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Transient or persistent norovirus infection does not alter the pathology of *Salmonella typhimurium* induced intestinal inflammation and fibrosis in mice

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ABSTRACT

Murine noroviruses (MNV) are currently the most prevalent viruses infecting mouse research colonies. Concurrent infection of research mice with these viruses can dramatically alter the experimental outcome in some research models, but not others. In this report, we investigated the effect of MNV1 and MNV4 on a murine model of intestinal inflammation and fibrosis induced by *Salmonella typhimurium* infection in C57BL/6 mice. Subsequent co-infection of these mice with MNV1 or MNV4 did not lead to major changes in histopathology, the inflammatory response, or the fibrotic response. Thus, MNV does not substantially alter all gastrointestinal research models, highlighting the importance of investigating potential alterations in the research outcome by MNV on an individual basis. We hypothesize that this is particularly important in cases of research models that use immunocompromised mice, which could be more sensitive to MNV infection-induced changes.

1. Introduction

Murine noroviruses (MNV) are non-enveloped, positive-strand RNA viruses that belong to the *Norovirus* genus in the *Caliciviridae* family. Most noroviruses infect humans and cause the vast majority of sporadic cases and outbreaks of infectious nonbacterial gastroenteritis worldwide in people of all ages [1–3]. However, noroviruses infecting mice [4–6], cattle [7–9], pigs [10,11], sheep [12], a lion cub [13], and a dog [14] have also been described. The first murine norovirus, MNV1, was isolated in 2003 from immunocompromised mice [4] and shown to replicate in macrophages and dendritic cells *in vitro* and *in vivo* [15–18]. We and others have since shown that MNV1 also causes a transient infection in immunocompetent inbred and outbred mice [5,6,18,19]. Peak viral titers are seen between 1 and 3 days post-infection (dpi) in the intestine, mesenteric lymph nodes, and spleen, with viral clearance by day 7 [5,18]. Like the human noroviruses, multiple MNV strains have been isolated. Of the ones that are characterized (*e.g.* MNV4, CR6), many persistently infect wild-type animals and viral genomes can be detected in the small intestine, mesenteric lymph nodes and feces of infected animals 1–2 months post-infection [5,6]. Despite the different biological phenotypes, of the 15 strains analyzed in one report, all cluster in one genogroup and form one serogroup [5].

MNV is the most prevalent virus in research mice [20]. Serologic analysis of mice from research colonies in North America and Europe demonstrated that 2–64% of all mice

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had antibodies against MNV1 [19–22]. Similar prevalence rates (22–23%) are seen in Japan and South Korea after RT-PCR analysis of murine fecal samples [23,24] with a lower rate of genome positive samples (13%) found in the cecum [25]. Eradication of MNV from research facilities requires complete depopulation, a potentially large financial burden for the research community [26]. Therefore, the impact of MNV for biomedical research is actively being investigated. Early indications suggest that MNV has the ability to alter the outcome of some research investigations but will likely require evaluation on a case-by-case basis. To date, striking abnormalities in Paneth cell function were observed in mice with gene-trap-mediated disruptions of Atg16L1, a Crohn's disease susceptibility marker, when infected with the CR6 MNV strain but not MNV1 [27]. More subtle histopathologic changes have been described in immunocompromised [16,17] and immunocompetent mice [27]. In one report, MNV4 altered antigen presentation by dendritic cells and was associated with exacerbation of a bacterial-induced inflammatory bowel disease model in *Mdr1a*−/− mice [28]. In another model, previous infection of BALB/cByJ mice with MNV-G led to an increased mouse parvovirus genome load in tissues and increased duration of mouse parvovirus shedding [29]. In contrast, previous infection of C57BL/6 mice with MNV1 and/or CR6 had no effect on vaccinia virus and influenza A virus-induced $CD8^+$ T-cell and antibody responses or lethality [30]. Similarly, MNV infection did not impact murine cytomegalovirus (MCMV) titers or reactivation, but decreased $CD8^+$ T-cell responses to immunodominant MCMV epitopes [31]. Furthermore, CR6 infection does not alter Friend retrovirus-induced immune responses (T cell, NK cell and antibody responses) or the course of infection (viremia, titers, pathology) [32]. These varying responses of MNV in research outcomes led Ammann et al. [32] to suggest that MNV co-infections might be more likely to alter responses to infections in the gastrointestinal tract. To better understand the impact of MNV on a research model of inflammation and intestinal fibrosis, we investigated the effect of the transient MNV1 and persistent MNV4 strains on a *Salmonella typhimurium*-induced intestinal fibrosis model in C57BL/6 mice. Treatment of mice with streptomycin followed by infection with *S. typhimurium* ΔaroA produces a persistent bacterial infection of the cecum [33]. Subsequent intestinal colitis, characterized by transmural inflammation, hypertrophy of the muscle layers, and excessive extracellular matrix deposition, produces an inflammatory disease phenotype similar to inflammatory bowel disease in humans [33]. However, no major effect of MNV1 or MNV4 on the histopathology and inflammatory response were observed in this model. Thus, we propose that MNV is more likely to affect research outcomes in intestinal models using immunocompromised mice.

