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ANNOTATED BIBLIOGRAPHY ON PATHOGENESIS OF SHIGELLOSIS

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PREFACE

The *Specialized Bibliography Series* is part of the larger effort to facilitate exchange of information and to establish an information network in the field of diarrhoeal diseases -- an effort being carried out by the International Diarrhoeal Disease Information Service and Documentation Centre (DISC) of the ICDDR,B. The present issue, the seventh of the Series, includes 133 papers (66 abstracted) on pathogenesis of shigellosis. This is a subject of high current importance, and the reason for selecting the topic is explained in the introduction.

This is not an exhaustive bibliography on the topic. The bibliography was compiled from the available resources, and it is possible that inadvertent omissions may have occurred.

We hope the present bibliography will contribute towards generating greater interest and awareness in this field, and will facilitate user access to existing knowledge. Copies of articles abstracted and cited in this bibliography are available from DISC to interested persons/organizations. We will consider this attempt successful if the bibliography helps diarrhoeal disease researchers and practitioners. Suggestions for improvement of a future edition will be appreciated.

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CONTENTS

INTRODUCTION

Shigellosis is a complex infection involving both small and large bowel. The pathogenesis of shigellosis, following ingestion of bacteria, requires invasion and multiplication in the epithelial cells of the colonic mucosa. The resulting fever, diarrhoea and dysentery are caused by the host response to the various virulence determinants of *Shigellae*.

There are three broad aspects of investigation in the pathogenesis of *Shigella* infection; namely (1) characterization and biochemical study of toxin(s) and other virulence factors, (2) clinical investigation in patients with *Shigella* infection and (3) investigation on genetic determinants of virulence.

The most controversial aspect of the pathogenesis of shigellosis concerns the involvement and role of cell-free protein toxins. In cytotoxic activity, the first step is its binding to the cell surface receptor, which in HeLa cells has been partially characterized as a lysozyme sensitive glycoprotein. Then the toxin is internalized by an energy dependent process called receptor-mediated endocytosis. The toxin inhibits peptidyl elongation at the 60 S ribosome and an immediate cessation of protein synthesis in both prokaryotic and eukaryotic cells follows. The *in vivo* correlates of this capability is not known. The mechanism of the secretory enterotoxin effect is not clear. Although *Shigella* toxin resembles the action of cholera toxin or *Escherichia coli* heat-labile toxin in the rabbit jejunum, there is conflicting evidence regarding its ability to activate adenylate cyclase. Based on the *in vivo* effects of *Shigella* toxin on the central nervous system and in the ligated rabbit ileum, it can be concluded that similar cytotoxic action works on vascular endothelium in the central nervous system and on the ileal epithelial cells. Although *Shigella* toxin might cause diarrhoea, still epithelial cell penetration seems to be very important in the genesis of the disease. In tissue culture model it was found that cell penetration requires active metabolic participation from both the bacterium and the cell it enters.

Inhibition of attachment is presumably the major mechanism by which secretory immunoglobulin A (IgA) gives protection against shigellosis. Secretory IgA also prevents some of the manifestations of *Shigella* infection, by reacting with toxin and preventing their absorption through the intestinal mucosa. Once bacteria have penetrated the tissue, systemic host factors come into play. They encounter not only antibody and complement but also phagocytic cells. Several serum factors participating in opsonization have been identified. The alternate pathway of complement fixation is required for efficient heat-labile opsonization of *Shigellae*, but some opsonization also occurs through the classical pathway of activation.

Recently, interest has been focused on the role of plasmid in the biological properties of *Shigellae*. A series of studies has shown that all four *Shigella* species causing dysentery carry a large plasmid, of 180 to 210 kilobase pairs in length, that is functionally homologous with respect to the ability to penetrate epithelial cells of the colon, the first step in the pathogenesis of dysentery. Loss of this plasmid correlates with loss of invasive ability and reinsertion of these plasmids into these avirulent *Shigella* restores virulence. The mechanism of the plasmid-mediated invasive ability is not known but appears to involve the synthesis of several outer membrane proteins. All virulent *Shigella sonnei* strains carry the

genes for form I, O-antigen synthesis on the same 180 kilobase pair plasmid that encodes invasive ability. Elimination of this plasmid was accompanied with irreversible transition to the avirulent form II state. Recently, a small plasmid of *Shigella dysenteriae* 1 has also been found to be involved in the O-antigen expression.

