Norwalk Virus Infection and Disease Is Associated with ABO Histo–Blood Group Type

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Some people are resistant to Norwalk virus (NV) infection; however, the factor(s) responsible for resistance or susceptibility to NV infection has not been identified. This study investigated the relationship between a person’s ABO histo–blood group type and the risk of NV infection and symptomatic disease after clinical challenge. ABO phenotypes were identified by using serum samples from volunteers who participated in an NV challenge study (n = 51). Individuals with an O phenotype were more likely to be infected with NV (odds ratio [OR], 11.8; 95% confidence interval [CI], 1.3–103), whereas persons with a B histo–blood group antigen had decreased risk of infection (OR, 0.096; 95% CI, 0.16–0.56) and symptomatic disease (OR, 0; 95% CI, 0–0.999). This is the first report demonstrating an association between a genetic factor and the risk of NV infection and symptomatic disease.

“Norwalk-like viruses” (NLVs) are a major cause of epidemic and sporadic cases of acute gastroenteritis in adults and children in the United States [1]. Despite 30 years of study, the replication and pathogenesis of the prototype NLV, Norwalk virus (NV), remains poorly understood, in part because of the lack of a tissue culture system or an animal model of infection. Most knowledge of NV infection comes from the epidemiology of outbreaks and from volunteer challenge studies.

The initial NV challenge studies described a subset of volunteers who were resistant to infection. Parrino et al. [2] reported that 6 of 12 individuals challenged with NV developed clinical signs of infection, including vomiting and/or diarrhea, whereas the other 6 persons were asymptomatic. When the same 12 individuals were rechallenged with the same NV inoculum 1–2 years later, the individuals who were ill previously suffered clinical disease again, whereas those who were asymptomatic after the initial challenge remained resistant to clinical disease. Thus, previous exposure to NV that resulted in clinical illness did not confer protection against rechallenge in that study; yet, under the same conditions, resistance to clinical illness persisted in a subset of the volunteers [2]. Subsequent NV challenge studies also found an absence of NV infection in 12.5%–40% of volunteers [3, 4]. A genetic control of susceptibility and/or resistance to NV infection was proposed, but the basis for this control has remained elusive [2].

The ABO histo–blood groups are one set of cell antigens that have been associated with susceptibility to other gut pathogens [5]. We suspected that an individual’s ABO phenotype may play a role in NV susceptibility, because in recent studies [6, 7] hemagglutination by NV-like particles (VLPs), which are structurally and antigenically similar to virions, occurred in all samples of type O human red blood cells (RBCs) but in very few samples of type B human RBCs. On the basis of these observations, we hypothesized that the uninfected volunteers who were challenged with NV would express the type B antigen.

Methods

Case definition. A series of experimental infections (n = 51) with NV containing stool filtrates (8FIIs) were performed during 1985–1990 at Baylor College of Medicine (Houston) [8]. NV infection was defined as a ≥4-fold increase in NV-specific serum antibody titer (determined by ELISA) or NV-antigen shedding (determined by sandwich ELISA, RIA, or reverse-transcription polymerase chain reaction) [8]. Asymptomatic infection was defined as the absence of vomiting and/or diarrhea and a low overall symptom score (abdominal cramps, chills, body ache, headache, nausea, and fever) [8]. Nine (18%) of the 51 volunteers challenged with NV...
were classified as uninfected because they did not have any clinical signs of infection, a rise in convalescent NV-specific antibody titer, or detectable NV-antigen shedding. Thirteen of the remaining 42 infected volunteers had asymptomatic infection with an increase in NV-specific antibodies in convalescent serum samples, with or without antigen shedding, and without clinical [8].

Determination of ABO histo–blood group type. RBCs from volunteers were not available for direct ABO typing. Therefore, serum samples stored at −70°C were thawed and used for ABO back-typing using A1A2BO Referencell reagents, in accordance with the manufacturer’s instructions (Immucor). Fifty-microliter aliquots of each individual’s serum samples were combined with 25 μL of each cell type. Cells were pelleted by centrifugation for 1 min at 1000 g and then resuspended in serum. Individuals were considered to be positive for an ABO type if their serum did not hemagglutinate cells of that type. All serum samples tested did not hemagglutinate type O cells, and control serum samples hemagglutinated reagents, as expected.

