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# Antigenic Relationships Between the Enteroinvasive *Escherichia coli* O Antigens O28ac, O112ac, O124, O136, O143, O144, O152, and O164 and *Shigella* O Antigens

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Enteroinvasive *Escherichia coli* strains have been found in the following O serogroups: O28ac, O112ac, O124, O136, O143, O144, O152, and O164. The biochemical reactions of these enteroinvasive *E. coli* strains are often similar to those of *Shigella* strains, and identification may be difficult. The problem of differentiation and identification is further complicated by the sharing of antigenic components. The antigenic relationships between these O antigens and those of all the established and provisional *Shigella* serovars were examined. O antigen identity was demonstrated between *E. coli* O124 and *Shigella dysenteriae* 3 and between *E. coli* O152 and provisional *Shigella* serovar 3341:55. Reciprocal relationships were found between *E. coli* O112ac and *S. dysenteriae* 2, *S. boydii* 1, *S. boydii* 15, between *E. coli* O136 and *S. dysenteriae* 3, and between *E. coli* O164 and *S. dysenteriae* 3. A one-way relationship was demonstrated between *E. coli* O136 and *S. boydii* 1. The remaining enteroinvasive *E. coli* O serogroups showed no significant antigenic relationships with any *Shigella* serovar.

Certain *Escherichia coli* serogroups cause diarrhea that clinically resembles dysentery. The O serogroups which so far have been found to contain these dysentery-producing strains include O28ac, O112ac, O124, O136, O143, O144, O152, and O164. *E. coli* O124 and O164 have been reported to cause both large and small outbreaks of diarrhea (4–6, 8–10, 13). The remaining serogroups of the enteroinvasive *E. coli* (EIEC) have been reported in many countries in sporadic cases of dysentery-like disease and occasionally in small outbreaks (7, 11, 12).

There is extensive sharing of antigenic components among the *Enterobacteriaceae*, and many examples exist for *Shigella* serovars and *E. coli* serogroups. Some *Shigella* O antigens are identical to *E. coli* O antigens, and others are closely related (2, 14). This sharing of antigens often complicates the serological identification of *E. coli* and *Shigella* spp.

In this study we investigated the antigenic relationships between the EIEC serogroups and all the *Shigella* serovars, including the provisional serovars.

## MATERIALS AND METHODS

**Bacterial strains.** Test strains for *E. coli* serogroups O28ac, O112ac, O124, O136, O143, O144, O152, O164 and for all the *Shigella* serovars, including the provisional serovars (3), were from the Division of Enteric Pathogens culture collection. The serovars and a list of the descriptive literature for each of the EIEC strains are given in Table 1.

**Preparation of antisera.** O antigen vaccines were prepared by harvesting the growth from two nutrient agar slopes in 0.7% saline and heating it at 100°C for 2.5 h. After centrifugation, the heated organisms were suspended in 15 ml of saline, and commercial Formalin was added to a final concentration of 0.3%. Rabbits were immunized by intravenous injections of 0.5, 1.0, 2.0, 2.0, and 2.0 ml at 5-day intervals, 40 ml of blood was taken at 5 and 10 days after the final injection, and the rabbits were exsanguinated 5 days later. The sera from all three bleedings were pooled.

**O antigen suspensions for agglutination tests.** O antigen suspensions for agglutination tests were prepared by heating overnight broth cultures at 100°C for 30 min. Commercial Formalin was added to a final concentration of 0.3%.

**Agglutination tests.** O antisera for *E. coli* O28ac, O112ac, O124, O136, O143, O144, O152, and O164 were tested against the O antigen suspensions of all the *Shigella* strains, and O antigen suspensions of *E. coli* O28ac, O112ac, O124, O136, O143, O144, O152, and O164 were tested against the O antisera of all the *Shigella* strains.

Agglutination tests were performed in plastic microtiter agglutination trays which were incubated at 50°C for 16 h.

**Absorption studies.** When cross-reactions were found, reciprocal absorptions were performed to determine the relationship between the strains.

Serological cross-reactions were considered significant only if the cross-reaction titer was greater than or equal to 1/16 of the homologous titer.

