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 (EAEC) 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 EAE 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 EAEC, 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...
TABLE 1. HEp-2 adherent E. coli strains isolated from HIV-infected adults with and without diarrhea

| Adherence pattern and genotype | No. of infected adults | p
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients (%)</td>
<td>Controls (%)</td>
</tr>
<tr>
<td>LA+</td>
<td>18 (20.4)</td>
<td>5 (5.7)</td>
</tr>
<tr>
<td>EPEC, eaeA+ , BFP+ , EAF+</td>
<td>142/30</td>
<td>0</td>
</tr>
<tr>
<td>non-EPEC serogrouped, eaeA+ , BFP+, EAF+</td>
<td>3/40</td>
<td>0 NS</td>
</tr>
<tr>
<td>non-EPEC serogrouped, eaeA+ , BFP+, EAF+</td>
<td>1/30</td>
<td>5 NS</td>
</tr>
<tr>
<td>AA+</td>
<td>28 (31.8)</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>EAST/1 (astA)+</td>
<td>112</td>
<td>0</td>
</tr>
<tr>
<td>EAST/1 (astA)+, AAFII (astA)+</td>
<td>3</td>
<td>0 NS</td>
</tr>
<tr>
<td>EAST/1 (astA)+, AAFII (astS)+</td>
<td>3b</td>
<td>0 NS</td>
</tr>
<tr>
<td>EAST/1 (astA)+, AAFII (astA)</td>
<td>111k</td>
<td>2k</td>
</tr>
<tr>
<td>DA+</td>
<td>13 (14.7)</td>
<td>11 (12.5)</td>
</tr>
<tr>
<td>AFA+</td>
<td>2</td>
<td>2 NS</td>
</tr>
<tr>
<td>SFA+ , PAP+</td>
<td>1</td>
<td>2 NS</td>
</tr>
<tr>
<td>PAP+</td>
<td>1</td>
<td>1 NS</td>
</tr>
<tr>
<td>AFA+ , SFA+ , PAP−</td>
<td>9</td>
<td>6 NS</td>
</tr>
</tbody>
</table>

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 < 0.05). 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
serotype. 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 astA DNA probe
(b). 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 172-2 (31). 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.

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

e Colony hybridization assays with the astA DNA probe (18) confirmed all of
the PCR results.

f Two of the colonies hybridized with EAECC with a strong LA pattern and harbored
EAECC with a moderate LA pattern.

g Patient with HC and HUS; all of the EAECC isolates studied produced
verotoxin (Stx2 according to PCR assays).

h 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.

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

j 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.

k All EAggEC isolates from these three subjects hybridized with the EAggEC
DNA probes (1) under low-stringency conditions.

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

m None of the strains produced verotoxin or hemolysin.

n The DAEC isolates from one patient harbored astA.

o The DAEC isolates from six patients harbored astA.

(31) in the 22 patients harboring astA-positive EAggEC or DAEC. Antimi-
icrobial susceptibility tests were carried out, and accordingly, the 22 patients harboring EAECC with virulence factors (9 with

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

<table>
<thead>
<tr>
<th>No. of CD4 cells/μl</th>
<th>Cases with EAEC</th>
<th>Cases with no EAEC</th>
<th>Assessment(s) (no. of cases) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with virulence factors</td>
<td>with no virulence factors</td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>5+ (7)</td>
<td>1+ (3)</td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>5+ (9)</td>
<td>1+ (2), 2+ (1)</td>
<td></td>
</tr>
<tr>
<td>&lt;75</td>
<td>5+ (7)</td>
<td>2+ (5)</td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>5+ (7), 4+ (2)</td>
<td>2+ (3), 1+ (3)</td>
<td></td>
</tr>
<tr>
<td>&lt;125</td>
<td>5+ (5), 3+ (1)</td>
<td>3+ (3), 2+ (1)</td>
<td></td>
</tr>
<tr>
<td>&lt;150</td>
<td>5+ (5), 3+ (1)</td>
<td>3+ (3), 2+ (1)</td>
<td></td>
</tr>
<tr>
<td>&lt;175</td>
<td>5+ (2), 4+ (2)</td>
<td>2+ (1), 1+ (4)</td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>4+ (1)</td>
<td>1+ (4)</td>
<td></td>
</tr>
<tr>
<td>&lt;225</td>
<td>2+ (5)</td>
<td>2+ (5)</td>
<td></td>
</tr>
</tbody>
</table>

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 e 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.

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 negativity of cultures was associ-
ated 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 hemolyti-
cremic syndrome (HUS) (13, 15). The eight patients afflic-
ted 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 HEC
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 stx2 probe that reacts only with total cellular DNA.
These results indicated that the stx2 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 pro-
duced 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 EAEC. They did not hybridize with the eaeA (18) or EHEC (23) probes, even under low-stringency conditions. Southern blot analysis indicated that the stx2 gene was present on the chromosome. Plasmid profiling analysis and antimicrobial susceptibility testing indicated that strains from the seven patients were epidemiologically unrelated. Tax cycle sequencing (21, 30) showed that the B-subunit gene of the toxin stx2 was 100% homologous to the stx2 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 region) and expression of the HEp-2 adherin (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 region) and expression of the HEp-2 adherin (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.

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