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Virulence of *Escherichia coli* in Experimental Hematogenous Pyelonephritis in Mice

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Differences in nephropathogenicity between *Escherichia coli* strains were studied by following the kinetics of the viable count in the mouse kidney during 8 h after intravenous injection. Assuming as a reference point that at zero time 0.1% of the inoculum was lodged in the kidney, we found that strains fell into three main groups with different behavior patterns: in group I, the viable count fell and remained low; in group II, the viable count first fell and then rose after 4 h, reaching the level of the reference point within 8 h; in group III, the viable count rose rapidly and remained high. The kinetics of the viable counts were also studied in blood, spleen, and liver: group I and II strains behaved similarly; only in the kidney did group II strains show higher counts than group I strains. These data suggest that group II strains are specifically virulent for the mouse kidney. Group III strains also gave higher viable counts in blood and spleen but comparable counts in the liver, suggesting that group III strains are more generally virulent. Fifty percent lethal dose measurements confirmed the conclusion that group I strains are avirulent and group III strains are the most virulent. Possible relationships between behavior pattern and serotype are discussed.

Escherichia coli is by far the commonest infecting organism in urinary tract infections, especially in acute episodes, when it accounts for about 80% of the cases. Urinary bacteria commonly originate in the patient's own bowel, and infection occurs mostly via the so-called ascending route (4). There are, however, still two different views about the nature of the infecting strains. According to the "prevalence theory," the *E. coli* strains causing infection are those predominant in the feces (3, 12, 21), whereas according to the "special pathogenicity theory" the infecting *E. coli* strains belong to a select group with properties which specially allow them to infect the urinary tract. With regard to this special pathogenicity theory several authors have suggested a more or less positive correlation between nephropathogenicity and, for instance, serogroup (6), possession of K-antigen (13, 18, 19, 33), the amount of K-antigen present (9, 17, 24, 26), hemolysis (5, 8), adhesion (14, 31), serum sensitivity (13, 20), and dulcitol fermentation (13, 19, 33).

The purpose of the present study was to determine possible differences in nephropathogenicity between different *E. coli* strains in a more quantitative way. We found it impossible to measure 50% kidney infective dose, because unfortunately the dose required to develop pyelonephritis lay close to the 50% lethal dose (LD_{50}) so that many mice died from bacteremia within 48 h (10; unpublished data). Thus, we decided to measure the kinetics of the viable count in the mouse kidney after intravenous (i.v.) injection (10, 11, 23).

MATERIALS AND METHODS

Bacterial strains. Most of the 26 *E. coli* strains used in this study were isolated from domiciliary urinary infections in younger women in England and in The Netherlands. The strains were biotyped by the Enterotube system (Roche, Basel, Switzerland) and the API 20E system (API system S.A., Montalien Vercien, France). All strains were sensitive for a range of seven antibiotics. Serotyping was performed by standard techniques at the International Escherichia Centre, Copenhagen.

Behavior pattern determination in the mouse kidney. Strains were grown with agitation in nutrient broth (Oxoid, London, England) to yield 10^9 late-log-phase cells per ml and were centrifuged and resuspended in quarter-strength Ringer solution. At zero time 10 female Swiss mice (Swiss-Random, TNO, Zeist, The Netherlands), aged 8 weeks and weighing about 25 g, were injected i.v. in the tail with 2.5×10^8 cells of the *E. coli* strain to be tested, which were suspended in 0.5 ml of quarter-strength Ringer solution. At different times after injection, up to 8 h, two mice were killed. The kidneys were removed aseptically and homogenized separately in a total volume of 5 ml of quarter-strength Ringer solution (Thomas

Tissue Grinder, Philadelphia, Pa.). Serial dilutions of these suspensions were plated out on eosin-methylene blue agar plates (Oxoid), and the viable count per kidney was calculated as the mean of the viable counts of each of the four kidneys. Each strain was tested this way at least twice. The two remaining mice per experiment were observed to see whether they survived the inoculum.

Behavior pattern determination in mouse blood, spleen, and liver. The kinetics of the viable count in blood, spleen, and liver were determined as described for the kidney. Blood was sampled from the thoracic cavity after incision of the heart and collected in a drop of heparin (Thromboliquine, Organon Teknika, Oss, Holland). Spleens were homogenized in a total volume of 5 ml, and livers were homogenized in a total volume of 10 ml, of quarter-strength Ringer solution.

