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Children with the Le(a+b−) Blood Group Have Increased Susceptibility to Diarrhea Caused by Enterotoxigenic *Escherichia coli* Expressing Colonization Factor I Group Fimbriae▽

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Recent studies have shown that children with blood group A have increased susceptibility to enterotoxigenic *Escherichia coli* (ETEC) diarrhea and that Lewis blood group "a" antigen (Le^a) may be a candidate receptor for ETEC colonization factor (CF) antigen I (CFA/I) fimbriae. Based on these findings, we have attempted to determine if children with the Le(a+b−) phenotype may be more susceptible to diarrhea caused by ETEC, in particular ETEC expressing CFA/I and related fimbriae of the CFA/I group, than Le(a−b+) children. To test this hypothesis, we have determined the Lewis antigen expression in 179 Bangladeshi children from a prospective birth cohort study in urban Dhaka in which ETEC expressing major CFs such as CFA/I, CS3, CS5, and CS6 was the most commonly isolated diarrhea pathogen during the first 2 years of life. The Lewis blood group phenotypes were determined by a dot blot immunoassay using saliva samples and by a tube agglutination test using fresh red blood cells. The results indicate that Le(a+b−) children more often had symptomatic than asymptomatic ETEC infections ($P < 0.001$), whereas symptomatic and asymptomatic ETEC infections were equally frequent in Le(a−b+) children. We also show that children with the Le(a+b−) blood type had significantly higher incidences of diarrhea caused by ETEC expressing fimbriae of the CFA/I group than Le(a−b+) children ($P < 0.001$). In contrast, we did not find any association between the Lewis blood group phenotype and diarrhea caused by ETEC expressing CS6 or rotavirus.

Expression of Lewis or ABO histo-blood group types has been shown to be associated with different risks of enteric infections (4, 5, 12, 15, 24, 27), presumably through differential expression of cell surface glycoconjugates that are used as receptors for pathogens of the intestinal mucosa. The Lewis blood group antigens on the intestinal mucosa are synthesized through a group of glycosyltransferases, which insert fucose residues in type 1 and type 2 oligosaccharide precursors (21, 29, 30). The synthesis of Lewis antigens is dependent on the *FUT2* and *FUT3* genes. If both genes are functional, the phenotype will be Le(a−b+), i.e., the secretor type, whereas individuals in whom the *FUT2* gene is not expressed will have the Le(a+b−) phenotype, i.e., the nonsecretor type. Failure to express both *FUT2* and *FUT3* will result in Le(a−b−) (9).

A predisposition for obtaining dehydrating cholera has been seen in blood group O individuals (8, 12, 14, 19, 28). In contrast, our recent study showed that enterotoxigenic *Escherichia coli* (ETEC) diarrheal episodes were more common in children with blood group AB or A than in individuals with blood group O (24). We have also shown that colonization factor (CF) antigen I (CFA/I) expressed by ETEC binds to glycosphingolipids that are associated with blood group antigens, e.g., Le^a, that may be expressed on epithelial cells in the small intestine in humans (16). The glycosphingolipid binding capacity of CFA/I fimbriae resides in the major CfaB subunit protein (3, 8, 16). CFA/I was the first identified human-specific CF of ETEC bacteria (11). Subsequently, seven other genetically related fimbriae, CS1, CS2, CS4, CS14, CS17, CS19, and putative CF O71, denoted as the CFA/I group (1), have been shown to be related to CFA/I both in the structural subunits (26) and tip-localized minor adhesive subunits (1). A glycosphingolipid binding pattern similar to that of CFA/I has been demonstrated for CS1 and CS4 that might be due to related N-terminal sequences (3, 8, 16). In addition, in another study (25) we have also shown that the conserved regions of the CF subunit proteins (shared by the CFA/I group fimbriae) are likely to be responsible for the receptor binding, since monoclonal antibodies against this region prevented enterocyte binding and protected against challenge with ETEC expressing CFA/I and CS4.

In a recent longitudinal birth cohort (BC) study in Dhaka, we showed that ETEC was a major pathogen in children up to 2 years old and that a high proportion of symptomatic infections were caused by ETEC expressing the CFA/I group fimbriae (24). In this study, we present additional data to determine whether children with specific Lewis blood group antigen phenotypes, e.g., Le(a+b−) or Le(a−b+), have different susceptibilities to diarrhea caused by ETEC, in particular ETEC expressing the CFA/I group fimbriae, as well as susceptibilities to diarrhea caused by rotavirus.

