarrhea, weight loss (Figure 2 A) and decreased survival (Figure 2 B) beginning approximately 2 to 3 wk after AT. At 4 wk after AT, the study was terminated, and large bowel inflammation was evaluated by histopathology. Average percentage of weight change from baseline did not differ significantly at any time point between mice infected with MNV4 compared with mice without MNV4. Neither percent survival nor IBD score (Figure 2 C) differed between mice infected with MNV4 compared with those without MNV4. These data suggest that MNV4 infection does not alter the *H. bilis*-accelerated T-cell AT model of IBD.

Lack of MNV4-induced effect on typhlocolitis in the *Helicobacter*-free T-cell AT model of IBD. Because *H. bilis* infection in the T-cell AT model of IBD results in rapid and severe disease development, we reasoned that the severe disease might mask any subtle effects of MNV4 on inflammation. Therefore, we tested whether MNV4 infection altered this model of IBD in the absence of *H. bilis*. In addition, we determined whether the timing of MNV4 infection differentially influenced disease development by testing 2 infection time points relative to AT (that is, 7 d before and 3 d after AT) and compared these treatment groups with animals that received AT only.

In our initial study, control animals and mice infected with MNV4 after AT (AT–MNV group) began to lose weight at approximately 15 wk after AT, and the average percent weight change was significantly different between the 2 groups beginning at week 22 after AT until the end of study ($P \leq 0.026$ for each weekly comparison) (Figure 3 A) Mice administered MNV4 prior to AT (MNV–AT group) continued to gain weight throughout the study, and the average percent weight change was significantly different from control mice beginning at week 18 ($P = 0.0232$), at endpoint ($P = 0.0067$), and intermittently between these time points ($P \leq 0.0254$ for each weekly comparison). However, there were no significant differences between any of the groups when comparing survival (Figure 3 B) or IBD scores (Figure 3 C). Because the clinical assessment of weight loss suggested a potential difference between groups that was not supported by the survival and IBD scores, we performed 2 additional experiments to validate our findings. In the second study, the average percent weight change for all groups began to decrease beginning at approximately 10 wk after AT, with significant differences between control and AT–MNV groups at week 15 ($P = 0.0443$) and at endpoint only ($P = 0.0104$) (Figure 3 D). Similar to the initial study, there were no differences between any of the groups in survival (Figure 3 E) or IBD scores (Figure 3 F). In the final study, the average percent weight change was significantly different between the MNV–AT and control groups from week 8 through 11 ($P \leq 0.0405$ for each weekly comparison) and between the control and AT–MNV groups from week 11 until endpoint ($P \leq 0.035$ for each weekly comparison) (Figure 3 G). Again, neither survival (Figure 3 H) nor IBD score (Figure 3 I) differed between any of the groups. Collectively, the results from these 3 independent experiments indicate that MNV4 infection does not alter IBD in the T-cell AT model of colitis.

Lack of MNV4-induced effect on T-cell proliferation after AT. Cell expansion occurs in lymphopenic mice after AT with CD4^{+} or CD4^{+}CD45RB^{high} T cells.[11,25,32,34,39,42] To determine whether MNV infection altered T-cell proliferation after AT, splenic Thy1.1^{+}CD4^{+} T cells labeled with CFSE were adoptively transferred into recipient *Rag1*$^{-/-}$ mice that had been infected with MNV4 either 2 d before or 3 d after AT. Cells transferred into sham-infected mice served as controls. CFSE dilution of AT T cells in recipient splenocytes was evaluated by flow cytometry to determine whether T cells remained undivided or underwent spontaneous or homeostatic proliferation (Figure 4 A). The percentage of CD4^{+}Thy1.1^{+} T cells that remained undivided or that underwent spontaneous or homeostatic proliferation did not differ between MNV4-infected animals compared with sham-infected controls (Figure 4 B). These results indicate that MNV4 infection does not alter CD4 T-cell proliferation after AT.