Statistical analysis. Categorical data were analyzed using Fisher’s exact test (2 tailed). Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using SPSS software for Windows (version 10.0). When the Mantel-Haenszel estimate of the 95% CI could not be made by use of SPSS, an exact solution for the interval was calculated [9].

Results

The distribution of O (51%), A (35%), B (10%), and AB (4%) phenotypes was similar to that reported for the US population. The frequency of NV infection and clinical disease was examined for each ABO type (table 1). Persons with type O phenotype were significantly more likely to become infected with NV (table 2). However, individuals expressing type B antigen (B and AB phenotypes) had a decreased risk of infection. Moreover, the 3 type B individuals who were infected with NV did not develop symptomatic illness.

Discussion

This is the first report of an association between a person’s ABO phenotype and their susceptibility to a viral gut pathogen. Results reported here suggest that individuals with an O phenotype have an increased susceptibility to NV infection and that those expressing the type B antigen are more resistant to NV infection and symptomatic disease. Similar associations have been reported between ABO phenotypes and susceptibility to several bacterial gut pathogens. Numerous studies have shown that individuals with the type O phenotype are more susceptible to Vibrio cholerae and that they suffer more severe symptoms of cholera disease (reviewed in [10]). Susceptibility to Helicobacter pylori infection does not appear to be associated with the O phenotype, as shown by similar antibody seroprevalence in persons with O and those with non-O blood types. However, the increased density of H. pylori colonization and enhanced immune response in individuals with a type O phenotype may contribute to the preponderance of the type O phenotype among patients with duodenal ulcers [11, 12]. In addition, the B antigen was found less frequently among patients with a diagnosis of hemolytic uremic syndrome during an Escherichia coli O157:H7 outbreak in Sakai, Japan, than among a Japanese blood donor population [13]. Thus, our associations between ABO phenotype and susceptibility to NV may conform to a collective disease mechanism also utilized by bacterial pathogens. Alternatively, an ABH or related antigen(s) may be directly involved in NV binding to cells.

A, B, and O phenotypes are determined by the presence or absence of carbohydrate antigens on glycolipids and glycoproteins found on the surface of mucosal epithelia as well as RBCs. A and B antigens are made by enzymatic addition of N-acetyl-D-galactosamine or D-galactose, respectively, to an H antigen precursor. The ABH and other related carbohydrate antigens are highly expressed on gut cells in the gastric and duodenal regions [14, 15]. Their expression is regulated during enterocyte differentiation, with the most ABH expression at the villi [15]. This villus expression may be significant because the villi are the sites of the most NV-associated pathological changes, as has been observed during gut biopsies [2]. Furthermore, the VLPs from another calicivirus, rabbit hemorrhagic disease virus, also hemagglutinate human RBCs and bind to the H (type 2) carbohydrate antigen [16]. Results reported here that the type B antigen reduces the likelihood of NV infection and symptomatic disease suggest that the presence of the terminal α-galactose may in some way modify the NV ligand and make it cryptic to NV binding. The cryptic nature of some
protein-carbohydrate interactions has been described elsewhere for antibodies and their carbohydrate epitopes [17, 18].

As suggested by initial NV challenge studies, the presence or absence of a receptor or other genetic factor could explain the susceptibility or resistance of some individuals to NV infection and disease. Factors other than the presence of the B antigen must be important in prevention of infection and symptomatic disease in persons with A and O phenotypes who remained uninfected after exposure to NV. One possibility is that such uninfected individuals may not secrete ABH antigens (frequency of 20% among white subjects) [19]. An individual who secretes ABH antigens also expresses ABH antigens on a greater variety of precursor carbohydrates on gut cells. Unfortunately, without archived saliva or cell samples, the secretion status of our NV-challenged volunteers is one genetically determined factor that we are unable to examine at this time. In addition, the uninfected individuals may have short-term immunity to NV because of previous exposure to a homologous strain [4]. Furthermore, the NLVs are genetically diverse, and studies with different strains will be required, to determine whether these associations between ABO phenotype and NV infection and disease can be generalized to other strains. Identifying the NV receptor(s) required for cell binding may be useful in developing antiviral treatments for the elderly, immunocompromised patients, or others at greater risk of NV disease, as well as in providing insights for successful cultivation of NV in vitro.

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References