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TABLE 1. Anthropometric data

Study group	*n*	No. of males/ no. of females	Mean age ± SD	Mean wt ± SD (kg)	Mean ht ± SD (cm)
BC children	179	90/89	56.2 ± 2 mo	13.6 ± 2	97.0 ± 5
BC mothers	171	0/171	25.3 ± 5 yr		
Control children	112	68/44	13.4 ± 3 mo	8.9 ± 5	73.1 ± 2

TABLE 2. Subjects with different Lewis blood group phenotypes in the different study groups

Lewis phenotype	No. (%) of subjects in indicated study group[a]		
	BC children	BC mothers	Control children
Le(a−b+)[b]	105 (58.6)	99 (57.9)	66 (59.0)
Le(a+b−)	47 (26.3)	42 (24.6)	29 (25.9)
Le(a−b−)	27 (15.1)	30 (17.5)	17 (15.1)

[a] BC children were >4 years old (n = 179); BC mothers were >20 years old (n = 171); and the children in the control group were <24 months old (n = 112).
[b] A total of 17/66 of the subjects were found to have the Le(a+b+) phenotype using the RBC test but were Le(a−b+) using the saliva test. They have been included in the Le(a−b+) group since RBC expression of the Le(a+b+) phenotype eventually develops into Le(a−b+) later in life (9).

MATERIALS AND METHODS

Study population and sample collection. A prospective community-based study was conducted in an urban slum in Mirpur, Dhaka, from April 2002 to October 2004. The details of the study protocol have been described elsewhere (24). Briefly, 254 newborn children were enrolled in the study in the order that they were born and were followed for 24 months for active surveillance of ETEC diarrhea (24). After completion of the initial 2-year study period, 179 children in the BC were studied again to determine their Lewis blood group (age, >4 years). Red blood cells (RBC) and saliva samples were used for the determination of the Lewis blood group phenotype in the children from the BC. To evaluate if children below 2 years of age had a distribution of Lewis blood group phenotypes similar to that of the older children, we also analyzed the distribution of Lewis antigens in a group of 112 younger children (6 to 24 months old) from the same study area (Qadri et al., ongoing study). To compare the distribution of Lewis blood group phenotypes in children to that in adults, we studied 171 mothers of the BC children. Written informed consent was obtained from the parents of all children and from the participating mothers. The study was approved by the Ethical Review Committee of the International Centre for Diarrheal Disease Research, Dhaka, Bangladesh (ICDDR,B).

Lewis blood group typing. Lewis blood groups were typed using fresh whole blood in an agglutination test tube assay according to the manufacturer's instructions (Gamma Biologicals, Inc., Houston, TX). Briefly, a 3 to 4% suspension of washed RBC in phosphate-buffered saline was mixed with an equal volume of anti-Le^a and anti-Le^b antibodies, respectively, and incubated for 20 min at room temperature. After centrifugation, the cells in the tube were resuspended and examined for RBC agglutination. We also compared the Lewis blood group phenotypes determined in blood with an alternative approach that allows analysis of saliva samples by a dot blot immunoassay as previously described (23). Briefly, saliva samples were applied to nitrocellulose membrane strips and allowed to dry. After blocking with 1% bovine serum albumin, monoclonal anti-Le^a and anti-Le^b antibodies (Gamma Biologicals, Inc., Houston, TX), respectively, were added and incubated for 30 min at room temperature. After washing, the strips were incubated with secondary horseradish peroxidase-conjugated antibody and enzyme substrate (4-chloro-1-naphthol–H_2O_2) and read as described previously (23).

Blood group ABO typing. For ABO blood group typing, we used data from the BC study (24).

Detection of pathogens, CFs, and toxin profiles. Data regarding the children with ETEC and rotavirus infections during the first 2 years of life were obtained from the BC study (24). The CF and toxin profiles of the ETEC strain isolates were also derived from the study.