The ultimate goal in research on *Shigella* infection is its prevention. Elucidation of the pathogenic mechanism of the disease is one of the most effective ways by which that goal can be achieved. The dramatic advances in the field of immunology and genetics have strongly stimulated and facilitated research directed at the identification and characterization of the virulence determinants of *Shigellae* that enable them to invade the host and cause disease. Two factors add to the difficulty of identifying the virulence determinants of *Shigella*. First, more than one virulence factors are involved as evidenced by the number and complexity of the steps in the disease production. And hence the assessment of the relative significance of any of these factors to the establishment of infection is problematic. Second, most of the work on *Shigellae* is carried out with microorganisms growing *in vitro*, whereas the target is to explain a phenomenon exhibited when the microorganisms grow *in vivo*. Under these different environmental conditions, selection of genetypes or phenotypic change can result in deficiency of the required determinants of pathogenicity.

The major objective in preparing this bibliography has been to compile in a single presentation information on the pathogenesis of shigellosis. It was impossible to cover the whole of it, but attempts were made to select papers which might highlight previous important works and current research trends. This bibliography should thus provide the readers with a comprehensive survey of the present situation concerning the pathogenesis of shigellosis. Considerable efforts are currently being made worldwide to bring shigellosis under control and important achievements can be expected in the next decade. It is hoped that the present bibliography will be stimulating and useful to all those participating in this process.

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The Author Section contains citations arranged alphabetically by the first author and then by the title of paper. Co-authors' names also appear in alphabetical order along with a cross-reference to the first author (e.g. Akhtar Q see Kabir S). This will facilitate a search by co-authors' names.

Efforts have been made to present abstracts with all available information regarding the study's nature and objective, methods used, and the major findings and conclusions.

The bibliography is in English. A title in parentheses indicates that the paper is in another language.

PATHOGENESIS OF SHIGELLOSIS

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Adamus G, Romanowska E. Outer membrane proteins of *Shigella sonnei*. II. Comparative studies on virulent and avirulent strains of phase I. Arch Immunol Ther Exp (Warsz) 1980;28(4):553-8

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This study examines whether outer membrane proteins (OMP) isolated from *Shigella* are protective. The keratoconjunctivitis shigellosa test was used for demonstrating acquired immunity. Active immunization of guinea pigs and rabbits with OMP isolated from *Shigella flexneri* 3a and *S. sonnei* phase I protected the animals against keratoconjunctivitis shigellosa induced with the homologous or heterologous strain. Protection was also achieved in rabbits after passive immunization with anti-OMP immune serum. Active immunization with lipopolysaccharide of *S. flexneri* 3a did not protect rabbits against keratoconjunctivitis shigellosa. It is suggested that a vaccine preparation containing OMP may also protect humans against natural *Shigella* infection.

Akhtar Q see Kabir S

Ali S see Kabir S

Allen RC see Madonna GS

Amer S see Chugh TD

Austin S see Formal SB

Austin S see Hale TL

Banwell JG see Steinberg S

Banwell JG see Steinberg SE

Baron LS see Formal SB

Baron LS see Keren DF

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Beckman B see Flores J

Bendersky J see Olitzki L

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Bhogale SR, Sharma KD, Kamat RS. Role of heat labile antigens of *Shigella flexneri* in HeLa cell invasion. J Med Microbiol 1983 Feb;16(1):37-43

The role of surface antigens of *Shigella flexneri* in HeLa cell invasion was examined, using blocking antisera. A quantitative assay was employed to determine the number of adhering and intracellular bacteria recovered from HeLa cell monolayers incubated with 5 x 10^7 *S. flexneri*. *Shigella* cultures were preincubated with rabbit antiserum against live (ALS) or boiled (ABS) autologous antigen, as well as with ALS absorbed with boiled antigen (ALAS). Reduction of adhesion and invasion, apparently not mediated by bacterial agglutination, was obtained by ALS 1:200 (62 and 58%, respectively), and by ABS 1:200 (46 and 31%, respectively). ALAS did not block either process at the 1:200 dilution, but at a 1:20 dilution it reduced both by 72-73%. *S. flexneri* LPS had no blocking effect. These results show the importance of heat-labile antigens in the adhesion and invasion of HeLa cells by *S. flexneri*. It is speculated that antiserum against heat-stable antigens may block adhesion by steric hindrance.

