Typing of Intimin (eae) Genes in Attaching and Effacing Escherichia coli Strains from Monkeys

Attaching and effacing Escherichia coli (AEEC) strains cause histopathological alterations termed “attaching and effacing (A/E) lesions” (8). The ability to cause A/E lesions is encoded on a large bacterial chromosomal pathogenicity island, the locus of enterocyte effacement (LEE). The central portion of LEE encodes intimin (Eae, 94- to 97-kDa outer membrane protein) and Tir, the intimin receptor, which is translocated into the host cell membrane by the type III system (8). Differentiation of intimin alleles represents an important tool for AEEC typing in pathogenesis and epidemiological, clonal, and immunological studies, and it may also be a potential tool in routine diagnostics (1, 2, 3, 4, 12, 15). The 5′ portions of eae genes are conserved, whereas the 3′ regions are heterogeneous. This observation led to the construction of universal primers described by Blanco et al. (2, 4) for the already key strains showed nontypeable intimins (5). In order to ascertain whether these intimin subtypes were actually new ones, some of these strains were examined again by PCR using a set of new primers described by Blanco et al. (2, 4) for the already known intimins as well as for new eae variants β2, μ, ν, and ϵ. For comparison studies, the monkey strains were serotyped by the method described by Guineé et al. (6), and the previous results obtained for bfp by PCR, as well as BFP expression by Western blotting, were reconsidered in this study (5).

All 15 monkey Escherichia coli strains assayed were positive with universal primers EAE-1 and EAE-2 that generated PCR products obtained from the amplified 5′-conserved region of the eae gene. Six monkey AEEC strains presented identical serotypes and intimins (two O142:H6 α1, two O128:H2 β1, and two O127:H40 γ2/0 strains) to human enteropathogenic E. coli (EPEC), whereas eight strains showed new serotypes not previously found in human or animal AEEC with β1 (two O132:H31 strains), β2 (one O139:H14 strain and one O167:H6 strain), γ (one O26:H7 strain), α2 (two O49:H46 strains), and λ (one O33:H−) intimins. The remaining monkey strain, which belonged to serotype O167:H9 (β1), although it was not included among human EPEC serotypes, was characterized as an AEEC strain that caused an outbreak of gastroenteritis involving a large number (256 patients) of schoolchildren (11) (Table 1). The intimins α2, γ1, δ/κ, ζ, η, μ, ν, and ϵ were not found among the AEEC strains isolated from marmosets in Brazil. However, considering that only 15 strains were studied, the

### Table 1. Serotypes and intimin types of monkey AEEC strains isolated in Brazil

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>Status</th>
<th>Serotype</th>
<th>bfpA gene/BFP expression</th>
<th>Intimin subtype</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diarrhea</td>
<td>O142:H6</td>
<td>+/-</td>
<td>α1</td>
<td>Human EPEC serotype</td>
</tr>
<tr>
<td>1</td>
<td>Diarrhea</td>
<td>O142:H6</td>
<td>−/−</td>
<td>α1</td>
<td>Human EPEC serotype</td>
</tr>
<tr>
<td>1</td>
<td>Healthy</td>
<td>O132:H31</td>
<td>+/−</td>
<td>β1</td>
<td>New serotype</td>
</tr>
<tr>
<td>1</td>
<td>Healthy</td>
<td>O147:H9</td>
<td>−/−</td>
<td>β1</td>
<td>Human/overbreak</td>
</tr>
<tr>
<td>1</td>
<td>Diarrhea</td>
<td>O139:H14</td>
<td>−/−</td>
<td>β2</td>
<td>New serotype</td>
</tr>
<tr>
<td>1</td>
<td>Diarrhea</td>
<td>O167:H6</td>
<td>+/−</td>
<td>β2</td>
<td>New serotype</td>
</tr>
<tr>
<td>2</td>
<td>1 Diarrhea, 1 healthy</td>
<td>O127:H40</td>
<td>−/−</td>
<td>γ2/0</td>
<td>Human EPEC serotype</td>
</tr>
<tr>
<td>1</td>
<td>Diarrhea</td>
<td>O26:H7</td>
<td>−/−</td>
<td>ε</td>
<td>New serotype</td>
</tr>
<tr>
<td>1</td>
<td>Healthy</td>
<td>O49:H46</td>
<td>−/−</td>
<td>λ</td>
<td>New serotype</td>
</tr>
<tr>
<td>1</td>
<td>Healthy</td>
<td>O33:H−</td>
<td>−/−</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* New serotype represents a serotype not found in human or animal AEEC with the indicated intimin subtype in previous studies.
diversity of intimins found among these strains was relatively high.

In conclusion, this study indicates that nonhuman primates may represent a natural reservoir of EPEC serotypes pathogenic for humans.

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