Data analyses. Analyses were carried out using the statistical program SigmaStat 3.1. Significant differences were assessed using the chi-square test or Fisher's exact test. In this study, we compared the Le(a+b−) children with children from the Le(a−b+) group. The Le(a−b−) group was not included in the data analysis, since this group contained a mixture of individuals who are secretors but are Lewis negative (*FUT3*$^{-/-}$) and individuals who are nonsecretors (approximately 15%).

RESULTS

Study population and causative agents of diarrheal episodes. Lewis phenotypes were determined in 179 children over 4 years old (BC children) and also in 112 younger children (<2 years old) from the same area (Table 1). We also determined Lewis blood group phenotypes for 171 mothers of the BC children (Table 1). For the 179 BC children, we analyzed microbiological data from 860 stool samples that had been collected during diarrheal episodes occurring within the first 2 years of life (24); in these specimens, ETEC was detected in 20% of the samples and rotavirus in 9.4%.

The distribution of ETEC infections found among the 179 BC children in the present study was comparable to that of the children studied in the previous BC study (24). Based on the data analysis, ETEC was the cause of at least one diarrheal episode in 56% of the BC children (101 of 179 studied), and 37% (n = 66) of them had had one or more asymptomatic ETEC infection during the first 2-year study period. We were not able to detect any ETEC infections in only 7% (n = 12) of the BC children during the initial 2-year study period. Among the ETEC strains isolated from diarrheal stool samples, 47% expressed ST, 32% expressed LT/ST, and 21% expressed LT only; among the strains isolated from asymptomatic infection samples, 49.4% produced ST, 16.5% produced both LT and ST, and 34.1% expressed LT. Among the 13 CFs analyzed, CFA/I, CS3, and CS6 were the major ones identified in isolates from symptomatic and asymptomatic stool samples. The frequencies of ETEC strains that had been isolated from diarrheal stool samples and that expressed CFA/I, CS3 (alone or together with CS1 or CS2), and CS6 (alone or together with CS5) were 9.8%, 7.5%, and 23.0%, respectively; for asymptomatic stool samples, the corresponding frequencies were 7.7%, 4.0%, and 11.4%, respectively.

In addition, 40% (72/179) of BC children had been infected with rotavirus during the first 2 years of their lives.

Distribution of Lewis blood groups among children and mothers. Among the BC children, 59% were Le(a−b+), 26% were Le(a+b−), and 15% had the Le(a−b−) blood group phenotype as determined by blood and saliva tests (Table 2). The distribution of the Lewis blood groups in the BC children was comparable to that in the mothers (Table 2). To determine if Lewis antigens are also expressed by young children under 2 years of age, blood and saliva samples were analyzed from 112 control children. Among them, the frequencies of Le(a−b+) and Le(a+b−) were similar to those in the older children and adults, as determined by using the saliva test. However, when analyzing Lewis phenotypes with the RBC agglutination test, 17 of the young control children were found to have the Le(a+b+) phenotype. Since it has been shown that the Le(a+b+) RBC phenotype eventually converts to the Le(a−b+) phenotype by the age of 2 years (9), these children were included in the Le(a−b+) group (Table 2).

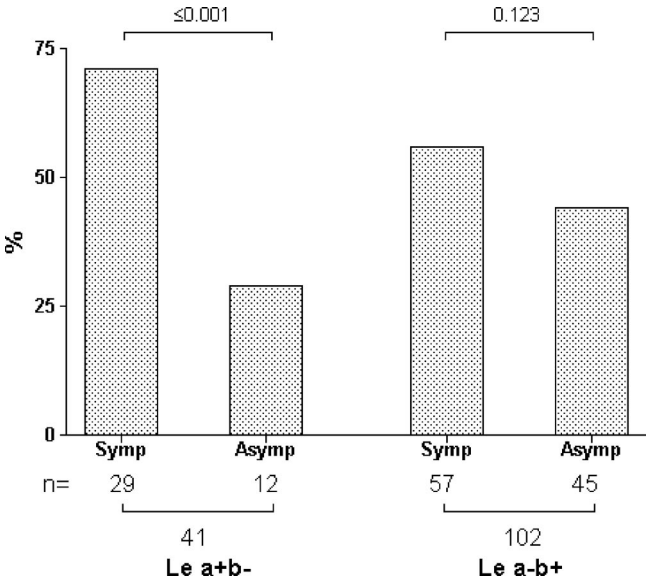

FIG. 1. Association between Lewis blood groups and symptomatic (Symp) and asymptomatic (Asymp) ETEC infections. The chi-square test was used to compare the symptomatically and asymptomatically infected children with different blood groups.