Determination of LD_{50}. Log-phase bacterial cells resuspended in quarter-strength Ringer solution were injected i.v. in serial dilutions into groups of 8-week-old male Swiss mice, each group containing six mice. Injection of quarter-strength Ringer solution on its own did not give rise to deaths. After 14 days the LD_{50}s were calculated according to the method of Spearmann and Kärber (7).

Serum sensitivity test. For estimation of the bactericidal activity of mouse serum, we followed the technique described by Taylor et al. (32), with some minor alterations. An overnight culture in nutrient broth was diluted in fresh nutrient broth and grown at 37°C with agitation to a density of about 5×10^8 cells per ml. This culture was washed once and resuspended in 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.4) to a density of 10^3 cells per ml. One milliliter of this suspension was added to 3 ml of pooled mouse serum and incubated at 37°C with agitation. Viable counts were measured 0, 1.5, and 3 h after incubation. Serum was collected freshly and sterility was checked.

Serum agglutinin determination. After incubation overnight, bacteria were scraped from a nutrient agar plate and suspended in 0.9% NaCl to a density of approximately 8×10^8 cells per ml.

Serial dilutions were made from freshly collected pooled mouse serum in 0.5 ml of saline. To each dilution 0.5 ml of the bacterial suspension was added, and agglutination was read after incubation overnight at 50°C.

RESULTS

Determination of the kinetics in the kidney. With the experimental model described above, it was possible to divide the *E. coli* strains into four different groups with distinct behavior patterns. These behavior patterns are shown in Fig. 1 through 4. In these figures each line represents one *E. coli* strain. The arrows at zero time do not represent an exact viable count in the kidney; they indicate a reference point, based on the assumption that at zero time 0.1% of the inoculum was lodged in the kidney.

Figure 1 shows the behavior pattern of group I strains. The viable count per kidney of these strains fell rapidly and remained low for 8 h at approximately 10^4 living cells. In Fig. 2 the viable counts are shown for the strains belonging to group II. There was, again, a comparable decline in the viable counts per kidney; however, after about 4 h they began to rise and reached the level of the reference point by 8 h.

In Fig. 3 the pattern of group III strains is shown; it differs from the first two patterns in that the viable count per kidney in this group rose rapidly and remained high at 10^6 to 10^7 living cells. Mice injected with these strains often died quickly (dashed lines).

Figure 4 shows the pattern of group IV strains. Here the viable count per kidney was relatively high after 15 min and remained approximately at the level of the reference point. The group IV pattern lies between the patterns of groups II

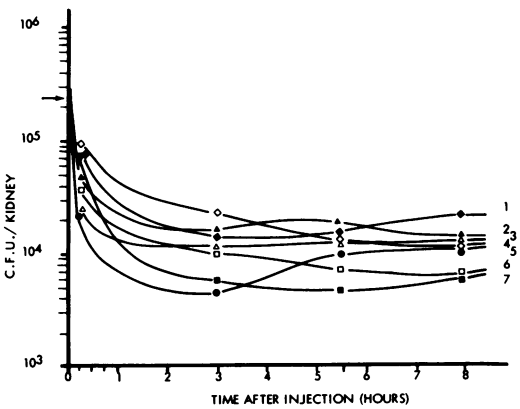

FIG. 1. *Behavior patterns in the mouse kidney of group I strains after i.v. injection. CFU, Colony-forming units.*

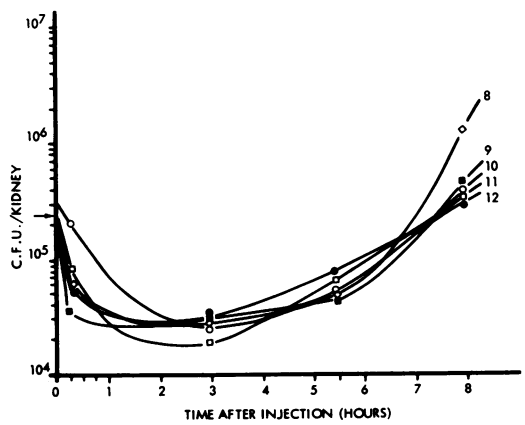

FIG. 2. *Behavior patterns in the mouse kidney of group II strains after i.v. injection. CFU, Colony-forming units.*

