INFECTION AND IMMUNITY, Aug. 1972, p. 104-111

Vol. 6, No. 2
Printed in U.S.A.

Virulence of *Shigella flexneri* Hybrids Expressing *Escherichia coli* Somatic Antigens

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Received for publication 19 April 1972

The genes controlling either *Escherichia coli* somatic antigen 8 or 25 were conjugally transferred to virulent *Shigella flexneri* 2a recipients to determine whether the aquisition of these antigens would affect the virulence of the resulting hybrid. A high proportion of such hybrids were found to be rough and hence were avirulent. Some smooth *S. flexneri* hybrids which replaced their native group antigens with *E. coli* factor 25 were still virulent in the animal models employed. All *S. flexneri* O-8 hybrids were uniformly avirulent. Our finding, that *S. flexneri* hybrids with the chemically divergent *E. coli* O-8 repeat unit are avirulent whereas some hybrids with the chemically related O-25 repeat unit retain virulence, suggests that the chemical composition and structure of the O side chain of somatic antigens may represent one determining factor for bacterial penetration of mucosal epithelial cells, the primary step in the pathogenesis of bacillary dysentery.

As with salmonellae (15), the synthesis and expression of O antigens by *Shigella flexneri* is generally considered to be of importance for the maintenance of a virulent state. The precise role, however, that smooth somatic antigens play in the pathogenesis of bacillary dysentery remains unelucidated at the present time. The structure and composition of smooth lipopolysaccharide components may be of significance in the primary step of the *S. flexneri* infective process, namely the penetration of bacteria into mucosal epithelial cells. Likewise, O antigens may serve as protection against host cellular destructive mechanisms after bacterial penetration is achieved. Such protection would allow for further multiplication by virulent bacilli which is a necessary factor for establishment of clinical disease.

Previous studies from this laboratory have been concerned with the genetic control of *S. flexneri* 2a virulence (2, 4, 5, 7). Based on analyses of hybrids derived from matings between *Escherichia coli* K-12 Hfr donors and virulent *S. flexneri* 2a recipients, at least two chromosomal regions have been identified as affecting virulence. A locus termed *kcpA*, which affects the ability of *S. flexneri* to provoke keratoconjunctivitis in guinea pigs and to penetrate mucosal epithelial cells, has been positioned close to the *purE* locus in the *lac-gal S. flexneri* chromosomal segment (4). Similar genetic hybridizations have also revealed that the *xyl-rha S. flexneri* 2a chromosomal region is involved in the conservation of virulence. Such hybrids retain the ability to penetrate the intestinal mucosa but, after doing so, have a decreased capacity to multiply (5).

Previous studies have also established loci on the chromosome of *S. flexneri* 2a which determine O antigen specificities. *S. flexneri* 2a group antigen genes have been mapped near the *his* operon, whereas the type-specific II antigen locus is situated near the *pro* chromosomal marker (3, 14). The finding of a *his*-linked O antigen locus in *S. flexneri* parallels the results reported for smooth somatic antigen loci of *Salmonella* species and *E. coli* (11, 13, 22).

This similarity of chromosomal position for antigen genes of *E. coli* and *S. flexneri* has enabled us to construct, by intergeneric hybridization techniques, *S. flexneri* derivatives which express *E. coli* antigenic characteristics rather than their native serotype. Our purpose for preparing such hybrids was to determine whether they would retain their ability to cause infection despite being altered in their antigenic structure. The present report summarizes findings from such studies on *S. flexneri* hybrids which have inherited either the O-25 or O-8 somatic antigen of *E. coli*.