TABLE 1. Antigenic relationships between EIEC serogroups O28ac, O112ac, O124, O136, O143, O144, O152, and O164 and *Shigella* serovars

| EIEC serovar | Original designation | Antigenic relationship |
|---|---|---|
| O28acK73H− | Katawijk | None |
| O112acK66H− | Guanabara H194 | *S. dysenteriae* 2 (a,b-a,c)<br>*S. boydii* 1 (a,b-a,c)<br>*S. boydii* 15 (a,b-a,c) |
| O124K72H30 | 227 | *S. dysenteriae* 3 (identical)<br>Provisional *Shigella* serovar 3615.53 (a,b-a,c) |
| O136K78H− | 1111.55 | *S. dysenteriae* 3 (a,b-a,c)<br>*S. boydii* 1 (one way) |
| O143K?H− | 4606.58 | None |
| O144K?H− | 1624.56 | None |
| O152K?H | 1184.68 | Provisional *Shigella* serovar 3341.55 (identical) |
| O164K?H− | 145/46 | *S. dysenteriae* 3 (a,b-a,c) |

## RESULTS

The antigenic relationships found are summarized in Table 1.

Significant cross-reactions were found between *E. coli* O112ac, O124, O136, O152, and O164 and certain *Shigella* serovars (Tables 2–6). The remaining *E. coli* serogroups showed no significant cross-reactions with any *Shigella* serovars.

The test strain of *E. coli* O112ac showed a reciprocal relationship of a,b-a,c variety with *S. dysenteriae* 2, *S. boydii* 1, and S. *boydii* 15.

The O antigen of *E. coli* O124 was identical to that of *S. dysenteriae* 3 and also showed a reciprocal relationship of the a,b-a,c variety with the provisional *Shigella* serovar 3615.53.

*E. coli* O136 showed a reciprocal relationship of the a,b-a,c variety with *S. dysenteriae* 3. The test strain of *S. boydii* 1 was agglutinated by *E. coli* O136 antiserum, but a reciprocal reaction was not found. Reciprocal absorptions showed that the O antigen of *E. coli* O152 was identical to that of the provisional *Shigella* serovar 3341.55.

*E. coli* O164 showed a reciprocal relationship of the a,b-a,c variety with *S. dysenteriae* 3.

## DISCUSSION

EIEC infections in humans produce bloody diarrhea and pyrexia, a disease that may be clinically indistinguishable from that caused by *Shigella* infections. EIEC produce this disease by invading the epithelial cells of the large

TABLE 2. Antigenic relationships between EIEC serogroup O112ac and *Shigella* serovars

| Serum | Absorbing suspension | Titer vs antigen suspension | | | |
|---|---|---|---|---|---|
| | | O112ac | *S. dysenteriae* 2 | *S. boydii* 1 | *S. boydii* 15 |
| O112ac | None | 25,600 | 25,600 | 1,600 | 3,200 |
| | *S. dysenteriae* 2 | 1,600 | <100 | <100 | <100 |
| | *S. boydii* 1 | 6,400 | 3,200 | <100 | 400 |
| | *S. boydii* 15 | 12,800 | 6,400 | 400 | <100 |
| *S. dysenteriae* 2 | None | 3,200 | 6,400 | 200 | 800 |
| | O112ac | <100 | 6,400 | <100 | <100 |
| *S. boydii* 1 | None | 200 | <100 | 800 | <100 |
| | O112ac | <100 | <100 | 1,600 | <100 |
| *S. boydii* 15 | None | 400 | 200 | 200 | 3,200 |
| | O112ac | <100 | <100 | <100 | 3,200 |

TABLE 3. Antigenic relationships between EIEC serogroup O124 and *Shigella* serovars

| Serum | Absorbing suspension | Titer vs antigen suspension | | |
|---|---|---|---|---|
| | | O124 | *S. dysenteriae* 3 | 3615.53 |
| O124 | None | 1,600 | 3,200 | 400 |
| | *S. dysenteriae* 3 | <100 | <100 | <100 |
| | 3615.53 | 6,400 | 3,200 | <100 |
| *S. dysenteriae* 3 | None | 800 | 1,600 | 400 |
| | O124 | <100 | <100 | <100 |
| 3615.53 | None | 400 | 400 | 3,200 |
| | O124 | <100 | <100 | 400 |

