Nitazoxanide was commenced at a dose of 500 mg twice daily. It was well tolerated with no adverse effects. Initially, an improvement in frequency and severity of diarrhea was noted. Results of PCR analysis of stool specimens became negative for norovirus; however, the MS2 bacteriophage used as an internal control added to the patient sample was also not detected by PCR, indicating inhibition of PCR. Dilution of the sample 1:100 prior to nucleic acid extraction overcame the inhibition, and both the norovirus and internal control became detectable by PCR in the diluted patient sample. Overall, 1% of feces samples that are tested in our laboratory demonstrate PCR inhibition, but consistent PCR inhibition over a series of samples, as seen in this patient, has not been seen before. Treatment with nitazoxanide was, therefore, discontinued after 12 months because of no clinical response and persistent detection of norovirus by PCR (after dilution). We believe that nitazoxanide was not inhibiting Taq polymerase within the PCR, as PCR analysis of feces specimens was consistently inhibited after nitazoxanide therapy was discontinued.

Subsequent to treatment with nitazoxanide, a 12-week trial of ribavirin and pegylated interferon alfa and, separately, a further trial of enteral immunoglobulin via a jejunostomy failed to eradicate norovirus infection.

This case demonstrates the lack of efficacy of nitazoxanide in treating chronic norovirus in an immunocompromised patient, despite previous reports of benefit [1]. Further clinical studies are required to establish the place of nitazoxanide in the treatment of norovirus in immunocompromised patients. Furthermore, the mechanism of the consistent PCR inhibition in this case is unclear. If it is a disease-specific effect, then it may be important to ensure that an internal control is being used in all fecal molecular assays for patients with XLA, to prevent false-negative results due to PCR inhibition.

**Note**

**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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**Reply to Kempf et al**

To the Editor—In their correspondence, Kempf et al report the use of nitazoxanide, a broad-spectrum antimicrobial agent, for the treatment of a chronic norovirus infection in an immunocompromised patient with X-linked agammaglobulinemia. Such individual case reports are valuable because of the lack of approved antivirals for norovirus infections and the increasing reports of enhanced norovirus-associated morbidity and mortality in immunocompromised patients [1].

Nitazoxanide has previously been shown to reduce the duration of symptoms in immunocompetent individuals and to clear infection in a single immunocompromised patient with chronic myeloid leukemia [2]. In contrast, despite noting an initial improvement in diarrhea frequency and severity, Kempf et al report that nitazoxanide treatment failed to eradicate norovirus infection in their patient. Several follow-up experimental treatments also failed to clear the infection, including ribavirin, which has been reported to have mixed success in the treatment of chronic norovirus infections [3].

The mechanism of action of nitazoxanide against norovirus is currently unknown. For other viruses, it has been shown to be 2-fold, involving inhibition of cellular processes that are required for viral infection and potentiation of the innate immune response [4]. Therefore, the disparity among the results of these individual reports suggests that the success of nitazoxanide treatment could be both virus and host specific and, possibly, influenced by the immune status of the patient. To compare these factors, it is essential that future studies of this kind report the genotype and, ideally, the entire viral genome sequence of the infecting virus, alongside a comprehensive description of the patient’s immune status. This additional information will prove invaluable in determining which factors may influence the likelihood of success of the experimental treatments.

Until recently, the lack of a robust and reproducible cell culture system for human noroviruses has presented a major barrier to the development and characterization of antivirals and molecular studies of human norovirus replication. Two recent advances now offer the opportunity to further investigate potential therapeutic approaches and potential strain-specific resistance phenotypes. The demonstration that immortalized B cells allow for limited norovirus replication in cell culture [5] provides one such experimental system. However, the breakthrough development of a stem-cell–derived human enteroid system that supports the replication of multiple human norovirus strains [6] now provides the opportunity to determine the molecular mechanism of action of nitazoxanide and whether its activity is genotype specific. This system also holds great potential for research and development of...
other, more-effective antiviral treatments for acute and chronic human norovirus infections.