MATERIALS AND METHODS

Bacterial strains. The characteristics of strains used are summarized in Table 1. Two *E. coli* Hfr donors

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TABLE 1. *Bacterial strains*[a]

Strain	Species	Characteristics										Antisera agglutination				Mating polarity
												Shigella		*E. coli*		
		lac	*ara*	*rha*	*xyl*	*mal*	*str*	*fuc*	*his*	*nic*	*asp*	group B	Type II	O-8	O-25	
17-14-2	*Shigella flexneri* 2a	−	−	−	−	−	R	−	−	−	−	+	+	−	−	F^-
17-14-18	*S. flexneri* 2a	−	−	−	−	−	R	−	−	−	−	+	+	−	−	F^-
W3703	*Escherichia coli*	+	+	+	+	+	S	+	+	+	+	−	−	NT[b]	+	Hfr
Hfr 59	*E. coli*	+	+	+	+	+	S	+	+	+	+	−	−	+	NT	Hfr
W1895	*E. coli*	+	+	+	+	+	S	+	+	+	+	−	−	−	−	Hfr

[a] Abbreviations: *lac*, lactose; *ara*, arabinose; *rha*, rhamnose; *mal*, maltose; *fuc*, fucose; *xyl*, xylose; *nic*, nicotinic acid; *asp*, aspartic acid; *his*, histidine; *str*, streptomycin; S, sensitive; R, resistant.
[b] Not tested.

(Fig. 1) were employed to transfer antigen determinants to *S. flexneri* recipients. *E. coli* O-25 strain W3702, originally described by Orskov and Orskov (13), expresses somatic antigen 25 and has lost its L type K antigenic factor. Genetic crosses have revealed a high frequency of *his* transfer and a linkage of the O-25 genes to the *his* operon (13). Also used as a donor was *E. coli* Hfr 59, 08:K27−, originally described by Schmidt (16), which expresses O antigen 8 and has lost its K antigen 27. Its polarity of chromosome transfer allows for the efficient introduction of the *his*-linked O-8 gene. In addition to these donors, *E. coli* K-12 Hfr W1895 was used in some crosses to exclude the presence of the *pro*-linked type-specific II antigen from hybrids. As recipients, we employed *S. flexneri* 2a *his*− strains 17-14-2 and 17-14-18. Although these strains are virulent, comparative studies with their immediate parent *S. flexneri* 2a M42-43 have shown their disease-provoking capacity to be slightly less severe; the reason for this is not known.

Bacteriophages. "Rough-specific" phages Ffm, BR60, 6SR, and BR2 were grown on a suitable "rough" strain of *S. flexneri*. Their "rough-specific" host range and the methods used for determining phage sensitivity spectra of bacterial strains have been described previously (6; and R. G. Wilkinson, P. Gemski, Jr., and B.A.D. Stocker, J. Gen. Microbiol., *in press*). A sensitivity to any of these phages was taken to represent a "rough" phenotype.

Media. Brain Heart Infusion (Difco), Penassay broth (Difco), and meat extract agar were used for routine cultivation of organisms. The composition of minimal medium used for selection and scoring recombinants has been described (1). When required, amino acids and other growth factors were added to the minimal medium at a final concentration of 25 μg/ml. Streptomycin, employed as a counter-selective agent against donor cells in some of the matings, was incorporated into selective media at a concentration of 500 μg/ml. Fermentation characters were scored either on MacConkey indicator medium which consisted of MacConkey agar base (Difco) supplemented with an appropriate carbohydrate at a concentration of 1%, or by culturing in phenol red broth (Difco) containing appropriate carbohydrate (1%).

