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The impact of primary and persistent cytomegalovirus infection on the progression of acute colitis in a murine model

Jerry L. Brunson [a,b,c], Felix Becker [a,d], Karen Y. Stokes [a,b,c,*]

[a] *Department of Molecular and Cellular Physiology, LSU Health Sciences Center, 1501 Kings Hwy, Shreveport, LA 71130, United States*
[b] *Center for Molecular and Tumor Virology, LSU Health Sciences Center, 1501 Kings Hwy, Shreveport, LA 71130, United States*
[c] *Center for Cardiovascular Disease and Sciences, LSU Health Sciences Center, 1501 Kings Hwy, Shreveport, LA 71130, United States*
[d] *Department of General and Visceral Surgery, University Hospital Muenster, Germany*

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Abstract

Cytomegalovirus (CMV) infects 60–100% of the population worldwide. CMV has been implicated in many diseases through the induction of inflammation. Inflammatory bowel disease (IBD) affects over 1 million Americans annually. IBD, in particular ulcerative colitis, has been associated with CMV infection. Here we use a murine model to test if both primary and persistent CMV infections exacerbate colitis. C57Bl/6J mice were injected with Mock inoculum or murine CMV (mCMV) 4 d (primary infection) or 6 wks (persistent infection) before inducing colitis. Colitis was induced by administering 3% DSS (dextran sodium sulfate) in the drinking water for 6 days. Distilled water was given to controls. Disease activity index (DAI), derived from scores for stool consistency, body weight loss, occult blood, and rectal bleeding, was recorded daily. DAI increased early with DSS treatment in Mocks when compared with water-treated controls. This was accelerated by both primary and persistent mCMV and appeared to be primarily due to the earlier appearance of gross bleeding vs. their Mock controls. Mocks reached similar DAI values by day 6. Myeloperoxidase was modestly elevated in the mCMV 4 d-DSS over the Mock 4 d-DSS, however there was no such synergism in the 6 wk groups. Histology was comparable in Mock and mCMV groups. Taken together our findings show that mCMV accelerated the development of acute colitis although a milder model of colitis may be needed to better delineate the impact of the virus on disease progression. Further work focusing on disruption of barrier function and bleeding may help determine the underlying mechanisms.

Keywords: Ulcerative colitis; Dextran sodium sulfate; Cytomegalovirus; Inflammation; Gross bleeding; Disease activity index

1. Introduction

Inflammatory bowel disease (IBD) is a general term for autoimmune diseases of the intestines, which are characterized by chronic or recurrent inflammation. There are two main types of IBD, ulcerative colitis (UC) and Crohn's disease (CD). It is estimated that 1–1.3 million people are affected by IBD in the United States with peak onset ranging in age from 15 to 30 [1,2]. CD can affect any part of the gastrointestinal tract. Inflammation is transmural and discontinuous within the intestinal wall and surrounded by healthy intestinal tissue. Occurrence rates of CD have been increasing worldwide particularly in developing countries; however, it is more common in the developed world [3]. UC is limited to the colon and only affects the inner mucosal layer. The disease is intermittent with periods of mild or no symptoms, interspersed with periods where symptoms are more severe. UC has a higher prevalence (7.6 to 246 cases per 100,000 persons per year) than CD (0.03 to 15.6 cases per 100,000 persons per year). Similar to CD, UC incidence rates are higher in

* Corresponding author at: Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130-3932, United States. Tel.: +1 318 675 8420; fax: +1 318 675 6005.
E-mail address: kstoke@lsuhsc.edu (K.Y. Stokes).

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developed countries particularly those in northern Europe and north America [4]. This is due in part to the western lifestyle and diet [5].

The prevailing understanding of IBD initiation is that host genetic factors and environmental factors lead to immune cell activation (i.e. T-cells, mucosal dendritic cells) against normal microbial flora of the intestines resulting in the development of chronic colonic inflammation. The primary causative agent has not been identified; however, many possibilities are being investigated including *Yersinia enterocolitica* [6] and *Mycobacterioum paratuberculosis* [7,8]. The establishment of chronic inflammation can result in the recruitment and activation of other pathogens which can result in exacerbation of IBD through the upregulation of NF-κB and inflammatory cytokines and chemokines. This includes human cytomegalovirus (HCMV), which is associated with exacerbated IBD [9,10].