Binder HJ, Whiting DS. Inhibition of small-intestinal sugar and amino acid transport by the enterotoxin of *Shigella dysenteriae* I. Infect Immun 1977 May;16(2):510-2

This paper describes the effect of *Shigella dysenteriae* 1 enterotoxin on the nonelectrolyte transport in the rabbit ileal mucosa. Both 10 mM galactose and 5 mM L-alanine absorptions were significantly impaired in enterotoxin-exposed rabbit ileal mucosa compared with control mucosa. L-alanine influx was not impaired in both cholera enterotoxin induced as well as hyperosmolarity induced secretory processes. These findings provide evidence that exposure of rabbit ileal mucosa to *Shigella* enterotoxin results in diminished absorption of both sugar and amino acid.

Binder HJ see Donowitz M

Blackmon B see Maurelli AT

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Pathogenesis of Shigellosis

Branham SE, Carlin SA. Studies with *Shigella dysenteriae* (Shiga) 1. Infection and toxin action in mice. J Infect Dis 1948;83:60-5

Brawner TA see Osato MS

Bridgewater FAJ, Morgan RS, Rowson KEK, Wright GP. Neurotoxin of *Shigella shigae;* morphological and functional lesions produced in central nervous system of rabbits. Br J Exp Pathol 1955 Oct;36:447-53

Brown JE, Ussery MA, Leppla SH, Rothman SW. Inhibition of protein synthesis by Shiga toxin: activation of the toxin and inhibition of peptide elongation. FEBS Lett 1980 Aug;117(1):84-8

Brown JE, Rothman SW, Doctor BP. Inhibition of protein synthesis in intact HeLa cells by *Shigella dysenteriae* 1 toxin. Infect Immun 1980 Jul;29(1):98-107

The effects of purified Shiga toxin preparation on both macromolecular synthesis and membrane functions in HeLa cells were investigated. The Shiga toxin purified to near homogeneity from all lysates of *Shigella dysenteriae* 1 inhibited protein and deoxyribonucleic acid (DNA) synthesis in intact HeLa cells. Inhibition was dependent on toxin concentration and time of incubation. A minimal latent period of 30 min was observed with saturating doses of toxin. Ribonucleic acid synthesis, uptake of α-aminoisobutyric acid, and maintenance of intracellular K^+ concentrations were not affected until well after maximal inhibition of protein and DNA synthesis. These results indicate that Shiga toxin did not cause gross membrane damage or exhaust adenosine triphosphate supplies and that inhibition was not due to loss of precursor pools or interference in the uptake of precursors. The inhibitory effect of the toxin was heat sensitive and was prevented by antibody neutralization. Several cytotoxic components were separated by polyacrylamide gel electrophoresis of the purified toxin preparation; all inhibited protein and DNA synthesis equally.

Brown KJ, Tannock GW, Eyres RA, Elliot RB, Lines RB, Lines DR. Colonization by *Salmonella typhimurium* and *Shigella flexneri* III of the gastrointestinal tract of mice treated with beta-2-thienylalanine and streptomycin. Antonie Van Leeuwenhoek 1979;45(4):531-46

Calabi O. *In-vitro* interaction of *Shigella flexneri* with leukocytes and HeLa cells. J Infect Dis 1970 Jul-Aug;122(1 & 2):1-9

For further studies on the pathogenesis of shigellosis, experiments were carried out to obtain information on the interaction of phagocytic cells with *Shigella flexneri.* It was found that leukocytes from guinea pigs showed no significant phagocytic and bactericidal activity *in vitro* for virulent *S. flexneri* organisms, though nonpathogenic strains of *Escherichia coli* were rapidly phagocytized and killed. These findings and histopathologic observations in preconditioned guinea pigs and in the natural host, the monkey, suggest that leucocytes do not function effectively as an antimicrobial defense system in acute shigellosis. Observations on interactions of virulent and avirulent strains of *S. flexneri* with HeLa cell monolayers, used as a model for intestinal epithelial cells, suggest that cellular invasion can be used as a condition for virulence. Furthermore, it is suggested also that the HeLa cell model is not applicable to nonpathogenic strains of *E. coli,* since these organisms are rapidly phagocytized and killed.

