ABO and P1 Blood Group Antigen Expression and stx Genotype and Outcome of Childhood Escherichia coli O157:H7 Infections

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P1 and ABO antigens and bacterial stx genotypes might influence the risk of developing hemolytic uremic syndrome (HUS) after Escherichia coli O157:H7 infections. We determined ABO status and P1 antigen expression in 130 infected and 17 uninfected children, and we determined the stx genotype of the infecting isolate. P1 expression was weakly and directly related to HUS risk (P = .04), but this risk did not extend to the group with the greatest P1 expression. P1 expression remained constant as HUS evolved. The ABO frequency was similar in all groups. These associations were not affected by the stx genotype. stx1/stx2 Es. coli O157:H7 strains were more commonly associated with HUS than were stx1/stx2 strains (P = .21), and 1 child with HUS was infected with a rare stx1/stx2 isolate. In the present study, ABO antigens and stx genotypes were not major determinants of the outcome of E. coli O157:H7 infections, and P1 expression did not protect against the development of HUS.

Escherichia coli O157:H7 cause diarrhea and bloody diarrhea. A subset of patients infected with E. coli O157:H7 develop hemolytic uremic syndrome (HUS). HUS is a thrombogenic microangiopathy believed to be precipitated by Shiga toxin (Stx) absorbed from the gut that was produced by E. coli O157:H7 [1]. HUS occurs ~1 week after the onset of diarrhea [2]. Presumably, Stx binds to vascular endothelial cells via globotriaosylceramide (Gb3), the glycosphingolipid receptor for the Stx B subunit [3]. Subsequent toxin-mediated injury to the endothelium causes microvascular injury that leads to hemolysis, thrombocytopenia, and extraintestinal injury, especially in the kidneys.

Various erythrocyte antigens have been proposed to either play a role in the development of Stx-mediated HUS or modulate the severity of resulting HUS. For example, the P1 erythrocyte antigen carries a terminal Galα1–4Gal residue capable of binding and neutralizing Stx1 and 2 in vitro [4], and it has been proposed that the binding of Stx by erythrocytes through the P1 antigen might prevent circulating toxin from binding to and injuring endothelial cells. In one study, children with HUS whose erythrocytes expressed the P1 antigen had less-severe courses than those whose erythrocytes were P1 negative, and children with postdiarrheal HUS had a significantly lower expression of the P1 blood group antigen on their erythrocytes, compared with control children with renal disease but without a history of HUS [5]. The theory that erythrocytes could express toxin-binding glycolipids is also supported by the observation that the erythrocytes of children with HUS have lower Gb3 levels than do erythrocytes of children with diarrhea secondary to Shiga toxin–producing E. coli (STEC) who do not develop HUS [6]. However, several subsequent epidemiological studies, mostly of children infected during outbreaks, have failed to associate the expression of the P1 blood group antigen with diminished risk for the development of HUS after infection with E. coli O157:H7 [7–10].

Stx also binds to a synthetic trisaccharide, Galα1–3Galβ1–4N-acetylglucosamine [11]. This trisaccharide epitope is expressed on cell surfaces of lower mammals but not on human cells and is the target of the naturally occurring “anti-Gal” human antibodies that have made xenotransplantation problematic [12, 13]. This trisaccharide is also identical to the B blood group antigen, minus a fucose linked to the subterminal galactose. Indeed, Shimazu et al. [14] found that children with B blood type who were infected during a large E. coli O157:H7 outbreak in Sakai, Japan, in 1996 experienced a lower HUS rate than did infected children with different blood types. The B blood group antigen
was proposed to protect against the development of HUS in children, possibly by binding circulating toxin.