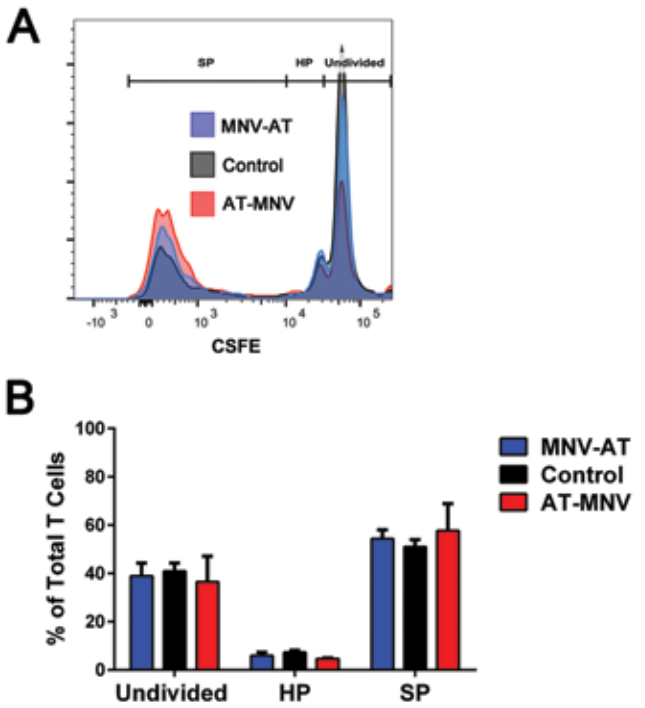

Figure 4. Effects of MNV4 on CD4^{+} T-cell proliferation after AT into *Rag1*$^{-/-}$ mice. Mice were infected with MNV4 at 2 d before AT of CSFE-labeled CD4^{+} T cells ($n = 3$, MNV–AT), 3 d after AT ($n = 3$, AT–MNV), or were mock infected ($n = 4$, control). (A) CD4^{+}Thy1.1^{+} splenic cells were evaluated for CFSE expression at 5 d after AT to assess whether cells were undergoing spontaneous or homeostatic proliferation or remained undivided (a representative sample from each group is shown; histogram overlays are normalized to the total area under the curve). (B) MNV4 does not alter CD4^{+} T-cell proliferation compared with uninfected controls. Data are given as mean ± SEM (error bars). AT, adoptive transfer; HP, homeostatic proliferation; SP, spontaneous proliferation.

Inflammation is induced in extracolonic tissues of mice that received T-cell AT. During the evaluation of MNV4 infection in the T-cell AT model of IBD without *H. bilis*, we observed inflammation independent of MNV4 infection status in several extracolonic organs of the mice (Table 1 and Figure 5) Clinically, the inflammatory lesions were most evident as multifocal ulcerative dermatitis, blepharitis (unilateral or bilateral; affecting both upper and lower eyelids), and occasional respiratory signs, such as increased respiratory rate and effort at rest and delayed recovery of normal respiration after handling. Histologically, in addition to inflammation in the cecum and colon, inflammation was noted in other digestive and nondigestive tissues, including the lungs, esophagus, stomach, small intestine, and liver.

Table 1. Incidence of extracolonic inflammation in mice in the T-cell AT model of IBD without *H. bilis*

	Study 1			Study 2			Study 3		
	MNV–AT	Control	AT–MNV	MNV–AT	Control	AT–MNV	MNV–AT	Control	AT–MNV
Skin	2/12	4/12	4/12	1/13	3/17	1/14	0/12	2/12	0/12
Eyelids	2/12	4/12	1/12	2/13	2/17	4/14	4/12	6/12	3/12
Lung	11/11	12/12	11/11	13/13	17/17	13/14	12/12	12/12	12/12
Stomach	11/11	12/12	11/11	13/13	16/17	13/14	12/12	12/12	11/12
Small intestine	10/11	11/12	11/11	nd	nd	nd	nd	nd	nd
Esophagus	9/11	12/12	11/11	12/13	15/16	8/10	7/11	12/12	5/12

nd, not done

Data are provided as the number of mice with inflammation / total number of mice available for analysis
Data for skin and eyelids are based on clinical observation; those for lung, stomach, small intestine, and esophagus were based on histologic evaluation