Association of ETEC infections with Lewis blood groups. We compared the distributions of symptomatic and asymptomatic ETEC infections in Le(a−b+) and Le(a+b−) children in the BC. A comparison showed that the Le(a+b−) children were more prone to having symptomatic (n = 29; 71%) rather than asymptomatic (n = 12; 29%; $P \leq 0.001$) ETEC infections (Fig. 1). In contrast, in the Le(a−b+) children, the prevalence of symptomatic (n = 57; 56%) and asymptomatic (n = 45; 44%) ETEC infections did not differ significantly (Fig. 1).

Association between Lewis blood group phenotypes and ETEC CFA/I and toxin profiles. When comparing infections with ETEC expressing CFA/I as a single fimbria in children with different Lewis antigen phenotypes, there was a trend for higher frequencies of symptomatic infections in the Le(a+b−) children than in the Le(a−b+) children (P = 0.075). When analyzing infections with ETEC expressing additional fimbriae of the CFA/I group alone, i.e., CFA/I, CS14, and CS17, there was a significantly higher incidence of symptomatic ETEC infections in children with the blood group Le(a+b−) antigens than in those with blood group Le(a−b+) antigens (P = 0.032) (Table 3). This correlation was even higher when including children infected with ETEC expressing CFA/I group fimbriae in combination with CS3, i.e., CS1 plus CS3 and CS2 plus CS3 ($P < 0.001$) (Table 3). Diarrhea caused by ETEC expressing CFA/II fimbriae (i.e., CS3, CS1 plus CS3, or CS2 plus CS3) was also significantly more prevalent in Le(a+b−) children than in Le(a−b+) children (10/41 versus 5/102; P = 0.002). However, when studying the relationship between infections with ETEC expressing CS5 plus CS6 or with CS6 only and Lewis blood group phenotypes, we did not find any significant relationship either with symptomatic or asymptomatic infections (Table 3). Similarly, no association between the Lewis blood group phenotype and the toxin profile of the ETEC strains isolated from the BC children was found (data not shown).

Lewis blood groups of mothers and overall ETEC infections among children. It was observed that children of mothers with the Le(a−b+) phenotype (n = 99) had symptomatic infections more often than asymptomatic infections (63% versus 37%; $P < 0.001$). For children of mothers with the Le(a+b−) phenotype (n = 42), no difference was found between symptomatic and asymptomatic infections (54% versus 46%; the P value was not statistically significant). Comparable distributions of ETEC expressing major CFs (CFA/I, CS3, or CS6-ETEC) were found in children with diarrhea whose mothers had different Lewis phenotypes (the P value was not statistically significant) (data not shown). In addition, we did not find any association between the Lewis blood groups of the mothers and the toxin profiles of ETEC isolated from the children.

Association of ETEC infection with Lewis and ABO blood groups. Analysis of the ABO blood group distribution in the BC children showed that 37% had blood group O, 25% had blood group A, 29% had blood group B, and 9% had blood group AB, which is comparable to the distribution in the gen-

TABLE 3. Association between Lewis blood group phenotypes of the BC children and symptomatic and asymptomatic infections with ETEC expressing different CFs

CF (toxin ratio) expressed by ETEC	Type of ETEC infection	No. (%) of children with indicated phenotype		P^b
		Le(a+b−) (n = 41)[a]	Le(a−b+) (n = 102)	
CFA/I (LT:ST:LT/ST = 0:36:5)	Symptomatic	7 (17)	6 (6)	0.075
	Asymptomatic	9 (22)	19 (19)	NS
CFA/I group fimbriae (CFA/I, CS14, and CS17) (LT:ST:LT/ST = 8:55:10)	Symptomatic	11 (27)	11 (11)	0.032
	Asymptomatic	13 (32)	38 (37)	NS
CFA/I group including strains co-expressing CS3 (CFA/I, CS14, CS17, CS1+CS3, and CS2+CS3) (LT:ST:LT/ST = 10:69:18)	Symptomatic	18 (44)	16 (16)	<0.001
	Asymptomatic	16 (40)	47 (46)	NS
CFA/II group (CS3 only, CS1+CS3, and CS2+CS3) (LT:ST:LT/ST = 2:15:12)	Symptomatic	10 (24)	5 (5)	0.002
	Asymptomatic	5 (12)	9 (9)	NS
CS6 (CS6 only and CS5+CS6 strains) (LT:ST:LT/ST = 3:38:18)	Symptomatic	8 (20)	17 (17)	NS
	Asymptomatic	9 (22)	25 (25)	NS