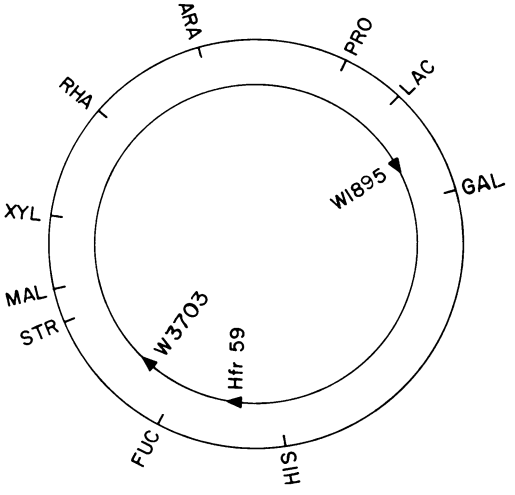

FIG. 1. *Chromosomal map of Escherichia coli.*

Mating procedures. Donor and recipient strains were grown overnight at 37 C in either Penassay broth or Brain Heart Infusion broth without agitation. On the next day, 10-ml portions of each culture were washed three times with 0.85% saline and suspended in 1 ml of saline (about 5×10^9 cells per ml). At this time, 0.1 ml of the donor cell suspension and 0.1 ml of the recipient cell suspension were plated separately (for controls) or together (for mating) on an appropriate selective medium. After incubation for 2 days at 37 C, isolated recombinant clones were picked and purified twice by streaking on the original selective medium.

Antigenic analysis of hybrids. The presence of *S. flexneri* somatic antigens and *E. coli* somatic antigens 25 and 8 was scored by slide agglutination tests performed on purified recombinants. The *S. flexneri* 2a type-specific II antigen was detected by the use of absorbed type-specific II antiserum (Difco). An organism was considered to have only *Shigella* group factors if it agglutinated in unadsorbed *S. flexneri* 2a antiserum or in *S. flexneri* Y antiserum (3, 4 group antigens) and failed to agglutinate in type-specific II serum. *E. coli* somatic antigens 25 and 8 were scored by agglutination in their respective typing sera (Difco). Failure of O-8 and O-25 hybrids to agglutinate in unadsorbed *S. flexneri* 2a serum was considered to be evidence of a complete loss of *S. flexneri* serological specificity. With certain selected hybrids, this conclusion was confirmed by the preparation of antisera in several rabbits and subsequent performance of agglutinin-absorption tests.

Guinea pig keratoconjunctivitis test. Details of the guinea pig keratoconjunctivitis test have been described (4, 9, 20). Strains considered to be virulent enter the corneal epithelial cells and cause keratoconjunctivitis. A drop of cell suspension was deposited into the conjunctival sac of a guinea pig, and the animal was observed daily for 72 hr. A reaction was considered to be positive if the cornea became opaque and conjunctivitis developed.

Oral infection of guinea pigs. Guinea pigs of the Hartley or Walter Reed strain weighing 300 to 400 g were used. They were deprived of food for 4 days and then fed by stomach tube 5×10^7 to 1×10^8 challenge organisms suspended in 10 ml of broth. Immediately after challenge, 1 ml of tincture of opium was injected intraperitoneally. The animals were sacrificed 24 hr after challenge. Virulent strains produce ulcerative lesions of the intestine and subsequent death in animals. Animals fed avirulent cells survive and do not exhibit any bowel lesions (7).

Fluorescent antibody and histological studies. Fluorescent-antibody examination of frozen tissue sections was carried out as previously described (7). Tissues for histological study were fixed in neutral buffered Formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin.

RESULTS

Isolation of S. flexneri hybrids which express E. coli somatic antigens. *E. coli* Hfr strains W3703 (antigen 25 donor) and Hfr 59 (antigen 8 donor) were mated with virulent *his*$^-$ *S. flexneri* 2a recipient strains 17-14-18 and 17-14-2, selections being made on minimal medium for hybrids which inherited *E. coli* genes controlling histidine biosynthesis. After 2 days of incubation at 37 C, His$^+$ hybrids were purified by streaking on the same selective medium and were scored for acquisition of various nonselected markers. The results of such analyses (Table 2) indicate a high genetic linkage of the determinants for *E. coli* somatic antigens 25 and 8 with the *his* chromosomal marker. Approximately 75% of the His$^+$ hybrids derived from the W3703 donor agglutinated in factor 25 serum and about 35% of the His$^+$ hybrids obtained from matings with Hfr 59 expressed antigen 8. As noted in Table 2, these hybrids did not inherit other nonselected markers from the donor strains with any significant frequency. Further analysis of the serological properties of His$^+$ hybrids is presented in Table 3. Their slide agglutination properties were tested in *E. coli* monospecific factor 25 or 8 serum, unadsorbed *S. flexneri* 2a serum, *S. flexneri* monospecific type II serum and *S. flexneri* Y serum containing agglutinins for group factors, 3,4. With hybrids derived from matings with the O-25 *E. coli* W3703 donor, three agglutinin classes could be scored. Class C, in which cells agglutinated only in *S. flexneri* antisera (type II and Y) represents His$^+$ hybrids which did not inherit *E. coli* determinants, this serological pattern being typical of the parental recipient strains. Among those hybrids which inherited antigen 25, two serological patterns were distinguished. Class A hybrids agglutinated strongly in factor 25 serum and in the *S. flexneri* type 2 serum but were unreactive in the group factor *S. flexneri* Y serum. It appears therefore that these hybrids had replaced their native group antigens with the *E. coli* O-25 antigen in the process of recombinant formation. The type-specific II antigen, whose gene(s) maps near the *pro* locus (distal to the *his* region), remains unaltered and is still expressed in such hybrids. With class B hybrids, agglutination was detected in all three sera, although it was noticeably weak