The comparative virulences of Stx1 and 2 have also been proposed to affect disease outcome in *E. coli* O157:H7 infections. The amino acid sequence of Stx1 is almost identical to that of Stx produced by *Shigella dysenteriae* type 1, but it differs from the sequence of Stx2 at 44% of its amino acid residues. Although Stx1 and 2 contain some conserved sequences, they have different antigenic [15] and biological properties. In particular, Stx2 is considered to be the more potent of the 2 toxins in vitro [16] and in vivo studies [17]. Moreover, a significantly higher proportion of children infected with isolates that contained Stx2 alone developed HUS than did the children who were infected with isolates that contained Stx1 alone or Stx1 and 2 [18], and STEC containing the gene encoding Stx2 were overrepresented as the cause of severe illness in a study of 112 human isolates [19]. Of interest, Donohue-Rolfe et al. [20] determined that *stx1*/*stx2* *E. coli* O157:H7 strains were less virulent in a gnotobiotic pig model of infection than from a derivative from which the *stx1* gene was deleted.

We conducted a prospective analysis of risk factors for the development of HUS after *E. coli* O157:H7 infections since 1997. Herein, we examine the relationship between the expression of ABO and P1 erythrocyte antigens in the host and the *stx* genotype of the infecting strain on the development of HUS in children infected with *E. coli* O157:H7.

### Subjects and Methods

**Subjects.** The *E. coli* O157:H7–detection network has been described elsewhere [21]. In brief, 46 participating laboratories in 4 states (Idaho, Oregon, Washington, and Wyoming) detect *E. coli* O157:H7 by screening stools on sorbitol-MacConkey agar. Sorbitol nonfermenting *E. coli* are then tested to determine whether they express the O157 lipopolysaccharide antigen, by use of commercial reagents. These laboratories then notify us of all patients exposed to *E. coli* O157:H7 strain 86-24 [24] were selected as the cause of severe illness in a study of 112 human isolates [19]. Of interest, Donohue-Rolfe et al. [20] determined that *stx1*/*stx2* *E. coli* O157:H7 strains were less virulent in a gnotobiotic pig model of infection than from a derivative from which the *stx1* gene was deleted.

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**Case definitions.** Infected children were considered to have HUS if they had a hematocrit <30%, with microangiopathic changes on smears of peripheral blood; a platelet count <150,000 cells/mm³; and a serum creatinine concentration above the upper limit of normal for age. Infected children were considered to have uncomplicated illness if they did not develop HUS. Children with postdiarrheal HUS precipitated by *E. coli* O157:H7 who presented to the Children’s Hospital and Regional Medical Center (CHRMC), Seattle, were also enrolled and studied if they had not been enrolled during the pre-HUS stage.
ABO blood group antigens and stx genotype) and the relative risk that a child would develop HUS.

Results

Subjects enrolled. Between April 1997 and March 2001, erythrocytes from 148 subjects were analyzed. These included 106 and 25 children with uncomplicated infection and HUS, respectively, and 17 control subjects (table 1). The 25 children with HUS included 7 children who were admitted to the CHRMC at the time of HUS diagnosis but who were not enrolled prior to the development of HUS. The isolate from 1 of the 106 subjects with an uncomplicated infection had no evidence of stx genes on either PCR or on Southern hybridization. This patient therefore was not included in these analyses, because of concern that this infection might not have been related to the toxigenic properties of E. coli O157:H7 [27, 28].

P1 antigen and development of HUS. Children with uncomplicated infection, children with HUS, and healthy control subjects had similar distributions of the P1 blood group antigen (P = .33, Fisher’s exact test). One subject had a P1 level of 1+, and this person was included in the 2+ group for purposes of analysis. When the larger control group reported by Taylor et al. [5] was substituted for comparison purposes for our healthy control subjects, the distribution of the P1 blood group antigen was not statistically different (P = .23, Fisher’s exact test; figure 1). There was also no difference when the distribution of P1 antigen expression was compared with that of 2 larger control groups recruited in Seattle for population-based studies [29]. These patient groups (ages 18–40 years) had ethnic distributions similar to the group of infected children in this study.