Pulmonary lesions consisted of pyogranulomatous foci composed of numerous multinucleated giant cells and multifocal accumulations of neutrophils, pulmonary artery medial hyperplasia, moderate perivascular and peribronchiolar inflammation, medial and epithelial hyperplasia, and foci of acidophilic macrophage pneumonia (Figure 5 A through B and data not shown). The distal esophagus had proliferative lymphohistiocytic and neutrophilic esophagitis (Figure 5 C), and the stomach showed hyperplasia and early delamination of the mucosa near the limiting ridge of the nonglandular area (data not shown), and the glandular stomach had moderate mucosal lymphocytic accumulations (Figure 5 D). Mild lymphocytic and proliferative enteritis was present in the duodenum (Figure 5 E). The liver showed mild inflammation and early fibrosis in centrilobular and periportal regions, with periportal lymphocytic and lesser fibrosing cholangiohepatitis (Figure 5 F). Although the primary purpose of this mouse model is to study IBD, these findings demonstrate that inflammation of nontarget tissues can occur in these mice and may need to be considered.

Discussion

MNV is a highly prevalent virus in research mice and can cause persistent infections.[4,14,19,43] The virus has a tropism to infect many cell types, including dendritic cells, macrophages, and B cells; more recently it was discovered that MNV can infect T cells and a rare type of intestinal epithelial cells, tuft cells.[12,22,27,48,50] Taken together, these characteristics of MNV support the possibility that infection might alter immune signaling, inflammatory responses, and disease outcomes in laboratory mice. The results from our laboratory and others have been variable, with some mouse models showing differences due to MNV infection,[5,6,15,16,18,23,29,35,40,41,46] whereas other mouse models show minimal to no effects after infection.[2,9,13,14,18,28]

Previous work by our laboratory investigated the effects of MNV infection on various mouse models of inflammatory diseases, such as IBD.[17,28,29] IBD is a chronic inflammatory disorder of the gastrointestinal tract and includes both ulcerative colitis and Crohn's disease. Although the precise pathogenic mechanisms of IBD are unknown, many experimental mouse models of IBD are available to help dissect the complex interactions between host genetics, immune cell function, and the gut microbiota under both regulatory and inflammatory conditions.[10,24,36,49] Studies of MNV infection in various mouse models of IBD have revealed that infection can exacerbate IBD progression in *Mdr1a*$^{-/-}$ mice infected with *H. bilis* but does not induce changes in IBD scores or incidence of colon cancer in *Smad3*$^{-/-}$ mice when compared with uninfected mice.[28,29] In *Il10*$^{-/-}$ mice, the genetic background strain of the mouse contributed to whether MNV caused changes in disease progression. *Il10*$^{-/-}$ mice on a C3H/HeJBir background but not a C57BL/6J background showed changes in colonic inflammation after infection.[5,17] In *Atg16L1* mutant mice, infection with MNV was necessary—along with a Crohn's disease susceptibility gene (*Atg16L1*), commensal bacteria, and dextran sodium sulfate treatment—to cause intestinal disease resembling various aspects of disease in patients with Crohn's disease.[6,51] Therefore, to expand on these previous findings regarding MNV infection and mouse models of IBD, we here determined the effect of MNV infection on the CD4^{+}CD45RB^{high} T-cell AT model of IBD.

The CD4^{+}CD45RB^{high} T-cell AT model of IBD is a widely used model of colitis that highlights the importance of the balance between effector cells that cause inflammation and regulatory cells that control inflammation. In this model, naïve CD4^{+}CD45RB^{high} T cells are injected into syngeneic, lymphopenic mice and cause colitis when they robustly proliferate in response to gut microbes in the absence of regulatory T cells that suppress inflammation and that are normally present in mature T-cell populations (CD4^{+}CD45RB^{low}).[24,26,42] Therefore, coadministration of naïve (CD4^{+}CD45RB^{high}) and mature (CD4^{+}CD45RB^{low}) T cells together—or administration of the mature T-cell population alone—to recipient lymphopenic mice does not result in colitis.[3,34,42] Given that this model is driven by the administration of naïve CD4^{+}CD45RB^{high} T cells, that CD4^{+} T cells recognize antigens in the context of MHC class II molecules present on professional antigen-presenting cells including macrophages, dendritic cells, and B cells, and that MNV has a tropism to infect macrophages, dendritic cells, B cells, and T cells,[12,22,50] we hypothesized that MNV infection would alter the CD4^{+}CD45RB^{high} T-cell AT model of IBD. MNV infection did not significantly change weight loss (with some exceptions at a few time points), survival, or IBD scores compared with uninfected mice both with the bacterial accelerator of inflammation, *H. bilis*, or without it. We also evaluated the proliferation of CD4^{+} T cells after AT in the context of MNV infection. Reportedly, cell expansion occurs in lymphopenic mice after AT with CD4^{+} or CD4^{+}CD45RB^{high} T cells.[11,25,32,34,39,42] Cell proliferation can be characterized as spontaneous proliferation, which occurs rapidly in

