

Immunocompetent Adults from Human Norovirus Challenge Studies Do Not Exhibit Norovirus Viremia

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Noroviruses (NoV) are among the leading causes of acute gastroenteritis worldwide (1). There is also limited knowledge of the pathogenesis of human NoV infection. Children, immunocompromised adults, and gnotobiotic pigs and calves used as models of human NoV infection all can develop NoV viremia (2–7), suggesting that NoV infection may not be limited to the gut. However, no data on NoV viremia in immunocompetent adults exist.

To determine whether immunocompetent adults develop NoV viremia, we tested samples from NoV-infected adults who participated in two challenge studies, which are described fully elsewhere (8, 9). On the basis of our power calculations, we chose 38 serum samples from 20 subjects (13 Norwalk virus [NV]-infected subjects and 7 Snow Mountain virus [SMV]-infected subjects). On average, samples were from day 4.6 postchallenge, the same as the average day of peak viral shedding in stool. NV-infected subjects had a median of 3 consecutive samples tested per subject (range, 1 to 3), selected from samples taken during the peak of viral shedding in stool. SMV-infected subjects had 1 sample tested per subject, selected from the day of peak viral shedding in stool. Our sample was large enough to detect an absence of viremia with more than 95% power, $\alpha = 0.05$, and assuming a null hypothesis of at least 15% of subjects with viremia, a lower rate than that seen in pediatric NoV or gnotobiotic models (2, 3, 6, 7). Serum samples were taken on the days of greatest viral shedding in stool, as quantified by reverse transcription-PCR (RT-PCR) (10), because a past study identified higher fecal NoV load in children with viremia, leading us to assume that days of highest NoV shedding in stool would be most likely to be associated with viremia (3). All samples were stored at -80°C prior to testing.

Viral RNA was extracted from 140 μl of 20% stool suspension or 140 μl of serum using QIAamp viral RNA minikit (Qiagen, Valencia, CA), following the manufacturer's instructions. A stool sample with known high NoV titer, a NoV-seeded serum sample, and 140 μl molecular-grade water were used as positive and negative extraction controls. *In vitro*-transcribed NV and SMV standards were used for quantification (10). RT-quantitative PCR (RT-qPCR) was performed with a One-Step RT-qPCR kit (Qiagen, Valencia, CA), as described by Kirby et al. (10), using NV- and SMV-specific primers and probes whose sequences have been previously published (10, 11). All reactions were performed in duplicate on a Bio-Rad CFX96 system (Bio-Rad, Hercules, CA). Amplification data were collected and analyzed with the Bio-Rad CFX Manager software (Bio-Rad, Hercules, CA). We repeated RNA extraction and PCR testing on 10 samples to verify our findings. We also performed a dilution series of NoV in serum samples to determine the limit of detection for our assay, which we estimated to be approximately 1×10^1 genomic equivalence copies (GEC) per reaction mixture or 6×10^2 GEC per ml of serum.

NoV was not present at detectable levels in any samples. The

subjects had a mean age of 28 years (standard deviation [SD] = 9.5), 50% were female, 44% were Caucasian, and 39% were African American. Their stool median peak viral titer was 1.2×10^8 genomic equivalence copies/g stool (interquartile range [IQR], 1.6×10^7 to 6.9×10^8), and their median day of peak NoV shedding in stool was day 4 postchallenge (IQR, 4 to 5). These data indicate that an adult can shed high levels of NoV in their stool yet still have no detectable NoV in their blood. This result suggests that an adult with a healthy immune system is capable of preventing NoV from crossing from the gastrointestinal tract into the bloodstream, and viremia may be associated with primary infection. Some limitations of this work include the lack of a true NoV-positive serum sample (such as one from a child or immunosuppressed individual) to use as a positive control, a long sample storage time, and the possibility that NoV may be present but below the limit of detection. This report warrants further investigation into the mechanisms by which NoV infects the bloodstream of susceptible populations yet remains absent in the blood of healthy adults.

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