TABLE 2. *Inheritance of Escherichia coli antigens 25 and 8 by Shigella flexneri 2a*[a]

Donor strain	Recipient strain	Donor selected character	No. analyzed	No. inheriting *E. coli* antigen
E. coli W3703	*S. flexneri* 2a 17-14-18	His$^+$	47	36 (O-25$^+$)
	S. flexneri 2a 17-14-2	His$^+$	176	131 (O-25$^+$)
E. coli Hfr 59	*S. flexneri* 2a 17-14-2	His$^+$	247	88 (O-8$^+$)

[a] With the exception of crosses with donor W3703, in which about 7% of the *his*$^+$ hybrids also inherited *fuc*$^+$ (apparent early marker of W3703), none of these hybrids inherited other nonselected markers such as *lac*, *ara*, *rha*, *xyl*, or *mal* from the donors.

TABLE 3. *Serological properties of His+ Shigella flexneri hybrids obtained from matings with Escherichia coli Hfr strains W3703 and Hfr 59*

Donor strain	No. of His+ hybrids tested	Agglutinin class	*E. coli* O-25	*E. coli* O-8	*S. flexneri* type 2	*S. flexneri* Y (3, 4)	No. in each class
W3703	223	A	+	NT[a]	+	−	144
		B	+	NT	+	+	23
		C	−	NT	+	+	56
Hfr 59	247	A	NT	+	−	−	88
		B	NT	−	+	+	159

[a] Not tested.

in the *S. flexneri* group factor serum. These results suggest that class B hybrids may be diploid for the *his* chromosomal region, conserving their native *S. flexneri* group factors in addition to inheriting antigen 25 from *E. coli*. With His+ hybrids derived from the antigen 8 donor, *E. coli* Hfr 59, only two distinct agglutinin classes were evident (Table 3, bottom). Class A hybrids agglutinated only in factor 8 serum, being unreactive in any of the *S. flexneri* antisera. Their lack of reaction in type II serum, in contrast to the finding with antigen 25 recombinants, will be subsequently discussed. Class B hybrids behaved serologically as typical *S. flexneri* 2a.

Virulence of S. flexneri hybrids with E. coli antigenic characteristics. The Sereney test for keratoconjunctivitis (9, 20) was employed in initial screenings to assess the virulence of *S. flexneri* hybrids expressing *E. coli* antigens. This test determines the ability of organisms to penetrate epithelial cells of the cornea, causing keratoconjunctivitis. A provocation of keratoconjunctivitis by *S. flexneri* in turn reflects the ability of the organism to invade bowel epithelium, a necessary attribute for *S. flexneri* virulence (7). Both *E. coli* Hfr 59 and Hfr W3703 failed to evoke keratoconjunctivitis and hence were considered avirulent. When the 88 His+, antigen 8-positive hybrids derived from a mating with Hfr 59 (Table 3, class A, bottom) were so screened, none caused keratoconjunctivitis, thus indicating that they were avirulent. Subsequent testing of these hybrids for sensitivity to "rough-specific" bacteriophages revealed that 77 of the 88 were lysed by at least one of them. Thus, the avirulence of a high proportion of the O-8 hybrids could be due to their rough state. Nevertheless, the remaining 11 hybrids, which exhibited no sensitivity to the "rough-specific" phages and agglutinated strongly in O-8 antisera, still were avirulent.