[a] A total of 143/179 children with Le(a−b+) and Le(a+b−) blood groups had an ETEC infection; we excluded 24 Le(a−b−) and 12 non-ETEC-infected children from this analysis.

[b] Statistical analysis was done for the relationship between children with the Le(a−b+) phenotype and children with the Le(a+b−) phenotype. NS, not statistically significant.

TABLE 4. Association between ABO blood group, Lewis blood group, and ETEC diarrhea in BC children

Blood group	*n*	Ratio (%) of children with ETEC diarrhea/children with indicated phenotype[a]		*P*[b]
		Le(a−b+)	Le(a+b−)	
A	32	9/21 (43)	9/11 (82)	0.061
B	43	16/32 (50)	8/11 (73)	0.294
O	53	22/38 (58)	11/15 (73)	0.359
AB	15	10/11 (91)	2/4 (50)	0.154
Total	143	57/102 (56)	30/41 (73)	0.084

[a] A total of 143/179 children with Le(a−b+) and Le(a+b−) blood groups had ETEC infections; we excluded 24 Le(a−b−) and 12 non-ETEC-infected children from this analysis.

[b] Statistical analysis was done for the relationship between children with the Le(a−b+) phenotype and children with the Le(a+b−) phenotype.

TABLE 5. Association between rotavirus diarrhea and Lewis blood group phenotypes in BC children

Lewis phenotype	No. (%) of children:		*P*[a]
	With rotavirus infection (*n* = 63)	Without rotavirus infection (*n* = 89)	
Le(a−b+)	45 (71)	60 (67)	0.727
Le(a+b−)	18 (29)	29 (33)	0.727

[a] Statistical analysis was done for the relationship between children with the Le(a−b+) phenotype and rotavirus infection and for the relationship between children with the Le(a+b−) phenotype and rotavirus infection.

eral population of Bangladesh (24). We have previously shown that ETEC diarrheal episodes were more prevalent in children with blood group A or AB (24). When analyzing for the occurrence of ETEC diarrhea in BC children with different combinations of Lewis blood group phenotypes and ABO blood groups, we found that among the children with blood group A antigens, children with the Le(a+b−) phenotype were more prone to acquiring ETEC diarrhea than those with the Le(a−b+) phenotype (82% versus 43%), although this difference was not statistically significant ($P = 0.061$) (Table 4).

Association of rotavirus infections with Lewis blood group phenotypes. No relation between the incidence of rotavirus diarrhea and the Lewis phenotypes was observed in the children from the BC ($P = 0.727$) (Table 5).

DISCUSSION

Increased understanding of the susceptibilities to different types of ETEC strains and genetic factors of the host, e.g., different Lewis blood group phenotypes, might assist the development of effective vaccines for developing countries. In the present study, we demonstrate that the incidence of diarrhea caused by ETEC expressing the CFA/I group CFs was significantly higher for children with Le(a+b−) blood group than for Le(a−b+) children. This may be related to the recently reported capacity of *E. coli* expressing CFA/I, CS1, or CS4 fimbriae to bind to Le^a-terminated glycosphingolipids, while Le^b-terminated glycosphingolipids are not recognized by these CFs (16). Thus, the present findings support that ETEC bacteria expressing CFA/I or related CFs may bind to Le^a determinants expressed by intestinal epithelial cells. However, a significant relationship was not obtained for diarrhea caused by strains with CFA/I only, most likely due to the low numbers of this phenotype used in the analyses. On the other hand, diarrhea caused by CS6 expressing ETEC, ETEC with different toxin profiles, or rotavirus was not related to the Lewis blood group phenotype. Previous studies have shown that the host cell ligand of rotavirus is sialic acid containing glycoconjugates (10, 31), i.e., not related to Lewis blood group antigens. Furthermore, in recent in vitro studies, we have shown that CS6 antigen does not bind to any type of Lewis blood group determinants but instead to sulfatide (SO_3-3-β-1-galactosylceramide) (17), confirming the present clinical data regarding a lack of association between diarrhea caused by CS6 expressing ETEC and Lewis blood group phenotype (17).