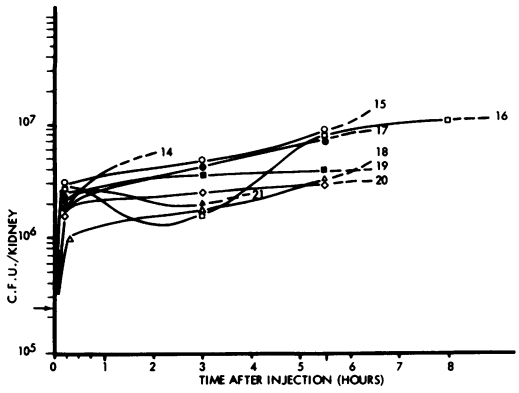

FIG. 3. *Behavior patterns in the mouse kidney of group III strains after i.v. injection. CFU, Colony-forming units.*

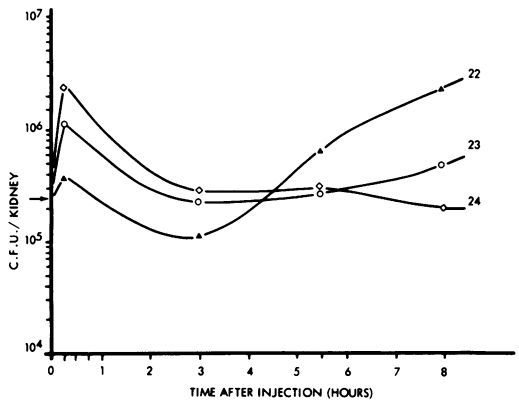

FIG. 4. *Behavior patterns in the mouse kidney of group IV strains after i.v. injection. CFU, Colony-forming units.*

and III but is distinct from both. It is not clear, however, whether this small group (only three strains) represents a specific pattern.

Figure 5 summarizes the behavior patterns in the mouse kidney of the three major groups (I, II, and III). Each line represents the mean of the various strains within one group. The differences between these three groups are very obvious.

Determination of the kinetics in blood, spleen, and liver. To find out whether these different behavior patterns in the mouse kidney (Fig. 5) are a reflection of differences in a specific virulence for the mouse kidney or merely a reflection of differences in a general virulence, we carried out similar experiments in which we followed the viable count in blood, spleen, and liver. The results are shown in Fig. 6, 7, and 8, respectively, each line representing the mean values of five strains belonging to one group (I, II, or III). Comparison of the kinetics of the viable counts in kidney, blood, spleen, and liver (Fig. 5 through 8) shows that group I and II strains only behaved differently in the kidney, giving similar counts in blood, spleen, and liver. These results indicate that the group II strains are specifically virulent for the mouse kidney. Furthermore, the group III strains gave higher counts than group I and II strains in kidney, blood, and spleen, indicating that group III strains show a more general virulence.

LD_{50} determination and killing rate. We also studied the LD_{50}s of these strains after i.v. injection (Fig. 9). The points in this figure represent the LD_{50}s of strains, arranged according to the behavior groups to which they belong. Most of the strains belonging to group I did not kill any mice, even after an injection of more than 10^9 cells. The group II strains showed LD_{50}s somewhat lower than 10^8 cells, whereas the strains belonging to group III showed LD_{50}s of about 10^7 cells or less. By statistical analysis with the Kruskal-Wallis test at the significance level $\alpha = 5\%$, the hypothesis that the LD_{50}s of groups I, II, and III are equal was rejected. According to the method of Dunn for multiple comparisons, only groups I and III could be shown to be significantly different (16). The small number of strains per group might explain why a significant difference between the LD_{50}s of groups I and II, on the one hand, and groups II and III, on the other, could not be demonstrated.

