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Chronic Diarrhea, Hemorrhagic Colitis, and Hemolytic-Uremic Syndrome Associated with HEp-2 Adherent *Escherichia coli* in Adults Infected with Human Immunodeficiency Virus in Bangui, Central African Republic

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In human immunodeficiency virus (HIV)-infected adults from the Central African Republic, the occurrence of chronic diarrhea due to HEp-2 adherent *Escherichia coli* (EAEC) harboring virulence markers (*eaeA*, BFP, EAF, *astA* determinant of EAST/1, positive FAS test, enteropathogenic *E. coli* O serogroup) was shown to be associated with AIDS. We also show that EAEC that produce verotoxin (Stx2) but do not harbor the genetic markers for classical enterohemorrhagic *E. coli* are involved in hemorrhagic colitis and hemolytic-uremic syndrome in patients with HIV.

The Central African Republic is strongly affected by the human immunodeficiency virus (HIV) epidemic (24). Nearly 72% of the adults hospitalized with AIDS present initially with chronic diarrhea (CD) (14). Between 1996 and 1999 we used phenotypic (14) and genotypic assays to study 88 HIV-infected adults hospitalized in Bangui and their matched controls to determine the clinical significance of diarrheagenic *Escherichia coli* (7, 8, 9, 10, 12, 16, 22, 25, 27, 29, 31, 32, 34, 35). The methods were as previously described (14). To be included in the study, the patients had to be HIV positive and aged 18 or over, have CD (3 or more loose watery stools per day for at least 14 days [3]), have *E. coli* in a stool sample, and give informed consent. Each patient was matched with a control recruited from among the neighbors and family members of the patient. The matching criteria dictated that the control be aged within 5 years of the patient's age and of the same sex. The recruitment criteria for the matched controls were as follows: testing positive for HIV antibodies, having had no diarrhea on the day of recruitment or during the previous month, and having *E. coli* in their stools on the day of recruitment. All controls gave informed consent to participate.

HEp-2 adherent *E. coli* (EAEC) (5, 28) with localized adherent (LA), aggregative adherent (AA), or diffuse adherent (DA) patterns were more common in the patients ($P < 10^{-5}$) than in the controls (Table 1). Some EAEC exhibited a strong LA pattern (16 patients versus no control) in which >20% of the randomly selected cells had attached bacteria (11, 19). These LA strains with a strong LA pattern were associated with CD, especially when the assays used to identify enteropathogenic *E. coli* (EPEC) virulence factors yielded positive results (*eaeA*, EPEC adherence factor [EAF] plasmid, bundle-forming pili [BFP] PCR, and fluorescent actin staining [FAS] test) ($P < 10^{-5}$), and all belonged to known EPEC O serogroups ($P = 0.0001$). The isolation of enteroaggregative *E. coli* (EAggEC) was strongly correlated with the presentation of CD ($P < 10^{-5}$). The difference in the isolation rates of EAEC strains exhibiting DA between patients and controls was only significant when the presence of the *astA* gene encoding EAST/1 was considered ($P = 0.016$); *astA* was located on 7- to 40-kb plasmids.

Interestingly, all of the enteric bacteria isolated from 42 patients (86% of the 49 patients with severe immunodepression) harboring EAEC with virulence factors were *E. coli* (Table 2). In contrast, in the 39 patients who had no EAEC or harbored EAEC with no virulence factor (Table 2) and in controls (data not shown), *E. coli* never represented more than 50% of the isolated enteric bacteria. This strongly suggests that some EAEC strains are diarrheagenic pathogens. Thus, colony hybridization assays under high-stringency conditions were carried out retrospectively on archived filters prepared from stools streaked onto nonselective medium to determine the percentage of colonies that harbored *eaeA* and *astA*. These stool samples were taken from 24 patients (7 carrying EPEC clones identified by the presence of *eaeA*, 13 harboring *astA*-positive EAggEC, and 4 harboring *astA*-positive diffusely adhering *E. coli* [DAEC]) and 12 controls. No hybridization was observed in controls. Results showed that 90 to 100% of the isolated bacteria hybridized with the *eaeA* probe (18) in the 7 patients carrying EPEC clones (100%) and with the *astA* probe

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TABLE 1. HEp-2 adherent *E. coli* strains isolated from HIV-infected adults with and without diarrhea[a]