Cantey JR. Shiga toxin - an expanding role in the pathogenesis of infectious diseases. J Infect Dis 1985 May;151(5):766-71

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Charney AN, Gots RE, Formal SB, Giannella RA. Activation of intestinal mucosal adenylate cyclase by *Shigella dysenteriae* 1 enterotoxin. Gastroenterology 1976 Jun;70(6):1085-90

Contrary to previous reports, *Shigella dysenteriae* 1 enterotoxin was found to activate mucosal adenylate cyclase, though the kinetic pattern was different from that of cholera enterotoxin. The effect of *Shigella* toxin on rabbit ileal mucosa was observed under various *in vitro* and *in vivo* conditions. It was found that a high substrate concentration of 1.5×10^{3}M, doses of *Shigella* toxin between 5.4 and 900 microgram of toxin protein and *in vivo* incubation times six and 18 h all increased adenylate cyclase activity by about 100%; though this rise in activity when seen with a comparable dose of cholera toxin was considerably less. Mucosal Na-K-ATPase activity was found to be unaffected by *Shigella* toxin. The authors suggest that the activation of adenylate cyclase system with resultant accumulation of cyclic 3'5'-adenosine monophosphate, may contribute to the alteration in fluid transport mediated by *Shigella* enterotoxin.

Collins HH see Keren DF

Collins HH, Jr see Sansonetti PJ

Corwin LM see Kim R

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Dack GM see Branham SE

Dammin GJ see Formal SB

Dammin GJ see Kinsey MD

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David M see Sansonetti P

Dawkins AT see DuPont HL

Day NP, Scotland SM, Rowe B. Comparison of an HEp-2 tissue culture test with the Sereny test for detection of enteroinvasiveness in *Shigella* spp. and *Escherichia coli*. J Clin Microbiol 1981 Mar;13(3):596-7

In this study the Sereny test was compared with a method that utilizes HEp-2 tissue culture model for detection of enteroinvasiveness in *Shigella* spp. and *Escherichia coli*. A good correlation was observed between the two methods in tests carried out on 63 *Shigella* and *E. coli* strains. The HEp-2 test, being comparatively less expensive, was suggested for rapid screening of *Shigella* and *E. coli* strains for their invasive mechanism.

d'Hauteville H see Sansonetti PJ

Doctor BP see Brown JE

Doctor BP see O'Brien AD

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Donohue-Rolfe A see Keusch GT

Donowitz M, Binder HJ. Effect of enterotoxins of *Vibrio cholerae*, *Escherichia coli*, and *Shigella dysenteriae* type 1 on fluid and electrolyte transport in colon. J Infect Dis 1976 Aug;134(2):135-43

Donowitz M, Keusch GT, Binder HJ. Effect of *Shigella* enterotoxin on electrolyte transport in rabbit ileum. Gastroenterology 1975 Dec;69(6):1230-7

Shigella enterotoxin-stimulated intestinal secretion is described. Rabbit ileal mucosa exposed *in vivo* to *Shigella dysenteriae* 1 enterotoxin was studied *in vitro* in a modified Ussing chamber. Fluid and electrolyte accumulation occurred *in vivo* and net sodium secretion was present *in vitro* in the enterotoxin-exposed tissue in contrast to net sodium absorption in control mucosa. Short-circuit current (Isc) was similar in *Shigella* enterotoxin-exposed tissue compared with control tissue. The increase in Isc following addition of either theophylline or dibutyryl cyclic adenosine monophosphate was similar in enterotoxin-exposed and control mucosa. The addition of glucose resulted in a smaller increment of Isc in *Shigella* enterotoxin-treated tissue. Mucosal cyclic adenosine monophosphate levels in enterotoxin-exposed mucosa did not differ from those of control. These results indicate that the characteristics of rabbit ileal mucosa exposed to *Shigella* enterotoxin and cholera enterotoxin markedly differ, although both produce electrolyte secretion both *in vivo* and *in vitro*. These studies further suggest that, in contrast to its role in cholera enterotoxin-induced intestinal secretion, cyclic adenosine monophosphate may not be the mediator of *Shigella* enterotoxin stimulation of intestinal fluid and electrolyte secretion.

