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* *E. 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 globothiosylceramide (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-G” 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, 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 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 <10 years old who have such presumptive E. coli O157:H7 infections. After enrollment by the patients, blood is obtained for clinical and research purposes. In some cases, blood remaining from prior or subsequent clinical determinations was used for erythrocyte antigen determinations. A standardized questionnaire is administered to the caregivers of all subjects, to determine the timing of the onset of illness. Day 1 of illness is considered to be the first day of diarrhea.

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.

Episodes of HUS were classified as oligoanuric if there was <0.5 mL urine/kg body weight/h produced for ≥48 h during the HUS phase and as nonoligoanuric if urine output exceeded this level. Control erythrocytes were obtained from 17 children <10 years old who did not have inflammatory, hematologic, infectious, or nephrologic disorders and who underwent elective operations at the CHRMC.

Specimen handling and erythrocyte antigen determinations. Blood obtained by phlebotomy was evacuated into vacuum tubes. After centrifugation, the clotted pellet was sent on ice to the laboratory of one of the authors (A.E.S.) for erythrocyte antigen determinations. The P1 and ABO antigens were determined by use of macroscopic hemagglutination assays (Immucor), according to standard blood-banking protocols [22]. Indeterminate P1 antigen groupings were assessed microscopically. The person who performed the assays was not aware of the clinical status of the children from whom the specimens were obtained. All specimens for the present study were obtained from phlebotomies performed before any blood was transfused.

Toxin typing of infecting strains. stx genotypes were primarily determined by polymerase chain reaction (PCR). Frozen isolates were grown overnight in Luria Broth at 37°C; 45 μL of turbid culture was added to 5 μL of 0.1% Triton X-100 (Sigma) and boiled for 20 min; and 5 μL of the boiled lysate was added to 45 μL of PCR mix. The PCR mix contained dNTPs (200 μM of each nucleotide), 3 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.25 U DNA polymerase (Klenow fragment), and stx1 (5’-CGC TTT GCT GAT TTT TCA CA-3’) or stx2 (5’-GTT CCG GAA TGC AAA TCA GT-3’) or stx Assy (5’-GAG GAC CAG GAA GGC TCA TTA TGTCG CCT CCA ACA CAG-3’) or stx2 (5’-GTT CCG GAA TGC AAA TCA GT-3’) or stx2 (5’-CGG GTT CAT CTT TTC GTA AC CTC TGG CCA ACA CAG-3’) primers (50 pmol each primer). The PCR was performed in an iCycler (Bio-Rad Laboratories) by use of the following conditions: 25 cycles of denaturation (1 min at 94°C), annealing (1 min at 55°C), and elongation (2 min at 74°C) and 1 cycle of final elongation (7 min at 72°C). The resulting respective 207- or 205-bp amplicons were visualized by electrophoresis in 1% agarose–0.5× Tris-borate–EDTA (TBE) gel and staining in ethidium bromide. E. coli O23:H11 strain TB231 [23], E. coli O157:H7 strain 87-01 [18], E. coli O103:H6 strain TB154 [23], and E. coli O157:H7 strain 86-24 [24] were the negative, stx1+/stx2−, stx1+/stx2+, and stx1−/stx2+ controls, respectively.

For 2 isolates with unusual genotypes, Southern hybridizations were performed to confirm the PCR genotyping. DNA was digested with Bam HI, electrophoresed in 1% agarose–0.5× TBE gel, and transferred to a nylon membrane (Osmonics). The immobilized DNA was probed under stringent conditions [25] with the inserts of pN37-19 and pNN111-19 to identify stxl and stx2, respectively [26]. E. coli O157:H7 strain 87-01 [18], E. coli O103:H6 strain TB154 [23], and E. coli O157:H7 strain 86-24 [24] were the stx1+/stx2+, stx1+/stx2−, and stx1−/stx2+ controls, respectively. DNA from E. coli HB101 [25] was the negative control.

Statistics. The association between 2 categorical variables was initially assessed by use of χ² tests. Some of the tables had expected values <5, which required that we apply Fisher’s exact test. For consistency, we reported only the Fisher’s exact test results for all categorical data. Logistic regression was used to assess the association between categorical variables (P1 and
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 toxicogenic 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 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 had similar distributions of the 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>
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<td>Race or ethnic group</td>
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<tr>
<td>White</td>
<td>15 (88)</td>
<td>88 (83)</td>
<td>21 (84)</td>
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<tr>
<td>Hispanic</td>
<td>0</td>
<td>8 (7)</td>
<td>2 (8)</td>
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<tr>
<td>Black</td>
<td>1 (6)</td>
<td>3 (3)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Asian or Pacific Islander</td>
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<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>
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<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>
</tbody>
</table>

**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 \( E. coli \) O157:H7 infection contrasts with a previously published report [5]. Taylor et al. [5] and other studies [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 \( E. coli \) 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

![Figure 2. Daily P1 blood group antigen expression and hemoglobin levels in 4 children with hemolytic uremic syndrome. ND, not determined.](image-url)
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

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