Adherence pattern and genotype	No. of infected adults		P^b
	Patients (%)	Controls (%)	
LA[c]	18 (20.4)	5 (5.7)	0.011
EPEC, *eaeA*$^+$, BFP$^+$, EAF$^+$	14[d,e,f]	0	0.0001
non-EPEC serogrouped, *eaeA*$^+$, BFP$^+$, EAF$^+$	3[e,f,g]	0	NS
non-EPEC serogrouped, *eaeA*$^-$, BFP$^-$, EAF$^-$	1[f,h]	5[f]	NS
AA[i]	28 (31.8)	2 (2.2)	$<10^{-5}$
EAST/1 (*astA*)$^+$	11[j,k]	0	0.0009
EAST/1 (*astA*)$^+$, AAFII (*aafA*)$^+$	3[k]	0	NS
EAST/1 (*astA*)$^+$, AAFI (*aggA, aggC*)$^+$	3[k,l]	0	NS
EAST/1 (*astA*)$^-$, AAFI (*aggA, aggC*)$^-$ AAFII (*aafA*)$^-$	11[k,m]	2[k]	0.021
DA[n]	13 (14.7)	11 (12.5)	NS
AFA$^+$	2[o]	2	NS
SFA$^+$, PAP$^+$	1	2	NS
PAP$^+$	1	1	NS
AFA$^-$, SFA$^-$, PAP$^-$	9[p]	6	NS

[a] The mean age of patients was 37 years, and 47% were male. The median CD4$^+$ cell count was 114 cells/μl in the 88 patients and 502 cells/μl in the matched controls ($P < 10^{-5}$). AIDS-related symptoms (4) were observed in all of the patients and none of the controls. Data represent the number of adults in whom the tested *E. coli* colonies displayed the indicated adherence pattern and genotype. The mean number of strains tested was 9.78 for the patients and 8.84 for the controls (not significant, $P = 0.57$). None of the nonadhering *E. coli* from the patients or controls were positive in PCR assays or with the *aafA* DNA probe (6). None of the strains were positive for heat-labile or heat-stable toxin.

[b] McNemar exact test. NS, not significant ($P > 0.05$).

[c] None of the LA strains hybridized with the *astA* probe produced by PCR amplification of the *astA* gene present in EAggEC strain 17-2 (31).

[d] Slide agglutination test performed with O antisera 26, 55, 86, 111, 114, 119, 124, 125, 126, 127, 142, and 157. EPEC serogroups: O26, 1 patient; O111, 8 patients; O126, 3 patients; O127, 2 patients. All of the patients harbored EPEC strains with a strong LA pattern.

[e] All of the strains studied were positive for the fluorescent actin staining (FAS) test.

[f] Colony hybridization assays with the *eaeA* DNA probe (18) confirmed all of the PCR results.

[g] Two of the patients harbored EAEC with a strong LA pattern and harbored EAEC with a moderate LA pattern.

[h] Patient with HC and HUS; all of the EAEC isolates studied produced verotoxin (Stx2 according to PCR assays).

[i] Strains from 27 patients and 2 controls were identified by the EAggEC DNA probe (1), which hybridized with plasmids ranging from 40 to 100 kb.

[j] None of the EAggEC strains isolated from one patient hybridized with the EAggEC DNA probe (1) even under low-stringency conditions.

[k] Colony hybridization assays with the *astA* probe and with the *aggA*-specific DNA probe generated by labeling the PCR product obtained from the *E. coli* 17-2 genomic DNA (32) confirmed all PCR results.

[l] All EAggEC isolates from these three subjects hybridized with the EAggEC DNA probe (1) under low-stringency conditions.

[m] HC and HUS were observed in seven patients harboring EAEC isolates with mixed adherence patterns (a combination of AA, LA, and DA patterns) and producing verotoxin (Stx2 according to PCR assays).

[n] None of the strains produced verotoxin or hemolysin.

[o] The DAEC isolates from one patient harbored *astA*.

[p] The DAEC isolates from six patients harbored *AstA*.

TABLE 2. Semiquantitative assessment of *E. coli* isolated on nonselective BCP medium according to the immunosuppression and the diarrheagenic *E. coli* in stools

No. of CD4 cells/μl	Assessment(s)[a] (no. of cases) for:	
	Cases with EAEC harboring virulence factors[b]	Cases with no EAEC or with EAEC harboring no virulence factors[c]
<25	5+ (7)[d]	1+ (3)
<50	5+ (9)[e]	1+ (2), 2+ (1)
<75	5+ (7)[f]	2+ (5)
<100	5+ (7), 4+ (2)[g]	
<125	5+ (5), 3+ (1)[h]	2+ (3), 1+ (3)
<150	5+ (5), 3+ (1)[i]	3+ (3), 2+ (1)
<175	5+ (2), 4+ (2)[j]	2+ (1), 1+ (4)
<200	4+ (1)	1+ (4)[k]
<225		2+ (5)[l]

[a] The percentage of *E. coli* isolated on the streaked BCP plate was estimated as follows: 1+, <30%; 2+, 30 to <50%; 3+, 50 to <70%; 4+, 70 to <100%; 5+, 100%.