Dubos RJ, Hoberman HD, Pierce C. Some factors affecting the toxicity of cultures of *Shigella dysenteriae*. Proc Natl Acad Sci USA 1942;28:453-8

DuPont HL, Hornick RB, Snyder MJ, Libonati JP, Formal SB, Gangarosa EJ. Immunity in shigellosis. II. Protection induced by oral live vaccine or primary infection. J Infect Dis 1972 Jan;125(1):12-6

DuPont HL, Hornick RB, Snyder MJ, Libonati JP, Formal SB, Gangarosa EJ. Immunity in shigellosis. I. Response of man to attenuated strains of *Shigella*. J Infect Dis 1972 Jan;125(1):5-11

DuPont HL, Hornick RB, Dawkins AT, Snyder MJ, Formal SB. The response of man to virulent *Shigella flexneri* 2a. J Infect Dis 1969 Mar;119(3):296-9

DuPont HL see Formal SB

DuPont HL see Levine MM

Eiklid K, Olsnes S. Animal toxicity of *Shigella dysenteriae* cytotoxin: evidence that the neurotoxic, enterotoxic, and cytotoxic activities are due to one toxin. J Immunol 1983 Jan;130(1):380-4

This paper describes the lethal effect on rabbits and mice of *Shigella dysenteriae* toxin and the ability of the toxin to induce fluid accumulation in rabbit ileal loops in relation to the cytotoxic activity. The relative concentrations of the three toxic activities were approximately the same in a crude toxin preparation and in purified, electrophoretically homogenous toxin.

The cytotoxic, lethal and enterotoxic activities were inactivated to essentially the same extent upon incubation for few minutes at 80°C and upon treatment with urea and trypsin. Graded precipitation of *Shigella* toxin in each case removed essentially the same fraction of the cytotoxic, lethal and enterotoxic activity. These data indicate that one molecular entity is responsible for the three biologic effects of *Shigella* toxin studies. After intravenous injection, the LD_{50} dose was estimated to be 2.2 ng/Kg in rabbits and 450 ng/Kg in mice. Guinea pigs and mice were significantly less sensitive. Mice were more sensitive to intraperitoneally injected toxin than to intravenous injected toxin.

Eiklid K see Olsnes S

Eiklid K see Reisbig R

Elliot RB see Brown KJ

Engley FB, Jr. Neurotoxin of *Shigella dysenteriae* (Shiga). Bacteriol Rev 1952 Sep;16:153-78

Eyres RA see Brown KJ

Falkow S see Formal SB

Flores J, Grady GF, McIver J, Witkum P, Beckman B, Sharp GWG. Comparison of the effects of enterotoxins of *Shigella dysenteriae* and *Vibrio cholerae* on the adenylate cyclase system of the rabbit intestine. J Infect Dis 1974 Oct;130(4): 374-9

This paper is a comparative study of the effects of *Shigella* enterotoxin with that of cholera enterotoxin on activation of intestinal adenylate cyclase in enhancing fluid secretion. Rabbit intestinal segments exposed to *Shigella dysenteriae* enterotoxin and/or *Vibrio cholerae* enterotoxin were used to compare fluid secretion by rabbit ileum in relation to intestinal adenylate cyclase, phosphodiesterase and cyclic 3'5'- adenosine monophosphate (AMP). Moreover, despite known cytotoxic properties of *Shigella* enterotoxin, prior treatment with this toxin did not prevent the intestine from responding to *V. cholerae* enterotoxin. Some similarity was observed in the gross appearance and kinetics of intestinal fluid secretion induced

by the two toxins, but the biochemical pathway was found to differ. *Shigella* enterotoxin applied *in vivo* or to *in vitro* intestinal preparations did not produce the marked increase in adenylate cyclase or cyclic AMP noted after the application of *V. cholerae* enterotoxin. The importance of the biochemical basis of *Shigella* toxin-induced secretion is stressed upon as a future means of identifying alternate pathways to intestinal secretion in diseased or normal states.