2. Materials and methods

2.1. Mice, bacterial strains, virus stocks

Specific pathogen-free female 8–10 week old C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME).

Salmonella typhimurium strain ΔaroA (a kind gift from G. Grassl, University of British Columbia, Vancouver, Canada) which is naturally resistant to streptomycin was grown in LB broth containing 100 μg/ml streptomycin at 37 °C. Infectious titers were determined by plating serial dilutions of the inoculum onto LB/streptomycin plates.

The plaque purified MNV1 isolate MNV1.CW3 [5] (referred herein as MNV1) was used at passage 6 for all experiments. MNV4 was a kind gift from R. Livingston (University of Missouri, Columbia) and was used at passage 2. A concentrated virus stock and corresponding mock lysate was generated as described [34] for both MNV strains and used in all experiments.

2.2. Animal studies

In three independent experiments, female C57BL/6 mice were divided into six groups. Animals in the three *S. typhimurium* infection groups received 20 mg streptomycin by oral gavage followed by infection with 2×10^6 colony forming units (cfu) of *S. typhimurium* ΔaroA in 100 μl 0.1 M HEPES buffer (pH = 8.0) 24 h later. Two days after the *S. typhimurium* infection, one group (St + M1) was co-infected orally with 2×10^7 plaque forming units (PFU) of MNV1 per mouse; a second group (St + M4) was co-infected 2×10^7 PFU of MNV4 per mouse; and a third group (St, or positive control) was orally given an equal volume of concentrated mock lysate. The additional three groups were not infected with *S. typhimurium* ΔaroA but received 20 mg streptomycin. The negative control group (no Tx) and MNV only groups were gavaged with 0.1 M HEPES buffer at the same time point as the *S. typhimurium* infection. Two days later, the MNV only groups were infected orally with 2×10^7 PFU MNV1 per mouse (MNV1 group) or 2×10^7 PFU MNV4 per mouse (MNV4 group), while the negative control group was orally given the same volume of mock lysate. Mice were euthanized 21 days post *S. typhimurium* infection. All animal experiments were conducted with the approval and oversight of the University of Michigan Committee on Use and Care of Animals in accordance with all federal guidelines.

2.3. Gross pathology and tissue collection

Serum, fecal pellets, small intestine, mesenteric lymph nodes, cecum and distal colon samples were collected 21 days post *S. typhimurium* infection, the time point of harvest. Cecum and distal colon were photographed, measured, and weighed. Cecal area was determined from digital photographs using NIH ImageJ in a region of interest (ROI) that included only the cecum. To control for differences in images, the cecal area was normalized against a 1 cm marker in the photographic image. Tissues were snap-frozen in liquid nitrogen and stored at −80 °C prior to analysis. Cecal contents were collected and serially diluted before plating onto LB-streptomycin plates to determine bacterial titers in the cecum.

2.4. Histology

Formalin-fixed and paraffin-embedded tissues were stained with Masson's trichrome by the University of