Role in Virulence of *Shigella flexneri* Antigens Derived from Lysogenic Conversion

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The virulence of *Shigella flexneri* var. Y (NTCC 4839), its lysogenic convertants characterized by antigens I, V, and 7, 8, and of a phage-free clone selected by means of antiphage serum was studied. The parent strain was avirulent in the guinea pig eye test and in the "mouse shigellosis" model, but chicken embryo tests indicated the presence of penetrating ability together with defective tissue-growing capacity. The lysogenic convertants failed to regain their virulence to the guinea pig eye and to the mouse, but showed an increased tissue-growing capacity for chicken embryos. The level of virulence of the phage-free derivative equaled that of the parent strain. We concluded that the terminal glucose component of O antigen, even of conversion origin, plays a role as one of the virulence factors in tissue-growing capacity.

Biochemical and genetic investigations of O-specific side chains and R core (7, 11) resulted in better advanced knowledge of cell wall antigens and their role in virulence. Medearis et al. (10) showed that virulence of *Escherichia coli* was associated with the number of sugar components in the antigen. The review of Roantree (14) contains numerous remarkable data concerning the virulence of *Salmonella*. Recently Valtonen (19) and Mäkelä et al. (9) made an attempt to determine the role in virulence of different O-specific side chains in *Salmonella typhimurium*. Gemski et al. (2) prepared conjugation hybrids between *Shigella flexneri* 2a and *E. coli* O8 and O25. Those hybrids carrying the O8 antigen became avirulent, whereas some part of the hybrids with the O25 antigen retained their virulence in the Serény test.

In the case of *S. flexneri*, the origin of most of the type antigens and group antigen 7, 8 are attributed to lysogenic conversion (2, 3, 5, 8, 12). Simmons (17, 18) and Seltmann (15) have shown that the chemical representatives of these factors are α-glucosyl groups. Mäkelä et al. (9), in a test of limited sensitivity, were unable to show that the antigens of lysogenic origin had any influence on virulence in the *Salmonella* group. In an early paper, Cooper et al. (1) demonstrated that 2a and 2b types of *S. flexneri* without their type antigens were relatively avirulent as compared with noncloned parent strains. In the case of *S. flexneri*, different methods for testing of virulence are available, and factors responsible for penetration and for tissue-growing capacity can be identified separately. The present study was stimulated by these technical and theoretical considerations.

**MATERIALS AND METHODS**

As parent strain, *S. flexneri* var. Y (NTCC 4839) was used. Its convertants were obtained by means of phages φ1 (12), PE5 (6), and f7, 8 (3). With phages PE5 and f7, 8, a double lysogenic convertant was also produced. The method of obtaining lysogenic convertants was published previously (6). During the course of selection with PE5 antiphage serum, a phage-free clone devoid of the converted antigen V was isolated from the convertent strain PE5. Strains UP511, UP515, and UP517 were type strains of this laboratory.

For slide agglutination, absorbed sera were used at a dilution of 1: 10. For tube agglutination, unabsorbed sera were used.

For testing virulence, the guinea pig eye test of Serény (16), the mouse shigellosis model (13) using BALB/c mice, and the chicken embryo test, performed on 10-day-old chicken embryos (13), were used.

**RESULTS AND DISCUSSION**

In the guinea pig eye test, the parent strain NTCC 4839, its lysogenic convertants, and the phage-free clone all gave uniformly negative results. The parent strain and the phage-free clone were not recoverable from the eyes after 24 h. However, most of the convertants could be reisolated within 48 h; convertant PE5 survived for 4 days in the conjunctival sac. Although prolonged survival may indicate an increase in virulence, because of the lack of pathomorphological changes, this result cannot be considered as positive.

Results obtained with the mouse shigellosis...
model also were negative: the mean infective dose values were above 10⁴ for all clones tested. The development of prolonged excretion of shigellae, similar to the Serény test, needs a complexity of virulence factors. Therefore, the role of antigenic conversion in virulence may be demonstrated only in the more sensitive, less complex virulence factor-requiring chicken embryo tests.

Table 1 shows the results of two kinds of chicken embryo tests. With allantoic injection, the mean lethal dose value of the parent strain was 10³. This level indicates retained penetrating capacity (13). When injected intravenously, the mean lethal dose value was found to be 10². This value reflects a defective tissue-growing capacity (13). On the other hand, convertants showed an increased virulence when infected by either the allantoic or the intravenous route. The virulence of convertant PE5 was especially enhanced as revealed by the intravenous test. The result was significant for both the parent strain and the culture devoid of the converted antigen (P < 0.05 and P < 0.01, respectively).

Finally, the selected phage-free clone exerted a virulence similar in level to that of the parent strain.

It is not known whether the increased virulence, shown in the allantoic test, had any special significance. It may be attributed to the increased tissue-growing capacity and not to an increased penetration ability. It should be noted that probably other factors in addition to the lack of antigens of conversion origin are responsible for the relative avirulence of the parent strain. Therefore, restoration to full virulence is not expected.

On the other hand, the lack of homogeneity of virulence of the convertants is noticeable; e.g., convertant PE5 exhibited a higher virulence than other convertants, including the double lysogenic convertant obtained with phages f7, f8.

### Table 1. Examination of Shigella flexneri var. Y NTCC 4839 and its derivatives in intravenous and intra-allantoic chicken embryo tests

<table>
<thead>
<tr>
<th>Culture</th>
<th>Mean lethal dose (intravenous test)</th>
<th>Mean lethal dose (intra-allantoic test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4839</td>
<td>95</td>
<td>1,800</td>
</tr>
<tr>
<td>4839 (dI)</td>
<td>50</td>
<td>250</td>
</tr>
<tr>
<td>4839 (f7, 8)</td>
<td>12</td>
<td>90</td>
</tr>
<tr>
<td>4839 (PE5)</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>4839 (f7, 8 + PE5)</td>
<td>65</td>
<td>195</td>
</tr>
<tr>
<td>4838 (-)</td>
<td>120</td>
<td>1,200</td>
</tr>
</tbody>
</table>

*Derivative lost its antigen V of conversion origin (PE5) on selection with antiphage serum.

### Table 2. Comparison in tube agglutination of some convertants of Shigella flexneri var. Y NTCC 4839 with standard type strains

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Strain</th>
<th>Reciprocal agglutination titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>UP511 (standard)</td>
<td>2,560</td>
</tr>
<tr>
<td></td>
<td>4839 (dI)</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>UP517 (standard)</td>
<td>1,280</td>
</tr>
<tr>
<td></td>
<td>4839 (f7, 8)</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>4839 (f7, 8 + PE5)</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>UP515 (standard)</td>
<td>1,280</td>
</tr>
<tr>
<td></td>
<td>4839 (PE5)</td>
<td>1,280</td>
</tr>
<tr>
<td></td>
<td>4839 (f7, 8 + PE5)</td>
<td>80</td>
</tr>
</tbody>
</table>

*Identical specificity of antigens of type strains and convertants proved by antigen absorption.

and PE5. This finding may be explained by both qualitative (configurational) and quantitative (α-glycosyl saturation) differences. Simple tube agglutination tests seemed to confirm the latter assumption (Table 2). As compared with antigenic type strains, the convertants showed a reduced agglutination titer. The only exception was convertant PE5. It should be noted that the identical antigen specificity of the converted antigens was proved earlier (6) by antigen absorption tests. Because of the differences in saturation revealed by tube agglutination, one wonders what comparative examinations could be done to judge whether qualitative, configurational differences in α-glucosyl groups exert an influence on virulence. This problem, particularly with regard to the nonrandom distribution of serotypes, should be considered.

### Literature Cited


