Resolution of diarrhea in an immunocompromised patient with chronic norovirus gastroenteritis correlates with constitution of specific antibody blockade titer

Bettina M. Knoll[1] · Lisa C. Lindesmith[2] · Boyd L. Yount[2] · Ralph S. Baric[2] · Francisco M. Marty[3]

Received: 5 November 2015 / Accepted: 7 January 2016 / Published online: 29 January 2016

Abstract Norovirus gastroenteritis in immunocompromised hosts can result in a serious and prolonged diarrheal illness. We present a case of chronic norovirus disease during rituximab-bendamustine chemotherapy for non-Hodgkin's lymphoma. We show for the first time a correlation between norovirus strain-specific antibody blockade titers and symptom improvement in an immunocompromised host.

Keywords Norovirus · Strain-specific antibody blockade titers · Immunocompromised host

Introduction

Noroviruses are the most common cause of non-bacterial gastroenteritis worldwide, with around 21 million cases occurring annually in the United States alone [1]. Three norovirus genogroups (I, II, and IV) infect humans, and are further organized into more than 30 genetic clusters [2]. A single genetic cluster, the GII.4 noroviruses, currently accounts for greater than 80 % of all infections in the US [3]. The major capsid protein of GII.4 strains is evolving rapidly, resulting in new epidemic strains with altered antigenic sites [3]. Usually a self-limiting disease of short duration, infection of immunocompromised hosts can result in serious and prolonged diarrheal illness [4].

Little is known about of the complex relationship between the large number of norovirus strains and host protective immunity. The lack of a cell culture or small animal model for human norovirus infection renders the study of neutralizing antibodies impossible. Measurement of the ability of an antibody to "block" binding of a virus-like particle (VLP) to a carbohydrate ligand can be used as a surrogate neutralization assay [3, 5]. Using this method it has been shown that an increased antibody blockade titer is associated with protection from infection and decreased symptoms in non-immunocompromised norovirus challenged volunteers [6, 7]. This phenomenon has not been studied in immunocompromised hosts. We present data showing that strain-specific antibody blockade titers do correlate with decreased symptoms and virus shedding in a patient with chronic norovirus disease in the setting of underlying immunocompromise due to lymphoma treatment.

Case

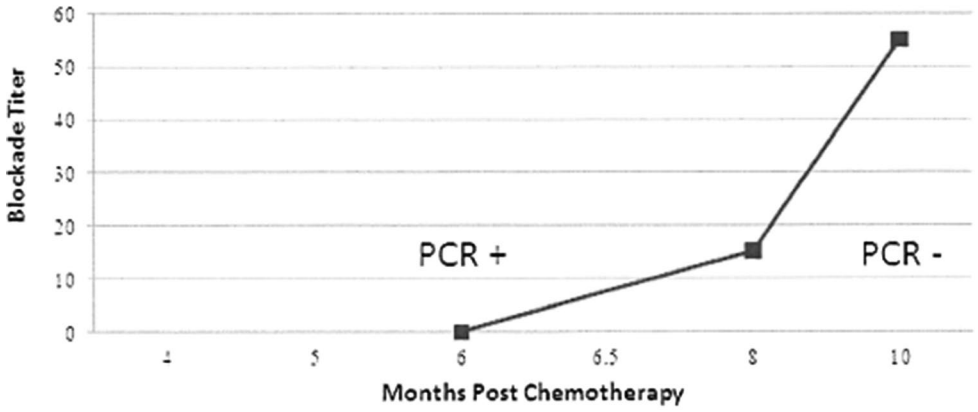
A 64-year-old female with follicular non-Hodgkin's lymphoma had been receiving monthly chemotherapy with rituximab-bendamustine when she developed persistent large volume watery diarrhea without associated fever, abdominal pain, nausea or vomiting a week before receiving her fifth out of six planned cycles of treatment. Colonoscopy, stool cultures for bacterial enteric pathogens and stool exam for ova and parasites were negative 2 weeks after the onset of diarrhea, but testing for norovirus by reverse transcriptase polymerase chain reaction returned positive (Fig. 1). The patient did not receive

✉ Bettina M. Knoll
Bettina.Knoll@wmchealth.org

[1] Division of Infectious Diseases, Department of Medicine, Westchester Medical Center, Valhalla, NY, USA

[2] Department of Epidemiology, University of North Carolina, Chapel Hill, NC, USA

[3] Division of Infectious Diseases, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA



Care:	Supportive	Nitazoxanide	IVIG	IVIG	Supportive	None
IgG (mg/dL)	611	605	NA	NA	NA	901
WBC (K/μL)	4.0	3.5	3.4	2.2	3.1	4.3
ANC (K/μL)	0.5	2.37	2.55	1.47	2.17	3.31
ALC (K/μL)	2.92	0.60	0.41	0.33	0.47	0.47