During behavior pattern determinations, we also observed differences between these three

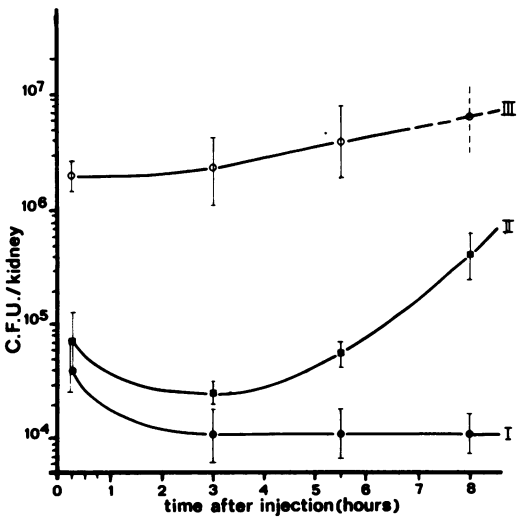

FIG. 5. *Mean of the behavior patterns in the mouse kidney of strains belonging to groups I, II, and III. CFU, Colony-forming units.*

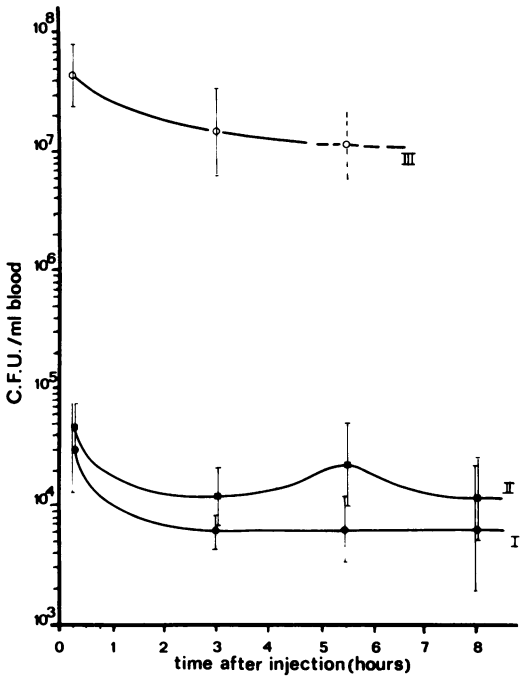

FIG. 6. *Kinetics of the viable count of group I, II, and III strains in the blood after i.v. injection. Mean of five strains per group. CFU, Colony-forming units.*

groups in survival times after i.v. injection of 2.5 $\times 10^8$ cells. Group I strains very rarely gave rise to deaths: no deaths were observed within 8 h, and only 10% of the mice were killed within 14 days. Group II strains killed 80% of the mice within 4 days and only 10% within 8 h. Group III strains killed most of the mice (90%) within 8 h after injection.

Geographical origin. The strains used in this study were mostly isolated from domiciliary infections in England and in The Netherlands. It was noticed that representatives of all four groups of strains with different patterns in the mouse kidney were found in both collections.

Serotyping of isolates. The results of the serotyping are shown in Table 1. All strains with the same serotype, even from different countries, fell into the same group. It is striking that all the O75 strains except one fell into group I, whereas all the O6 strains fell into groups II and III. With regard to the K antigens, all the O6:K2 strains were assigned to group II, but all the O6:K23 strains were assigned to group III. On the other hand, it should be noted that we found K− strains in all four groups. Furthermore, an O6:K− strain was also found in group II, the O75:K−:H5 strain belonged to group I, and both O18ac:K+ and O18ac:K− strains belonged to group III.

Biotyping of isolates. No clear correlation was found between the four behavior patterns and either the biotypes or the ability to ferment dulcitol.

Serum sensitivity and serum agglutinins. No bactericidal activity of mouse serum was observed. All the strains used in this study grew in vitro in mouse serum within 3 h. Nor were marked serum agglutinins observed in the sera of the mice used in this study. Against the O18ac:K+ strain, belonging to group III, the serum agglutinin titer was 1:16; for the other strains the titer was 1:2 or less.

DISCUSSION

The finding of three or possibly four distinct groups of *E. coli* strains with different behavior patterns in the mouse kidney is not inconsistent with the observations of Harle et al. (15) and MacLaren (23). They both found two different patterns in comparable experiments, but they tested only a few strains. Gorrill and co-workers (10, 11) only tested one strain.