TABLE 4. Antigenic relationships between EIEC serogroup O136 and *Shigella* serovars

| Serum | Absorbing suspension | Titer vs antigen suspension | | | |
|---|---|---|---|---|---|
| | | O136 | *S. dysenteriae* 3 | *S. boydii* 1 | *S. boydii* 15 |
| O136 | None | 6,400 | 400 | 3,200 | 200 |
| | *S. dysenteriae* 3 | 1,600 | <100 | <100 | <100 |
| | *S. boydii* 1 | 6,400 | 100 | <100 | <100 |
| | *S. boydii* 15 | 3,200 | 400 | <100 | <100 |
| *S. dysenteriae* 3 | None | 200 | 6,400 | 200 | <100 |
| | O136 | <100 | 1,600 | <100 | <100 |
| *S. boydii* 1 | None | <100 | <100 | 800 | <100 |
| | O136 | <100 | <100 | 800 | <100 |
| *S. boydii* 15 | None | 200 | 400 | 200 | 3,200 |
| | O136 | <100 | <100 | <100 | 800 |

TABLE 5. Antigenic relationships between EIEC serogroup O152 and *Shigella* serovars

| Serum | Absorbing suspension | Titer vs antigen suspension | |
|---|---|---|---|
| | | O152 | 3341.55 |
| O152 | None | 12,800 | 25,600 |
| | 3341.55 | <100 | <100 |
| 3341.55 | None | 1,600 | 12,800 |
| | O152 | <100 | <100 |

TABLE 6. Antigenic relationships between EIEC serogroup O164 and *Shigella* serovars

| Serum | Absorbing suspension | Titer vs antigen suspension | |
|---|---|---|---|
| | | O164 | *S. dysenteriae* 3 |
| O164 | None | 25,600 | 6,400 |
| | *S. dysenteriae 3* | 25,600 | <100 |
| *S. dysenteriae* 3 | None | 400 | 3,200 |
| | O164 | <100 | 3,200 |

bowel, leading to inflammation and ulceration of the mucosa (1, 4, 6).

As well as causing a dysentery-like disease, EIEC are often biochemically atypical and may resemble *Shigella* spp. in that they are nonmotile, anaerogenic, and non- or late-lactose fermenting (Table 7). This problem of biochemical misidentification can be remedied by the use of tests for β-galactosidase activity, lysine decarboxylation, and utilization of citrate in Christensen medium. The possibility of misidentification of EIEC as *Shigella* spp. increases if a strain is strongly agglutinated by antiserum for *Shigella* spp.

The antigenic relationships demonstrated (Table 1) show that the O antigens of many of the EIEC are identical to, or very closely related to, those of *Shigella* spp. Knowledge of the close antigenic relationships between the EIEC and *Shigella* spp. is valuable. If a laboratory is unable to carry out the additional biochemical tests mentioned above, then it may be difficult or even impossible to accurately identify EIEC.

TABLE 7. Important tests for the differentiation of *Shigella* spp. and EIEC

| Test | Reaction[a] of: | |
|---|---|---|
| | *Shigella* spp. | EIEC |
| Motility | − | +/− |
| Gas from glucose | −[b] | +/− |
| Lactose fermentation | −[c] | +/− |
| Sucrose fermentation | −[d] | d |
| Salicin fermentation | − | d |
| Christensen citrate | − | +/− |
| Sodium acetate | −[e] | +/− |
| Lysine decarboxylase | − | +/− |

[a] +/−, Most strains are positive; d, reactions vary from strain to strain.

[b] Some biotypes of *S. flexneri* 6, *S. boydii* 13, and *S. boydii* 14 are positive.

[c] *S. sonnei* frequently ferments lactose after incubation for more than 24 h. Positive strains of *S. flexneri* 2a and *S. flexneri* 9 have been described.

[d] *S. sonnei* may ferment sucrose after incubation for more than 24 h.

[e] Some biotypes of *S. flexneri* 4a may utilize sodium acetate.

From a clinical point of view, the misidentification of EIEC as *Shigella* spp. is of little importance, as both organisms produce a similar, if not identical, disease. However, from an epidemiological point of view, accurate differentiation between EIEC and *Shigella* spp. is essential.

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