Fig. 1 Norovirus GII.4.2009 polymerase chain reaction (PCR) negativity correlates with increased strain-specific blockade titer (*filled square*). Blockade titer is the reciprocal of the dilution of serum that blocked 50 % of GII.4.2009 binding to carbohydrate ligand. Measurements of total immunoglobulin G (IgG), white blood count (WBC), absolute neutrophil count (ANC) and absolute lymphocyte count (ALC) were not associated with symptom resolution

immunosuppressive medication after completion of her chemotherapy. Symptomatic treatment with loperamide 16 mg/day and tincture of opium 24 mg/day was used, leading to a reduction of stool counts from 10–15 to 5 per day. Attempts to taper treatment resulted in increased stool counts. Neither treatment with nitazoxanide 500 mg twice daily for 7 days [8] 5 months post chemotherapy, nor infusion of intravenous immunoglobulin (IVIG) 1 g/kg at 6 and 6.5 months post chemotherapy resulted in a change of stool pattern. The patient was able to slowly taper the amount of antidiarrheal medications 8 months after completion of chemotherapy, and was off of all antidiarrheal medications by 10 months.

Methods

Stool and serum samples were obtained 6 months after completion of the patients chemotherapy, prior to IVIG administration to evaluate for any immune response as the patient continued to be symptomatic and B cell reconstitution would be expected to begin between 3 and 6 months after her last rituximab dose [9]. Further samples were obtained to monitor for evolution of titers when symptom improvement and resolution were observed at 8 and 10 months.

To identify the infecting viral strain, RNA was extracted from a stool sample using the QIAamp Viral RNA Mini Kit from Qiagen and a cDNA copy made for norovirus RT-PCR using oligo dT as the primer. A strong RT-PCR band was visible by gel analysis with the GII primer set. This band was extracted and sequenced using norovirus capsid gene primers [10].

Surrogate neutralization assays were performed as described [3]. Briefly, patient serum or IVIG were serially diluted and mixed with VLP before adding the VLP to ligand coated plates and comparing the amount of VLP bound to gastric mucin in the presence of serum to the amount bound in the absence of serum. Reported titers are the reciprocal dilution of the percent serum needed to block 50 % of VLP binding.

Results

Six months after completion of chemotherapy patient's stool sample was positive for a genogroup II norovirus by RT-PCR. The infecting strain capsid sequence was 97 % homologous to norovirus GII.4-2009, the New Orleans strain [11]. A concomitant serum sample lacked the ability to block GII.4-2009 interaction with carbohydrate ligand in surrogate neutralization assays at 10 % serum (Fig. 1), as did aliquots of the administered IVIG batches (data not shown).

Eight months after chemotherapy completion patient's serum showed an increased blockade titer (EC50 15.3) that correlated clinically with the ability to taper the antidiarrheal medication. At 10 months patient's serum completely

blocked the GII.4.2009 VLP interaction with ligand (EC50 54.9), and she had returned to her normal stool pattern. A concomitant stool sample was negative for norovirus using the same RT-PCR protocol as for the 6-month stool sample. Total white blood count, absolute neutrophil and lymphocyte counts were not associated with symptom relief.

Discussion

We herein describe the emergence of norovirus strain-specific antibody blockade titers over time and their correlation with decreased clinical symptoms in a case of prolonged diarrhea in the setting of rituximab–bendamustine chemotherapy for non-Hodgkin's lymphoma.

Human mechanisms for protective immunity and clearance of noroviruses are not well defined, but cellular and humoral immunity are hypothesized to play a role in viral clearance [12]. Both pathways of immunity are disturbed after rituximab-bendamustine chemotherapy [9, 13]; rituximab can deplete B-cells for 3–12 months [9]. B cell recovery following rituximab in lymphoma is associated with a lasting preponderance of immature transitional B cells and a paucity of memory B cells [9]. This effect might explain the prolonged illness in our patient who continued treatment after the onset of her illness. No other cases of chronic Norovirus disease after bendamustin–rituximab chemotherapy have been described to date.

As in healthy volunteers, rising antibody blockade titers correlated with decreased diarrhea symptoms in our immunocompromised patient [6, 7]. This observation is of particular interest for patients after small bowel transplantation. In these patients it can be difficult to differentiate an alloimune reaction from a symptomatic norovirus infection, and subsequently to decide between augmentation versus reduction of immunosuppression. Histopathology may not definitively differentiate between either diagnosis as inflammation and crypt apoptosis can be found in both processes [14].