ABO type and development of HUS. Children with uncomplicated infection, children with HUS, and healthy control subjects, and the Seattle population-based control subjects [29] had similar distributions of ABO blood group antigens (P = .63, Fisher’s exact test). Because we were interested in the association of the presence of the B blood group antigen and the development of HUS, children with blood types B and AB were compared with children who expressed A and O blood group antigens. There was no evidence of association between ABO blood group and HUS development (P = 1, Fisher’s exact test; figure 1).

P1 antigen expression as microangiopathy evolves. Blood is subject to considerable shear stress as HUS evolves from gastrointestinal infection with E. coli O157:H7 [30], and it is possible that this shear stress might affect the expression of an erythrocyte surface antigen, such as P1. Therefore, we determined the degree of expression of the P1 antigen in 4 subjects whose erythrocytes were obtained on consecutive days between the day of enrollment and the day that they developed HUS, to determine whether evolving intravascular shear stress affects the expression of this antigen. P1 antigen did not change despite the evolution of profound hemolysis (figure 2).

Table 1. Characteristics of study subjects and the stx genotype of infecting isolates of Escherichia coli O157:H7.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy control subjects (n = 17)</th>
<th>Uncomplicated infection (n = 106)</th>
<th>Hemolytic uremic syndrome (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male:female</td>
<td>14:3</td>
<td>58:48</td>
<td>15:10</td>
</tr>
<tr>
<td>Age, mean years ± SD</td>
<td>4.3 ± 3.1</td>
<td>4.3 ± 2.6</td>
<td>3.4 ± 1.8</td>
</tr>
<tr>
<td>Race or ethnic group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>15 (88)</td>
<td>88 (83)</td>
<td>21 (84)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0</td>
<td>8 (7)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Black</td>
<td>1 (6)</td>
<td>3 (3)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Asian or Pacific Islander</td>
<td>0</td>
<td>5 (5)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Native American</td>
<td>1 (6)</td>
<td>2 (2)</td>
<td>0</td>
</tr>
<tr>
<td>stx Genotype of infecting isolates*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stx1+/stx2−</td>
<td>NA</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>stx1+/stx2+</td>
<td>NA</td>
<td>30 (29)</td>
<td>10 (40)</td>
</tr>
<tr>
<td>stx1−/stx2+</td>
<td>NA</td>
<td>75 (71)</td>
<td>14 (56)</td>
</tr>
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NOTE. Data are no. (%) of patients, except where noted. NA, data not available.

*In 1 uncomplicated infection, the patient’s E. coli O157:H7 isolate had no evidence of stx genes.
highest number of subjects from the HUS group (figure 1). We also performed a logistic regression, adjusting for stx and ABO blood group and adding P1 antigen in binary form (expressed vs. not expressed). P1 was statistically significant in the model (\(P = .012\)), with an estimated OR of 4.445 (95% CI, 1.203–16.423), which suggests that the presence of P1 on erythrocytes is associated with the development of HUS by an infected host.

**Discussion**

Our demonstration that expression of the P1 blood group antigen is directly, although weakly, associated with the risk of developing HUS after *Escherichia coli* O157:H7 infection contrasts with a previously published report [5]. Taylor et al. [5] and other studies [7–10] generated a hypothesis that expression of specific blood group antigens, such as ABO-B and P1, are protective against HUS. However, several more-recent analyses [7–10] have suggested that there is no association between P1 expression and infection outcome. The present study provides stronger data against a protective effect of P1, because it represents an analysis of children infected by a diversity of *E. coli* O157:H7 strains and not of children infected by a single strain, as in outbreaks [7, 9]. Also, in comparing previous studies of sporadic cases of HUS [5, 8, 10] in which the infecting isolates were not genotyped, we were able to examine the interaction between the P1 antigen as expressed by host erythrocytes, the ABO blood group, and the stx genotype of the infecting strain. It is possible that we overlooked some effect of genetic factors on the expression of blood group antigen and HUS risk in our mostly white population. However, it is noteworthy that race