When we consider the behavior of the three groups with the most obviously different behavior patterns in the mouse kidney (Fig. 5) and take into account the different kinetics of the viable counts in blood, spleen, and liver (Fig. 6 through 8) and the differences in LD_{50}s (Fig. 9) and survival times, we can draw the following

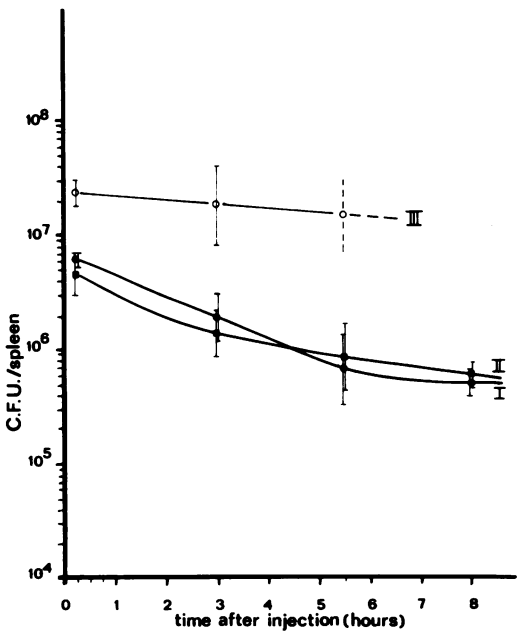

FIG. 7. *Kinetics of the viable count of group I, II, and III strains in the spleen after i.v. injection. Mean of five strains per group. CFU, Colony-forming units.*

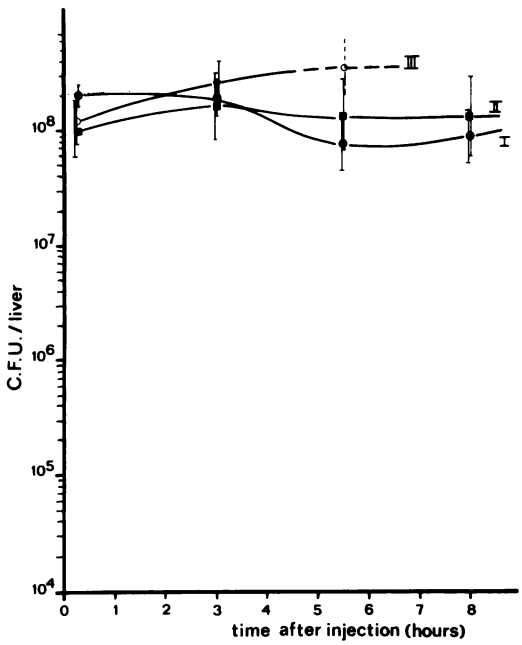

FIG. 8. *Kinetics of the viable count of group I, II, and III strains in the liver after i.v. injection. Mean of five strains per group. CFU, Colony-forming units.*

conclusions. The group I strains are avirulent, with relatively low counts in kidney, blood, and spleen, and they rarely kill the mice. The group II strains are more virulent, with rather high counts in the kidney 8 h after injection but relatively low counts in blood and spleen, and they often kill the mice. The group III strains are the most virulent of all, with high counts in the kidney, blood, and spleen immediately after injection, and they rapidly kill most of the mice. All three groups of strains gave similar high counts in the liver, presumably because of a rapid adhesion to the Kupffer cells in the liver (25). As shown in Results, it is likely that group II strains are specifically virulent for the mouse kidney, because in contradistinction to group I strains they can establish themselves and multiply in the kidney. However, group III strains show a more general virulence for mice, with high counts in kidney, blood, spleen, and liver, so that most of the animals die rapidly. This indicates that the mice are unable to cope with these strains, regardless of the reason.

The reason for the different behavior patterns still needs to be investigated. The differences in behavior patterns could not be explained by differences in sensitivity for mouse serum or by the presence of serum agglutinins against the *E. coli* strains used.