Sereney tests on *S. flexneri* hybrids which inherited *E. coli* antigen 25 revealed that some of these indeed retained the ability to penetrate epithelial cells. Screening tests were limited to class A hybrids (Table 3, W3703 donor) since this type of hybrid had apparently replaced *S. flexneri* group factors with the O-25 donor antigen. Six of 44 such hybrids provoked a positive Sereney test and, when tested with the rough-specific phages, scored as smooth. As in the case of the O-8 *S. flexneri* strains, many, but not all, of the avirulent O-25 hybrids appeared to be rough, showing sensitivity to at least one of the rough-specific phages employed; about 10% of the smooth hybrids were avirulent.

Upon reisolation of organisms from diseased eyes, the virulent cells were serologically indistinguishable from those employed in the initial challenge, being agglutinated only by *E. coli* O-25 and *S. flexneri* type II antisera. Although previous studies had revealed that the type II antigen was not essential for maintenance of virulence by *S. flexneri* 2a (4), we decided, nevertheless, to exclude this antigen from such O-25 hybrids. The type II antigen gene(s), which maps in the *lac-pro* region of the *S. flexneri* chromosome (3), can be readily removed by genetic hybridization of this region with the *E. coli lac-pro* segment. Thus, *E. coli* Hfr W1895 (Fig. 1) was mated with a virulent O-25, type II+ hybrid recipient, selections being made for hybrids which inherited the donor *lac*+ chromosomal segment. All 48 Lac+ hybrids so constructed were found to have lost their type II antigen, being agglutinated solely by *E. coli* O-25 antiserum and, as expected, remained virulent.

Serological characterization of S. flexneri O-25 hybrid strain 542-1-7. A detailed serological study was performed on O-25 hybrid strain 542-1-7, since this strain was chosen for further study of virulence properties. Antisera were prepared by immunizing seven rabbits with it, to insure that this strain lacked *S. flexneri* 2a agglutinogens. These sera had homologous agglutinin titers of at least 1:10,000. The *E. coli* parent O-25 strain also agglutinated to the homologous titer. On the other hand, the recipi-

ent parent strain, *S. flexneri* 2a 17-14-18, failed to react in these sera at a dilution of 1:40. Further experiments utilizing reciprocal agglutinin-adsorption techniques indicated that the hybrid strain was antigenically similar to the donor O-25 strain and bore no serological relationship with the recipient *S. flexneri* 2a strain. We therefore concluded that *S. flexneri* hybrid 542-1-7 expresses only the somatic antigens of *E. coli* O-25 strain W3703. Sereney tests with this strain revealed that it efficiently provoked keratoconjunctivitis and thus conserved this aspect of virulence.

Virulence of O-25 hybrid strain 542-1-7. Both the parent *S. flexneri* 2a strain 17-14-18 and the O-25 hybrid 542-1-7 were fed to groups of starved, opiated guinea pigs (2, 7) at a dose of about 10^8 cells. Animals were sacrificed 24 hr later, and their intestines were compared histologically to assess the extent of bowel damage.

Sections of the small intestine from guinea pigs fed the parent His$^-$ *S. flexneri* 2a strain 17-14-18 showed marked alterations of the mucosal architecture. These included villous cubbing with elongation and dilation of the glandular crypts, decreased numbers of goblet cells, increased focal sloughing of the epithelium, congestion and increased cellularity of the lamina propria. The large intestine was characterized by diffusely scattered areas of altered mucosal architecture (Fig. 2). There was an increase in the mucosal thickness. The epithelium lining the crypts showed nuclear hyperchromatism, increased numbers of mitoses, and decreased numbers of goblet cells. The surface epithelium was focally ulcerated with an associated acute inflammatory exudate. Occasional epithelial crypt abscesses and microabscesses in the mucosal lymphoid aggregates were observed. The lamina propria was congested, and diffusely scattered collections of polymorphonuclear neutrophils were present. Sections from both the small and large intestines of guinea pigs fed the hybrid *S. flexneri* O-25 strain 542-1-7 showed similar and essentially indistinguishable morphological alterations (Fig. 3). Control animals, fed either an avirulent strain or broth alone, showed normal small and large intestinal mucosal architecture without evidence of acute inflammatory reaction.