Formal SB, LaBrec EH, Kent TH, Falkow S. Abortive intestinal infection with an *Escherichia coli-Shigella flexneri* hybrid strain. J Bacteriol 1965 May;89(5): 1374-82

The mechanism of the apparent loss of virulence of an *Escherichia coli-Shigella flexneri* hybrid strain was studied. The parent *Shigella* strain caused a fatal enteric infection when fed to starved guinea pigs, and signs of dysentery followed its oral administration to monkeys. The hybrid strain failed to produce any apparent symptoms when fed to either of these species. The parent strain was shown to invade the intestinal mucosa of starved guinea pigs. This caused a severe inflammatory reaction in the lamina propria, which progressed to ulceration of the intestinal epithelium and resulted in death of the animal. The hybrid strain also invaded the intestinal mucosa and produced an inflammatory reaction. In this case, the inflammatory reaction subsided, the intestine returned to normal within 4 days after challenge, and the animal survived. Both fluorescent-antibody techniques and *in vivo* growth studies have shown that the hybrid strain cannot maintain itself in the intestinal mucosa. Preliminary studies have indicated that a similar situation also exists in the monkey. However the hybrid could evoke keratoconjunctivitis and invade HeLa cell in culture as well, as did the highly virulent parent strain. So it is concluded that the virulence of *dysenteriae bacilli* rests not only in the capacity to reach the lamina propria, but also in the ability to multiply in this region. The present work further emphasizes that results of indicator tests for the virulence of *Shigella flexneri* strain must be interpreted with caution.

Formal SB, Gemski P, Jr, Baron LS, LaBrec EH. A chromosomal locus which controls the ability of *Shigella flexneri* to evoke keratoconjunctivitis. Infect Immun 1971 Jan;3(1):73-9

By means of intergeneric conjugation between various *Escherichia coli* K-12 Hfr strains and *Shigella flexneri* 2a virulent recipients and by reciprocal transduction analysis with phage P1 *vir*, a locus was established on the genome of *S. flexneri* 2a which was found to be necessary for its penetration of epithelial cells as measured by Sereny test for keratoconjunctivitis. The locus, termed *kcpA* (in reference to its involvement in provoking keratoconjunctivitis), has been positioned between the *lac* and *gal* chromosomal markers and is cotransducible with the *purE* allele. Since *E. coli* K-12 hybridized with the *kcpA* allele of *S. flexneri* do not evoke keratoconjunctivitis, the authors conclude that other genetic loci might also be involved in the process. It is suggested that the knowledge of such loci potentially controlling virulence will prove useful in constructing safe, living, attenuated *Shigella* vaccines.

Formal SB, Baron LS, Kopecko DJ, Washington O, Powell C, Life CA. Construction of a potential bivalent vaccine strain: introduction of *Shigella sonnei* form I antigen genes into the *galE Salmonella typhi* Ty21 typhoid vaccine strain. Infect Immun 1981 Dec;34(3):746-50

Formal SB, DuPont HL, Hornick R, Snyder MJ, Libonati J, LaBrec EH. Experimental models in the investigation of the virulence of dysentery bacilli and *Escherichia coli*. Ann NY Acad Sci 1971;176:190-6

Formal SB, Dammin GJ, LaBrec EH, Schneider H. Experimental *Shigella* infections: characteristics of a fatal infection produced in guinea pigs. J Bacteriol 1958 May;75(5):604-10

A fatal enteric infection with ulcerative lesions in the colon of guinea pigs was established with a strain of *Shigella flexneri* 2a. To accomplish this, it was first necessary to deprive the animals of food for four days and to administer calcium carbonate before, and opium following the challenge suspension. Animals receiving this treatment succumb following oral challenges of *S flexneri* 2a (LD_{50} approximately 10^6 to 10^7 bacteria) but survive doses in excess of 10^8 cells of *Escherichia coli*. In animals succumbing after receiving live *S. flexneri* 2a, lesions of the intestinal mucosa were seen in the cecum and in the colon, consisting of isolated areas of ulceration of the mucosa, hemorrhage and infiltration with inflammatory cells. The inflammatory reaction was seen through the lamina propria and at times extending through to muscularis mucosae into the submucosa and the submucosal lymphoid tissue. The infection with *S. flexneri* that is described resembled the tissue response in humans in being limited to the colon. It is concluded that this method of enteric infection could be used as a laboratory model for future studies concerning immunity and pathogenesis of bacillary dysentery.