All the strains used in this study were isolated from urinary infections. On the assumption that mouse virulence is comparable to human virulence, it is tempting to speculate that the avirulent group I strains have had the opportunity to infect the urinary tract by a (temporary) decrease in the host defenses. By the same token the 075 strains, mainly found in group I, can fit into the prevalence theory, which seems to be supported by the finding of Mabeck et al. (22) that O75 strains rarely involved the kidney. On this line of the argument, the strains of the other more virulent groups can be considered to have infected the urinary tract in part because of their enhanced virulence, in accordance with the special pathogenicity theory. This latter suggestion is supported by the finding that all the O6 strains fell into groups II and III. Even supporters of the prevalence theory suggest that *E. coli* O6 may be especially pathogenic for the urinary tract (12, 29).

Furthermore, it is striking that all the O6:K23 strains fall into group III, whereas all the O6:K2 strains belong to group II. K23 has been found

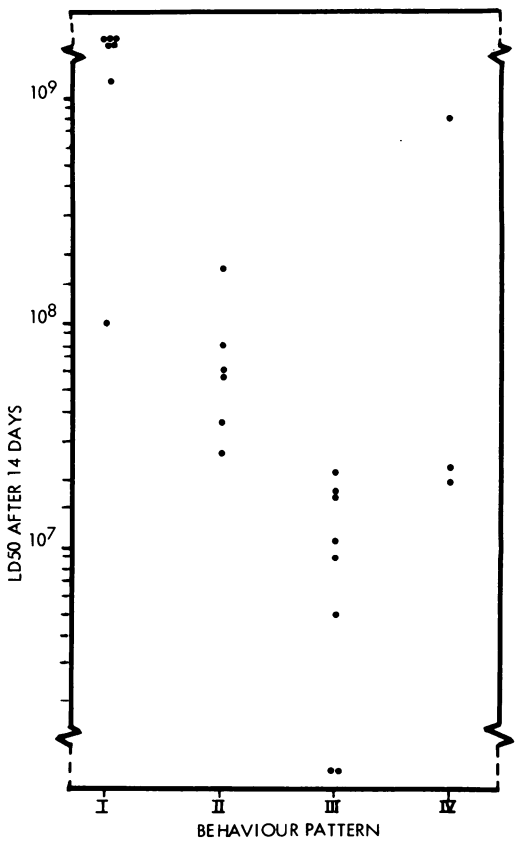

FIG. 9. *LD_{50}s of strains, arranged according to the behavior groups to which they belong.*

TABLE 1. *Serotypes of the strains studied, arranged according to the behavior groups to which they belong*

Behavior pattern	Serotypes
I	O1:K51:H7
	O117:K−:H−
	O75:K95:H5
	O75:K100:H5 (2)[a]
	O75:K100:H−
	Rough:K−:H27
	Rough:K+:H−
II	O4:K3:H?
	O6:K2:H1 (4)
	O6:K−:H?
	O75:K−:H5
III	O6:K23:H1 (3)
	O6:K23:H?
	O6:K+:H−
	O18ac:K+:H−
	O18ac:K−:H−
	O33:K+:H8
IV	O?:K3:H5
	Rough:K−:H2
	Rough:K+:H7

[a] Number in parentheses is number of isolates with the same serotype.

to cross-react with K13, indicating a close relationship between these antigens (28). O6:K13 has often been found in urinary tract infections (2, 30), especially in cystitis (18). On the other hand, O6:K2 strains are frequently found in cases of acute pyelonephritis (17, 18, 22). It should be interesting to see if additional strains of the same serotypes will behave similarly. Work along this line is in progress.

As far as the H antigens are concerned, we feel that the relatively high incidence of H1 strains in groups II and III and of H5 strains in group I is due more to the greater frequency of the O6-H1 and O75-H5 combinations in general than to the H antigen itself (1, 22, 27).

It should be emphasized here that correlations between behavior pattern in the mouse kidney and serotype do not necessarily mean that this pattern is determined by the antigenic structure itself. The pattern may be a character that can be traced by serotyping. It is not certain whether the strains used in this study came from cystitis or from pyelonephritis, because it is difficult to establish this on the basis of clinical data alone. In further studies it will be necessary to determine which strains have invaded the kidney and which have remained confined to the bladder.

Many authors suggest that not merely the presence of K-antigen but also the amount is important in nephropathogenicity (9, 17-19, 24, 26). The finding of K− strains in our virulent groups does not support this suggestion. Whether the amount of K antigen in K+ strains influences their virulence in our experimental model is under study.

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