[b] Out of a total of 49 such cases.

[c] Out of a total of 29 cases with no EAEC and 10 cases with EAEC harboring no virulence factors.

[d] Cases: 7 EAggEC involved in HC with HUS.

[e] Cases: 5 EAggEC, 2 EPEC, 1 non-EPEC serogrouped involved in HC with HUS, and 1 DAEC.

[f] Cases: 2 EAggEC, 4 EPEC, and 1 DAEC.

[g] 5+ cases: 7 EAggEC. 4+ cases: 1 EPEC and 1 DAEC.

[h] 5+ cases: 1 EAggEC and 4 EPEC. 3+ case: DAEC.

[i] 5+ cases: 1 EAggEC and 4 EPEC. 3+ case: EPEC.

[j] 5+ cases: 1 EAggEC and 1 DAEC. 4+ cases: 2 DAEC.

[k] Cases: 3 EAggEC and 2 DAEC.

[l] Cases: 1 EAggEC and 4 DAEC.

(*astA* PCR product from EAggEC strain 17-2 [31]) in the 22 patients harboring *astA*-positive EAggEC or DAEC. Antimicrobial susceptibility tests were carried out, and accordingly, the 22 patients harboring EAEC with virulence factors (9 with LA strains, 8 with AA strains, and 5 with *astA*-positive DA strains) received fluoroquinolones for 14 days. Seven days after the end of treatment, EAEC negativation of cultures was associated with complete resolution of diarrhea in 17 patients (77%; 9 with LA strains, 5 with AA strains, and 5 with DA strains). This observation provides additional evidence that these EAEC were etiologic factors of CD.

During this study, the Central African Republic was afflicted with epidemics of hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) (13, 15). The eight patients afflicted with both HC and HUS presented pure cultures of EAEC. Non-EPEC serogrouped LA clones producing both verotoxin (20) (Stx2 alone according to PCR tests) and hemolysin were isolated from the stools of one patient. All of the isolates were negative for the enterohemorrhagic *E. coli* (EHEC) plasmid marker *ehec-hly* (33) and for the PCR detection of EHEC and EPEC virulence genes. They did not hybridize with the EHEC probe (23) or the EAF probe (26) even under low-stringency conditions and were negative in the FAS test and for invasion in the HeLa cell gentamicin protection assay (2). They all harbored two plasmids (5 and 70 kb) that did not hybridize with an stx_2 probe that reacts only with total cellular DNA. These results indicated that the stx_2 gene was present on the chromosome. In the seven other patients, we isolated EAEC that produced the verotoxin (Stx2 alone according to the PCR analysis). These clones showed a mixed adherence pattern, predominated by AA. In six of these patients, isolates showed AA and also typical LA, and isolates from two patients produced hemolysin and gave negative results in the PCR analyses for the EHEC plasmid marker *ehec-hly* (33). In the seventh patient, isolates showed a combination of AA and LA patterns

and an intercalated DA pattern. All of the clones gave negative results by PCR for the detection of virulence markers associated with EHEC, EPEC, DAEC, and EAggEC. They did not hybridize with the *eaeA* (18) or EHEC (23) probes, even under low-stringency conditions. Southern blot analysis indicated that the stx_2 gene was present on the chromosome. Plasmid profile analysis and antimicrobial susceptibility testing indicated that strains from the seven patients were epidemiologically unrelated. *Taq* cycle sequencing (21, 30) showed that the B-subunit gene of the toxin stx_2 was 100% homologous to the stx_2 B gene from the O157:H7 strain EDL933 (17) and from the O157:H7 and O157:H^- strains recently isolated in the region (13, 15). Although these isolates did not contain the classical EHEC markers (such as the *eaeA* gene) and were negative in the FAS test, they can be classified as EHEC because they were all isolated from HC and HUS and all produced an Stx2. In immunocompetent subjects, Stx2 production alone does not confer human pathogenicity (27). The Stx2-positive EAEC described in this study are thought to colonize the intestinal mucosa as efficiently as the *eaeA*-positive EHEC. This may involve unknown adhesins (the HEp-2 adherence test is a useful tool in this case for identifying potential virulent strains of *E. coli*), or it may illustrate that Stx2-producing *E. coli* with reduced virulence have a greater potential for producing HC and HUS in HIV-infected persons with enteric immune defects than in healthy individuals.

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