Formal SB, Kent TH, Austin S, LaBrec EH. Fluorescent-antibody and histological study of vaccinated and control monkeys challenged with *Shigella flexneri*. J Bacteriol 1966 Jun;91(6):2368-76

Formal SB, Gemski P, Jr, Giannella RA, Austin S. Mechanisms of *Shigella* pathogenesis. Am J Clin Nutr 1972 Dec;25(12):1427-32

The relative roles of mucosal invasion and toxin production by a wild-type invasive-toxigenic strain of *Shigella dysenteriae* 1 in the provocation of disease was investigated through comparison with three mutants derived from it that were altered in both or either of these pathogenic properties. The results of studies on several animal models (rabbit ileal loop, fasted guinea pig and monkeys) indicated that the disease caused by a nontoxigenic, penetrating mutant was not easily distinguishable from that of the original toxin-producing parent strain. A nonpenetrating but toxigenic mutant and a double mutant lacking both the penetrating and toxin production capacity did not cause clinical disease. The results of this study did not identify a clear-cut role for the toxin in pathogenesis of dysentery by *S. dysenteriae* 1, but has emphasized the importance of mucosal invasion for establishment of disease.

Formal SB, LaBrec EH, Schneider H. Pathogenesis of bacillary dysentery in laboratory animals. Fed Proc 1965 Jan-Feb;24(1):29-34

Formal SB, LaBrec EH, Schneider H, Palkow S. Restoration of virulence to a strain of *Shigella flexneri* by mating with *Escherichia coli*. J Bacteriol 1965 Mar;89(3):835-8

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Formal SB see DuPont HL

Formal SB see Gemski P, Jr

Formal SB see Gots RE

Formal SB see Hale TL

Formal SB see Keren DF

Formal SB see Kinsey MD

Formal SB see Kopecko DJ

Formal SB see LaBrec EH

Formal SB see Levine MM

Formal S see Mathias JR

Formal SB see O'Brien AD

Formal SB see Rout WR

Formal SB see Sansonetti PJ

Formal SB see Schneider H

Formal SB see Takeuchi A

Formal SB see Thompson MR

Fujiwara T see Ogawa H

Gangarosa EJ see DuPont HL

Gangarosa EJ see Levine MM

Gemski P, Jr, Takeuchi A, Washington O, Formal SB. Shigellosis due to *Shigella dysenteriae* 1: relative importance of invasion versus toxin production in pathogenesis. J Infect Dis 1972 Nov;126(5):523-30

A comparison of the relative pathogenic importance of mucosal invasion with toxin production by *Shigella dysenteriae* 1 was performed. The pathogenicity of an invasive toxin-producing strain of *S. dysenteriae* 1 was compared with three mutants derived from the wild-type that were altered in both or either of these properties. Studies on several animal models indicated that the disease caused by a nontoxigenic invasive mutant is not easily distinguishable from that caused by the original toxin-producing parent strain. A noninvasive toxigenic mutant and a double mutant that was noninvasive as well as nontoxigenic did not cause clinical disease. On the basis of these findings it is suggested that the ability to penetrate and multiply in the colonic mucosa is relatively more important in causing disease, though the function of toxins in pathogenesis cannot be excluded.

Gemski P, Jr, Formal SB. Shigellosis: an invasive infection of the gastrointestinal tract. In: Schlessinger D, ed. Microbiology-1975. Washington, D.C.: American Society for Microbiology, 1975:165-9

Gemski P, Jr, Sheahan DG, Washington O, Formal SB. Virulence of *Shigella flexneri* hybrids expressing *Escherichia coli* somatic antigens. Infect Immun 1972 Aug;6(2): 104-11

By intergeneric hybridization techniques, *Shigella flexneri* 2a derivatives which express *Escherichia coli* antigenic characteristics rather than their native serotypes were constructed. The purpose of preparing such hybrids was to determine whether they would retain their ability to cause infection despite being altered in their antigenic structure. This 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*. 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. The findings, 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, suggest 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.

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 structures, whether they be a consequence of a smooth to rough mutation or of a distinct change in O repeat chemical structure.