Additional examination of diseased tissue with the fluorescent-antibody technique, using labeled antisera gainst *E. coli* O-25 or *S. flexneri* 2a, revealed that the degree of invasion of mucosal epithelial cells by the O-25 hybrid approximated that of the virulent *S. flexneri* parent 17-14-18

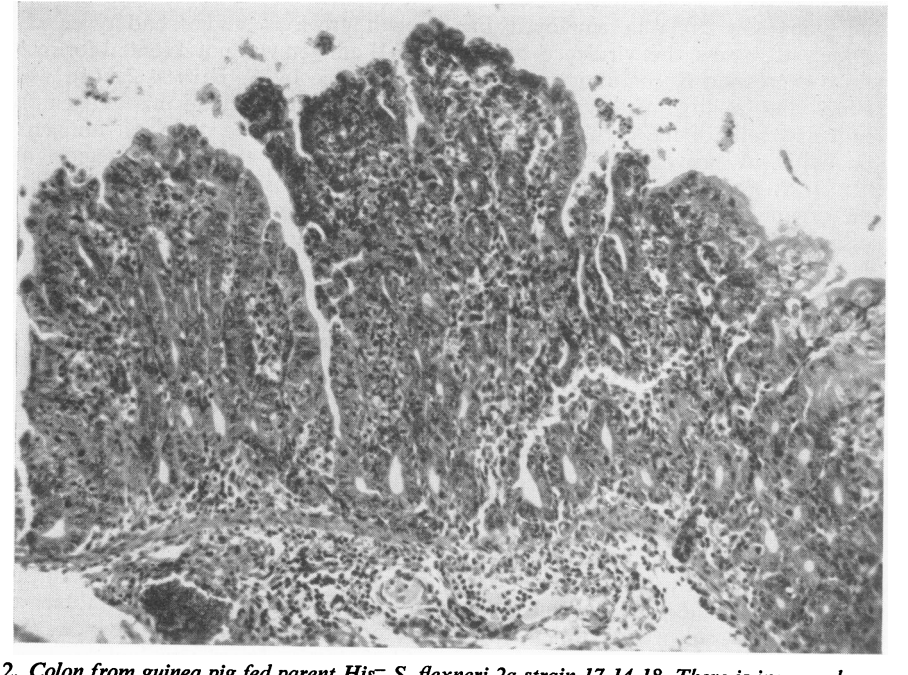

FIG. 2. *Colon from guinea pig fed parent His$^-$ S. flexneri 2a strain 17-14-18. There is increased mucosal thickness with architectural disarray. The lamina propria cellularity is increased due to polymorphonuclear neutrophil infiltration. Note the surface epithelial irregularity with focal areas of cellular slough. Hematoxylin and eosin stain. × 135.*