HCMV is a β-herpesvirus that infects a majority of the population worldwide. Infection occurs primarily during childhood and the virus establishes a lifelong persistent infection. In addition, HCMV can infect almost every organ system in the body including the intestines. 70% of patients with CD are seropositive for CMV although less than 5% of tissue and stool samples are CMV positive [11,12]. Evidence for a role of this virus in CD has been limited. However, the link between HCMV and UC is stronger [13–18]. The virus is prevalent in tissues of these patients [19] and reactivation of the virus in UC patients is associated with steroid-resistance during treatment [16]. The exact role of CMV in IBD is unknown. This virus may be recruited to inflamed intestinal tissue through the recruitment of immune cells (i.e. monocytes), and once present in the inflamed bowels will become reactivated due to inflammatory signaling (TNF-α, IFN-γ). Reactivated virus will in turn activate pro-inflammatory pathways and may also increase the permeability of the gut [20,21]. While murine CMV has been shown to exacerbate colitis in persistent/latent models of infection, there has been no side-by-side comparison of the impact of CMV on colitis during primary and persistent phases of infection.

This study sought to determine whether both primary and persistent CMV infection exacerbates colitis, so that it could be determined in future work if underlying mechanisms differ during these phases. We used the murine model of dextran sodium sulfate (DSS)-induced colitis, which results in acute colitis characterized by loose stool, bleeding, and weight loss. This is due to epithelial injury and a robust inflammatory response in the colon [22]. We induced colitis during either the primary or persistent murine CMV (mCMV) infection period to determine if we could detect worse disease score and histological changes.

2. Materials, methods & techniques

Wildtype C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Mice (3–5 wks old) were injected with Mock inoculum or mCMV (Smith Strain) at 3×10^4 PFU i.p. as described previously [23]. These mice were split into 2 cohorts based on time post-inoculation (p.i.) when colitis was induced: 4 d = primary infection, or 6 wk = persistent infection. To induce colitis, mice were given 3% DSS (molecular weight, 40 kDa; ICN Biomedicals, Aurora, OH, USA) in distilled water as their drinking water (ad libitum), while distilled water was given to control mice. Treatments were given for 6 days. $n = 4$ and 3 in the Mock 4 d + H_2O and Mock 6 wk + H_2O groups, and 6–8/grp in the other groups. Animal handling procedures were approved by the LSU Health Institutional Animal Care and Use Committee and were in accordance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health.

A previously validated disease activity index (DAI) monitoring was performed daily beginning on day 0 (just prior to DSS treatment) and continued through 6 days of DSS treatment. Several parameters were included in the assessment: stool (normal = 0; loose = 2; diarrhea = 4), occult blood as determined by a guaiac paper test (ColoScreen; Helena Laboratories Inc., Beaumont, TX, USA [24] (negative = 0; positive =2), gross blood (negative = 0, positive = 4) and weight change (compared the original body weight: <1% = 0; 1–5% = 1; 5–10% = 2; 10–15% = 3 and >15% = 4) [25].

At day 6, mice were weighed, and anesthetized with ketamine and xylazine i.p. Colons were harvested. In the 6 wk group, the harvested colons were weighed, and the colon length was measured. In all mice, samples of colon were placed in formalin for histology. The histological score was determined by a blinded observer using a previously published method [26], taking into account severity of inflammation, extent of injury, as well as crypt damage. The maximum possible score was 40.

A piece of colon was also taken for measurement of myeloperoxidase (MPO), as an indicator of inflammatory infiltrate. Colonic tissue was cleaned, weighed, and snap frozen for storage at −80 °C until subsequent analysis. The *o*-dianisidine method [27] was used for measurement of MPO activity and output was expressed as the amount of enzyme necessary to produce a change in absorbance of 1.0 unit per minute per gram of tissue (U/g tissue).

2.1. Statistical analysis

All values are reported as mean ± SEM. ANOVAs were performed using Fisher's post hoc test to compare experimental groups with a statistical significance set at $P \leq 0.05$.

3. Results

3.1. Primary mCMV infection

During primary infection (DSS administered at 4 days p.i.), the DAI of both Mock and mCMV DSS groups were