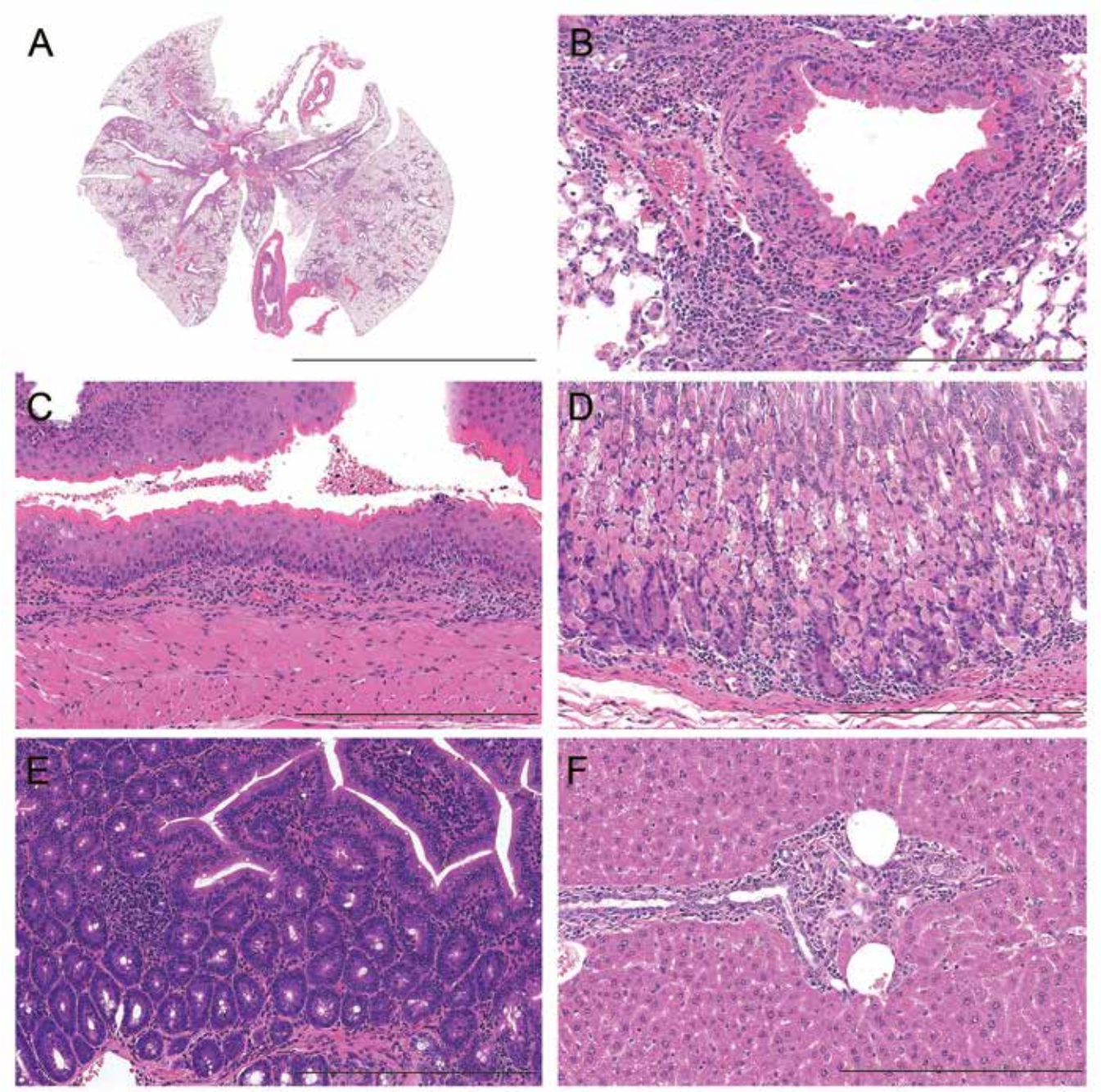

Figure 5. Lymphohistiocytic and neutrophilic inflammatory infiltrates present at the mucosal–submucosal interface and within the epithelium, lamina propria, and submucosa. (A) Moderately affected lung. (B) Bronchiole from lung in panel A, showing epithelial hyalinization and peribronchiolar and perivascular mixed inflammatory infiltrate. (C) Distal esophagus. Proliferative and lymphohistiocytic and neutrophilic esophagitis. (D) Glandular stomach. Mild to moderate mucosal lymphocytic accumulation. (E) Duodenum. Mild lymphocytic and minimal proliferative enteritis. (F) Liver. Mild periportal lymphocytic and minimal fibrosing cholangiohepatitis. Hematoxylin and eosin stain; scale bars: A, 100 mm; B through F, 250 µm.

severely lymphopenic hosts (for example, *Rag1*$^{-/-}$ mice), or homeostatic proliferation, which occurs more slowly and is more dominant in mildly lymphopenic hosts (for example, after irradiation).[11,25,31,32] As expected, the majority of the administered CD4^{+} T cells were undergoing spontaneous proliferation, but cell proliferation did not differ between MNV-infected mice and uninfected controls. This lack of a MNV-associated effect on CD4^{+} T-cell proliferation is in alignment with our histologic data, which showed no differences in IBD score between MNV-infected and uninfected mice.

Similar to our study, other work using the CD4^{+}CD45RB^{high} T-cell AT model has likewise occasionally revealed inflammation in extracolonic organs.[8,26,37,39,42] In our AT studies without the bacterial accelerator *H. bilis*, we observed that some animals began to develop clinical signs of disease, including alopecia, dermatitis, blepharitis, and respiratory abnormalities, beginning approximately 1 to 2 mo after AT. Significant differences in body weight between MNV-infected and uninfected control groups occurred at a few late time points and could not be explained by differences in colonic inflammation, given the lack of significant