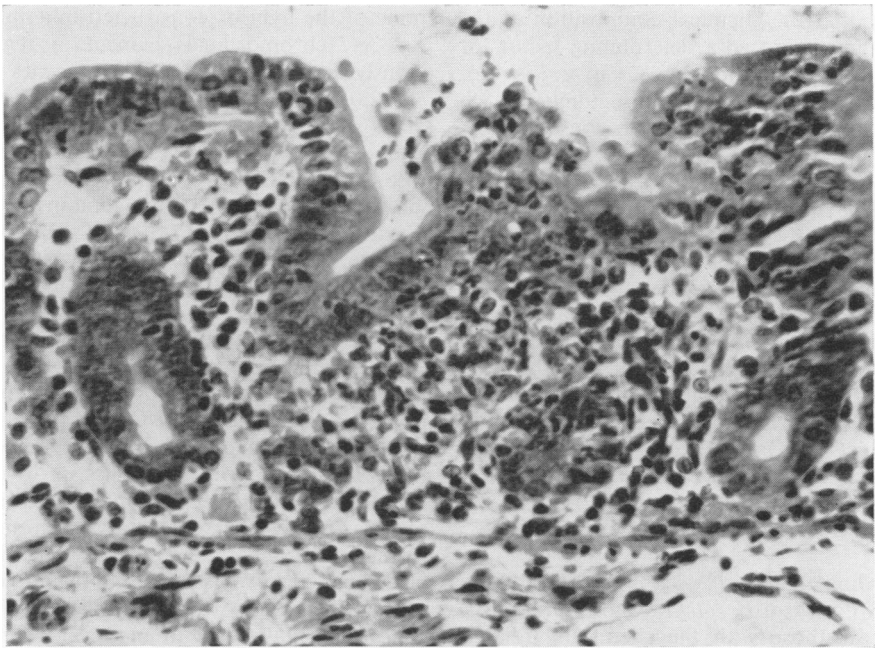

FIG. 3. *Colon from guinea pig fed hybrid S. flexneri strain 542-1-7 showing a focal aggregate of polymorphonuclear neutrophils in the lamina propria with ulceration of surface epithelium and luminal acute inflammatory exudate. Hematoxylin and eosin stain.* × *380.*

DISCUSSION

By employing techniques of intergeneric hybridization, in which *S. flexneri* 2a virulent recipients were mated with two smooth *E. coli* Hfr donors, we have prepared *S. flexneri* hybrids which have replaced their native O antigens with those expressed by the respective donors. Many of these hybrids were sensitive to rough phages and were avirulent. Among the O-8 and O-25 hybrids inferred to be smooth, only a proportion of those that had replaced their native antigens with *E. coli* antigen 25 retained virulence. None of these *S. flexneri* O-8 hybrids caused keratoconjunctivitis, and thus they had lost the attribute of epithelial cell penetration essential for the maintenance of virulence. Among the considerations for explaining the avirulence of these O-8 hybrids is the possibility that they represent some sort of rough type not detected by our scoring procedures, for example a semirough C type (10, 12). Attempts to establish this on the basis of phage sensitivity patterns have not been successful. If this is not the case, their avirulence could be accounted for in perhaps two other ways. They may have inherited an unidentified locus similar to *kcpA* (4) which controls the ability of organisms to penetrate epithelial cells.

Alternatively, the uniform avirulence of all the O-8 *S. flexneri* hybrids may indicate that the chemical composition and structure of the O repeat unit is indeed one determining factor for epithelial cell penetration by *S. flexneri*. The elegant studies of Simmons (21) have revealed that the group antigenic determinants of *S. flexneri* 2a consist of a *N*-acetylglucosamine-rhamnose-rhamnose repeat unit and that the attachment of α-glucosyl secondary side chains to a rhamnose of this repeat unit confers type II specificity. Similar studies on the chemical composition of *E. coli* O-8 strains have revealed that the immunodominant sugar of the O repeat unit is a D-mannose (17–19). This distinct difference in O repeat unit composition of the strains employed is further illustrated by our finding that those *S. flexneri* which replace their group antigens with the O-8 repeat unit do not express their type-specific II antigen. Since the genes controlling type II specificity are distal to the *his* chromosomal segment and were not altered in our hybridization procedure, it appears that the α-glucosyl side chains (type factor II) cannot be attached to the mannose residues of the O-8 repeat unit and hence can not be expressed by these hybrids. A similar situation was previously noted in studies dealing with the transfer of *S. flexneri* 2a antigens to *E. coli* K-12 recipients (3). Hybrids which contained the genes for type factor II did not express this antigen unless the genes for *S. flexneri* group factor were present (3). The