The present study investigating the relationship between Lewis blood group determinants and incidence of ETEC and rotavirus diarrheas was performed using a cohort of children in urban Dhaka who were followed for 2 years from birth. In a subgroup of the 254 children in the previous BC (24), 179 children who could be identified 2 to 3 years later had symptomatic and asymptomatic ETEC infections, CF and toxin profiles, as well as rotavirus infections that were comparable with those of all the children in the previous BC study (24).

Relationships between blood group antigens and diarrhea mediated by different enteric pathogens have been reported previously. We have also previously shown that ETEC diarrheal episodes were more prevalent in children with blood group A or AB (24). Marionneau et al. (20) showed that noroviruses bind to human gastroduodenal epithelial cells derived from individuals with the secretor phenotype [Le(a−b+)] but not from those with the nonsecretor phenotype. As nonsecretors lack α1,2-fucosyltransferase activity encoded by the *FUT2* gene, it was suggested that the α1,2-fucose epitope is essential for norovirus binding. The relationship between severe cholera and blood group O has been documented in several studies (8, 12, 14, 28). In a recent study, we found that ETEC diarrheal episodes were more common in children with blood group AB or A than in children with blood group O (24). A higher incidence of *Entamoeba histolytica*-associated diarrhea has been seen in individuals with blood group O or AB (13). The blood group distribution in the children in this study was comparable to the distribution in the general population of Bangladesh (24). No associations between the Lewis phenotypic expression and diarrhea caused by ETEC were found in different ABO blood groups.

The distribution of Lewis blood group phenotypes in young Bangladeshi children has not been determined earlier. The percentage of the Le(a−b+) phenotype was 59%, which is much lower than that in Caucasians (~70 to 80%) but similar to the Lewis antigen distribution in adult Indians (2, 6, 22) and that in the African population (9). An interesting observation in this study was that the distribution of the Le(a+b−) phenotype in Bangladeshi children and adults is higher than that seen in developed countries and this certainly could have implications for susceptibility to infections and vaccine interventions as well. Population-based studies are needed to better understand this relationship.

We observed that mothers and their children of over 4 years

of age showed similar expression patterns of Lewis antigen determinants both in blood and saliva samples. Interestingly, a similar distribution of Lewis phenotypes was found in children <2 years old when analyzing salivary but not RBC specimens. Thus, we observed that 26% of the children identified as having the Le(a−b+) phenotype by salivary tests were found to have the Le(a+b+) phenotype when analyzed using RBC. It has previously been reported that RBC expressing the Le(a+b+) phenotype in infants eventually will be converted into Le(a−b+) expression by 2 years of age (9). In addition, in the intestinal mucosa, Le^a and Le^b determinants are already present at birth, as evidenced by studies of glycosphingolipid expression in human meconium, which consists mainly of extruded epithelial cells from the developing fetal gastrointestinal tract (18).

When we analyzed the relationship between the mothers' Lewis phenotypes and the incidence of ETEC diarrhea in their children, it seemed to be the opposite of that seen between the Lewis phenotype and diarrhea in the children. We also observed that the Lewis phenotypes in the specimens from the mothers were not always the same as those in the children. No relationship was found between the Lewis phenotype of the mothers and diarrhea in children due to major CFs. It is believed that there is a changing pattern of expression of Lewis antigens at different stages of the lactation period (7) as well as with age in children (9). Further studies are needed to better understand the relationship between infection and the Lewis antigen profile in the pairs of mothers and children.

In conclusion, this study shows that children with the Le(a+b−) blood group phenotype have increased susceptibilities to diarrhea caused by ETEC expressing CFA/I group fimbriae. Further studies are needed to determine the relative importance of Lewis blood group phenotypes in relation to outcomes of ETEC infections and other enteric infections in different populations.

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