difference in IBD scores between groups at endpoint. Therefore, we speculate that the inflammation in these other extracolonic organs may have perhaps contributed to the differences in body weight observed at a few time points between groups in our studies. Histologically, we observed inflammation in several extracolonic organs, including the lung, esophagus, stomach, and liver. Adoptively transferred T cells were harvested from donor mice that were syngeneic to recipient mice, reducing the likelihood that the inflammation was caused by graft-versus-host disease. We speculate that we did not see the same extracolonic disease in adoptively transferred mice that received *H. bilis* due to the shorter study duration caused by the induced acceleration of inflammation in the colon. Interestingly, although the exact mechanism of the extracolonic inflammation in this animal model is unknown, people with IBD similarly occasionally exhibit extraintestinal manifestations affecting joints, skin, the hepatobiliary tract, lung, and eye.[47] The exact pathogenesis of the extracolonic inflammation in people is unknown, but one suggested possibility is a leaky intestinal barrier that allows the translocation of gut bacteria, thus triggering an immune response in other organs due to cross reactive epitopes between bacteria and self-antigens.[47] In contrast, others have reported that, although intestinal bacteria are indeed required for the development of colitis after AT of $CD4^+CD45RB^{high}$ T cells, the development of interstitial pneumonia is independent of intestinal bacteria given that lung inflammation occurred even in germ free conditions.[37] Therefore, although AT of $CD4^+CD45RB^{high}$ T cells to lymphopenic mice is a useful model for studying T-cell–mediated immune reactions in IBD, these results are a reminder that inflammation in other organs can occur and might serve as a model for extraintestinal inflammation in patients with IBD.[37]

In conclusion, we evaluated the effect of MNV infection on the $CD4^+CD45RB^{high}$ T-cell AT model of colitis both with and without a bacterial accelerator of disease, *H. bilis*. We demonstrated that MNV infection does not significantly alter IBD scores in this model, despite its reported ability to alter other models of IBD.[5,6,29] Considering that MNV is highly prevalent and even endemic in many research mice colonies, our findings add to knowledge regarding MNV infection and its potential effects on mouse models of inflammation.

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