Patients who undergo hematopoetic stem-cell transplantation (HSCT) can have prolonged diarrheal illness in the setting of norovirus infection [4]. A semi-quantitative polymerase chain reaction has been used as a marker to distinguish asymptomatic from symptomatic norovirus infection post-HSCT [4], however this method is flawed as high fecal viral loads have also been demonstrated in asymptomatic healthy volunteers after norovirus challenge [15]. Studies in immunocompromised hosts evaluating the incidence of asymptomatic norovirus shedding are currently lacking. The absence of strain-specific antibody blockade titers in immunocompromised hosts could suggest norovirus infection as the cause of diarrhea. Complete antibody blockade would favor asymptomatic shedding and a different cause of the diarrhea than norovirus infection.

Nitazoxanide use was associated with improvement in norovirus gastroenteretis in a single immunocompromised patient [8]. In our patient nitazoxande was ineffective. To evaluate whether immune reconstitution plays a role in symptom improvement when using off label or novel therapeutics for norovirus infection in immunocompromised hosts antibody blockade titers could be helpful. Administration of IVIG that was devoid of strain-specific blockade titer was also an ineffective treatment, supporting the association between strain-specific blockade IgG titer and symptom relief. These findings suggest that IVIG may not be an effective treatment for norovirus infection for newly emergent strains, as herd immunity to these strains will not yet have been established. Consequently, development of human monoclonal antibodies with cross-strain blockade activity may provide effective treatment for symptomatic norovirus infection, as recently suggested [3].

In conclusion, rising strain-specific antibody blockade titers correlated with symptom resolution in an immunocompromised patient with chronic norovirus disease. Treatment with rituximab or with other agents that impair B-cell responses may lead to protracted diarrheal illnesses.

Acknowledgments This work was supported by a grant from the National Institutes of Health, Allergy and Infectious Diseases AI056351.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 2011;17:7–15.
2. Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. Norovirus classification and proposed strain nomenclature. Virology. 2006;346:312–23.
3. Lindesmith LC, Beltramello M, Donaldson EF, et al. Immunogenetic mechanisms driving norovirus GII.4 antigenic variation. PLoS Pathog. 2012;8:e1002705 **(Epub 2012 May 17)**.
4. Roddie C, Paul JP, Benjamin R, et al. Allogeneic hematopoietic stem cell transplantation and norovirus gastroenteritis: a previously unrecognized cause of morbidity. Clin Infect Dis. 2009;49:1061–8.
5. Harrington PR, Lindesmith L, Yount B, Moe CL, Baric RS. Binding of Norwalk virus-like particles to ABH histo-blood group antigens is blocked by antisera from infected human volunteers or experimentally vaccinated mice. J Virol. 2002;76:12335–43.
6. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness. N Engl J Med. 2011;365:2178–87.

7. Reeck A, Kavanagh O, Estes MK, et al. Serological correlate of protection against norovirus-induced gastroenteritis. J Infect Dis. 2010;202:1212–8.
8. N Siddiq DM, Koo HL, Adachi JA, Viola GM. Norovirus gastroenteritis successfully treated with nitazoxanide. J Infect. 2011;63:394–7.
9. Anolik JH, Friedberg JW, Zheng B, et al. B cell reconstitution after rituximab treatment of lymphoma recapitulates B cell ontogeny. Clin Immunol. 2007;122:139–45.
10. Ando T, Monroe SS, Noel JS, Glass RI. A one-tube method of reverse transcription-PCR to efficiently amplify a 3-kilobase region from the RNA polymerase gene to the poly(A) tail of small round-structured viruses (Norwalk-like viruses). J Clin Microbiol. 1997;35:570–7.
11. Vega E, Vinjé J. Novel GII.12 norovirus strain, United States, 2009–2010. Emerg Infect Dis. 2011;17:1516–8.
12. Lindesmith L, Moe C, Lependu J, Frelinger JA, Treanor J, Baric RS. Cellular and humoral immunity following Snow Mountain virus challenge. J Virol. 2005;79:2900–9.
13. Hosoda T, Yokoyama A, Yoneda M, et al. Bendamustine can severely impair T-cell immunity against cytomegalovirus. Leuk Lymphoma. 2012.
14. Kaufman SS, Chatterjee NK, Fuschino ME, et al. Characteristics of human calicivirus enteritis in intestinal transplant recipients. J Pediatr Gastroenterol Nutr. 2005;40:328–33.
15. Atmar RL, Opekun AR, Gilger MA, et al. Norwalk virus shedding after experimental human infection. Emerg Infect Dis. 2008;14:1553–7.