possibility that the chemical composition of the O repeat unit is indeed a determining factor for epithelial cell penetration by *S. flexneri* is supported, to some degree, by our finding that *S. flexneri* expressing antigen 25 can conserve their penetrating ability and virulence. Although the chemical components of the O-25 lipopolysaccharide (LPS) layer have not been fully described, it has been established that rhamnose is present in its O repeat unit (8). In addition, our observation that *S. flexneri* O-25 hybrids continue to express type-specific antigen II indicates that the α-glucosyl side chains conferring this serological specificity can be functionally linked to the O-25 repeat unit, presumably to rhamnose as in *S. flexneri* 2a. Thus, although being serologically quite distinct, the O repeat units of the *S. flexneri* group factor and the *E. coli* O-25 antigen bear some similarity. This similarity may be reflected in the conservation of virulence by such O-25 hybrids.

The finding that *S. flexneri* hybrids with the chemically divergent *E. coli* O-8 factor are avirulent whereas those with the chemically related *E. coli* O-25 factor can conserve virulence parallels observations on the effect of different O side chains on the mouse virulence of *Salmonella typhimurium* (23). It has been shown that *S. typhimurium* hybrids, expressing group D antigens 9,12 rather than the native group B factors 4,5,12, were slightly less virulent, having an LD_{50} of about 10^6 rather than 10^5. In contrast, when *S. typhimurium* recombinants with group C factors 6,7 were similarly tested, an LD_{50} of over 10^7 was established (23). Thus, the expression of the 6,7 antigens, chemically quite distinct from the 9,12 or 4,12 repeat unit, seemed to have markedly affected the mouse virulence of these organisms.

Unlike these studies with *S. typhimurium* in which mortality of mice was employed to assess the level of pathogenicity of *S. typhimurium* hybrids, we employed a histological approach to study the degree of disease provoked by *S. flexneri* antigenic hybrids. Microscopic examination of bowel tissue from infected guinea pigs indicated that the mucosal changes caused by virulent *S. flexneri* O-25 hybrid 542-1-7 were not readily distinguishable from that evoked by the original parent. On the other hand, the avirulent strains produced no gross or microscopic changes in the bowel wall.

The rough phenotype which was observed in many of the *S. flexneri* O-8 and O-25 hybrids could be a consequence of inefficient attachment of the newly inherited *E. coli* O repeat units to the native *S. flexneri* LPS core. It is likely that the genes controlling the LPS core of hybrids are native *S. flexneri* genes, since, as noted in Table 2, none of the hybrids constructed inherited the *E. coli xyl* chromosomal segment, a region previously shown to include LPS core genes (*rfa* loci) (11, 22). Chemical studies on the core components of *S. flexneri* 2a and *E. coli* O-8, K-27$^-$ strains, although revealing that glucose, galactose, heptose, and KDO are common components, also indicate that *S. flexneri* core contains in addition *N*-acetylglucosamine (17–19, 21). This difference in core composition could result in inefficient attachment of the *E. coli* repeat unit to the *S. flexneri* core LPS.

The findings reported here and those from previous studies emphasize the importance of LPS layers for the conservation of virulence of enteric pathogens. Although some O repeat unit structure is essential for maintenance of optimal virulence by *flexneri*, the effect of changes in the chemical composition of such repeat units on pathogenesis of a classical bacillary dysentery syndrome awaits further study. In the present investigation, emphasis has been replaced on epithelial cell penetration, the primary step of this disease process. It appears that hybrids with an O repeat unit structure significantly distinct from that of *S. flexneri* are unable to penetrate epithelial cells. Although the biochemical and physical mechanisms involved in cell penetration by *S. flexneri* remain obscure, it is evident that mucosal epithelial cells can detect alterations in bacterial cell surface structures, whether they be a consequence of a smooth to rough mutation or of a distinct change in O repeat chemical composition.

ACKNOWLEDGMENTS

We are indebted to Randall T. Jones for kindly providing *E. coli* Hfr 59. We thank S. Austin and J. Schadt for excellent assistance.

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