Notes

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Staphylococcus epidermidis Small-Colony Variants Are Induced by Low pH and Their Frequency Reduced by Lysosomal Alkalization

To the Editor—We read with interest the article by Leimer et al, which reported that low pH induced Staphylococcus aureus small-colony variants (SCVs) and that phagosomal pH neutralization reduced the frequency of intracellular S. aureus SCVs [1]. SCVs are naturally occurring, slow-growing subpopulations that emerge in response to diverse environmental pressures [2–4]. They typically comprise a minor proportion of the source population and are common in chronic infections [5–9]. Although most reports focus on S. aureus SCVs, other organisms can form them. Particularly, Staphylococcus epidermidis SCVs are common in prosthetic joint infections [10]. To determine where the findings of Leimer et al apply to S. epidermidis, we assessed in vitro effects of low pH and intracellular growth on S. epidermidis colony phenotype.

S. epidermidis strain RP62A and 2 S. epidermidis isolates from prosthetic joint infections, IDRL-8933 and IDRL-8864, were studied. S. aureus strain 6850, previously shown to form SCVs under conditions of low pH [1], was used as a positive control. Bacteria were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum to a starting inoculum of 1.5 × 10^8 colony-forming units (CFU) at 37°C in 5% CO_2 for 5 days, with quantitative cultures performed on days 0, 3, and 5. pH values of 4.5, and 6.5 were achieved using buffers containing Na_2HPO_4 and citric acid at pH values of 2.6, 4.0, and 5.6, respectively. A pH of 7.4 was maintained by adding 50 mM HEPES. CFU were enumerated after overnight incubation on sheep’s blood agar (SBA) at 37°C, and colony phenotype was assessed after additional incubation overnight at room temperature. SCVs were identified on the basis of their size (Figure 1B). Thirty minutes after inoculation, each strain showed a normal colony phenotype, independent of pH. The frequency of SCVs increased over time at a pH of 4.0, ultimately reaching >15%, while at a pH of 7.4, SCVs were noted at a frequency of <8% (Figure 1A). At pH values of 5.5 and 6.5, there was an intermediate percentage of SCVs. These results show a positive correlation between low pH and SCV formation in 3 S. epidermidis strains, recapitulating the published findings that involved S. aureus [1].

To determine whether intracellular localization induces S. epidermidis SCV formation, human lung fibroblast MRC5 cells were infected at a multiplicity of infection of 1. MRC5 cells were washed daily with phosphate-buffered saline (PBS) and lysostaphin or daptomycin (to kill extracellular bacteria). Supernatants were monitored for sterility by plating to SBA plates. At 0, 3, 5, and 7 days after infection, host cells were lysed, and serial 10-fold dilutions of cell lysates were plated onto SBA to detect and quantitate intracellular bacteria. CFU and SCV frequency were measured as described above. Three hours after infection, a mean of 4% of viable intracellular bacteria had a SCV phenotype. The number of viable intracellularly persisting bacteria remained fairly constant over the course of infection (Figure 1D), while the frequency of SCVs increased in the intracellular environment, reaching an average of 23% after 7 days. The discrepancy between the numbers of viable intracellularly persisting bacteria in Leimer et al’s study versus ours may be explained by the lack of antibiotic added to the medium after daily washes. In addition, we examined bacterial intracellular persistence in human lung fibroblast cells, whereas Leimer et al used human lung carcinoma cells.

Our data suggested that low pH favors S. epidermidis SCV formation, as shown for S. aureus [1], and that the intracellular environment may have an analogous effect. The internal localization of persisting bacteria was analyzed by fluorescence microscopy. Intracellular bacteria were found to be localized within LAMP-2 antibody–positive vesicles (Figure 1C), suggesting that intracellularly persisting S. epidermidis organisms primarily reside within phagolysosomes. Samples stained with immunoglobulin G isotype control had negative results (not shown).