**Figure 1.** Distribution of P1 (A) and ABO (B) blood group antigens among study groups of patients with uncomplicated *Escherichia coli* O157:H7 infection (U), patients with hemolytic uremic syndrome (H), healthy control subjects (C), and “published” control subjects (P) [5, 22].
was not a risk factor for the development of HUS in 3 previous studies in the Pacific Northwest [21, 31, 32]. Furthermore, the distribution of blood groups in the published control population was similar to that found in an ethnically comparable population-based study from Seattle [29]. In addition, our sample size of 130 infected subjects is larger than that of any previous study that has examined P1 status and HUS risk. Finally, concerns that we studied these patients during acute illness, at a point when erythrocytes were subject to considerable shear stress, were addressed, because the P1 antigen was stable as hemolysis accelerated.

The lack of a protective role of the B blood group antigen in this diverse North American population is in contrast to the findings of Shimazu et al. [14], who studied an exclusively Japanese population involved in an outbreak. That study proposed that the B blood group antigen might bind circulating toxin, thus preventing circulating toxin from binding to Gb3 on endothelial cells. However, our data show that there is no statistical association between the B antigen and protection against HUS, even when the stx genotypes of infecting isolates are included in a multivariate model.

The relationship between the stx genotype of the infecting strain and disease outcome is complex. Although we demonstrated a trend toward the stx1+/stx2− genotype being associated more frequently with the development of HUS, the association did not attain statistical significance. Also, it is worth noting that 1 of the children with HUS was infected with a rare stx1+/stx2− isolate, which is found in only ~3% of clinical isolates [18]. To our knowledge, an stx1−/stx2− E. coli O157:H7 isolate has been recovered only once from a patient with HUS [33].

In summary, in this prospective study of childhood E. coli O157:H7 infections caused by different infecting strains, we determined that the degree of expression of the P1 antigen on host erythrocytes, the ABO blood type, and the presence of stx1 play nonprotective roles with respect to the development of HUS. In fact, our data suggest the possibility that the greater the expression of P1, the higher the frequency with which HUS occurs. This effect was largely confined to the group positive at a 3+ level of P1 expression. It is, however, difficult to draw conclusions about this association, because this association was not observed in the 4+ group. Therefore, in this North American population of children, the erythrocyte antigens studied and the

Table 2. Multivariate analysis for the association among stx genotype, ABO and P1 blood group antigens, and the risk of developing hemolytic uremic syndrome in children infected with Escherichia coli O157:H7.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Odds ratio (95% confidence interval)</th>
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<tr>
<td>stx1+/stx2− genotypea</td>
<td>1.986 (0.755–5.226)</td>
</tr>
<tr>
<td>B + AB blood groupb</td>
<td>1.195 (0.286–4.997)</td>
</tr>
<tr>
<td>P1 blood groupc</td>
<td></td>
</tr>
<tr>
<td>P1 (1+ and 2+)</td>
<td>3.282 (0.574–18.773)</td>
</tr>
<tr>
<td>P1 (3+)</td>
<td>6.285 (1.565–25.243)</td>
</tr>
<tr>
<td>P1 (4+)</td>
<td>3.087 (0.682–13.980)</td>
</tr>
</tbody>
</table>

a Reference group, stx1+/stx2−.
b Reference group, A + O.
c Reference group, O.
toxin genotypes observed in the infecting isolates are neither major nor absolute risk factors for the development of HUS after *E. coli* O157:H7 infection.

**Acknowledgments**

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**References**

6. Newburg DS, Chaturvedi P, Lopez EL, Devoto S, Hutto D, Tzipori S. *Escherichia coli* O157:H7 strains that express Shiga toxin (Stx) 2 alone are more neurotoxic for gnotobiotic piglets than are isolates producing only Stx1 or both Stx1 and Stx2. J Infect Dis 2000; 181:1825–9.