Gemski P see Griffin DE

Gemski P see O'Brien AD

Gemski P see Thompson MR

Gemski P, Jr see Formal SB

Gemski P, Jr see Levine MM

Giannella RA see Charney AN

Giannella RA see Formal SB

Giannella RA see Gots RE

Giannella RA see Kinsey MD

Giannella RA see Rout WR

Gilman RH see Koster F

Gladstone GP see van Heyningen WE

Golderman L, Rubinstein E. *Salmonella* and *Shigella* adherence to the intestine of mice. Isr J Med Sci 1982 Oct;18(10):1032-6

The *in vivo* adherence of (^{14}C) glucose-labeled *Salmonella* and *Shigella* strains to mice intestines was studied. The findings suggested that different intestinal segments may have different receptors for bacteria, regardless of the bacteria's pathogenicity. Lectin, a bacterial protein with mannose-binding characteristics, was seen to play a major role in the adherence process. *Salmonella* strains adhered significantly better to the small bowel mucosa than to the large bowel. *Shigella* strains adhered significantly better to the colonic than to the small bowel mucosa. A mannose-sensitive, lectin-bearing *Salmonella* strain adhered significantly better

to the jejunal mucosa than did mannose-resistant variant. A mannose-sensitive *Shigella* strain adhered significantly better to the colonic mucosa than did the mannose-resistant strain. The addition of a mannose derivative diminished, but did not abolish, the adherence of the mannose-sensitive strains. Adherence may depend, in part, on the presence of a mannose-sensitive lectin on the bacterial surface. Mannose derivatives can partially inhibit bacterial adherence to the intestinal epithelium. Though not very appropriate for reproducing the pathogenicity of *Salmonella* and *Shigella*, the mouse model studied was suggested to be useful for studying the adherence process, as well as the therapeutic measures that interfere with it.

Gots RE, Formal SB, Giannella RA. Indomethacin inhibition of *Salmonella typhimurium*, *Shigella flexneri*, and cholera-mediated rabbit ileal secretion. J Infect Dis 1974 Sep;130(3):280-4

Gots RE see Charney AN

Grady GF see Flores J

Grady GF see Keusch GT

Grady GF see McIver J

Griffin DE, Gemski P. Release of Shiga toxin from *Shigella dysenteriae* 1 by polymyxin B. Infect Immun 1983 Apr;40(1):425-8

The paper describes an efficient procedure for toxin release from *Shigella dysenteriae* 1 by treatment with polymyxin B. The amount of Shiga toxin released by lysis of cells was found to be dependent on the antibiotic concentration and the incubation time. Immunoblot characterization of the Shiga toxin released by exposure to polymyxin demonstrates its electrophoretic similarity to purified Shiga toxin and to Shiga toxin present in crude bacterial sonicate of *S. dysenteriae* 1 cells. Polymyxin treatment therefore offers an approach for rapid release of cell-bound Shiga toxin of high yields.

Hale TL, Sansonetti PJ, Schad PA, Austin S, Formal SB. Characterization of virulence plasmids and plasmid-associated outer membrane proteins in *Shigella flexneri*, *Shigella sonnei*, and *Escherichia coli*. Infect Immun 1983 Apr;40(1):340-50

The degree of homology shared by the virulence associated plasmids of *Shigella flexneri*, *S. sonnei* and enteroinvasive *Escherichia coli* was analysed. Biosynthetic activity of these plasmids in anucleate minicells in encoding plasmid-associated polypeptides of the bacterial outer membranes was also investigated. The 140-megadalton (Mdal) plasmids of *S. flexneri* (serotypes 1, 3 and 5) and enteroinvasive *E. coli* and the 120-Mdal plasmid of *S. sonnei* strains were cleaved with *Eco*RI and *Bam* HI restriction endonucleases. Considerable homology was evident in plasmids from *S. sonnei* strains, whereas only a few common fragments were observed among the *S. flexneri* and enteroinvasive *E. coli* plasmids. Nitrocellulose filter deoxyribonucleic acid (DNA) blot hybridization demonstrated a considerable complement of homologous sequences, despite variations in restriction sites. Minicell producing strains were obtained by N-metayl-N'-nitro-N-nitrosoguanidine mutagenesis. Retention of the invasive phenotype by minicells from invasive strains was demonstrated by transmission electron microscopy of infected HeLa cells. Sixteen polypeptides

were labeled when *S. flexneri* 5 minicells were incubated with ^{35}S methionine. In the invasive strains of *S. flexneri* 5, 14 of these plasmid coded polypeptides were associated with outer membrane while 9 polypeptides similar molecular weight were labeled in the outer membranes of invasive strains of *S. flexneri* 3, *S. sonnei* and *E. coli*. Seven of the *S. flexneri* 5 polypeptides were not labeled in a non-invasive strain which had sustained a large deletion in the virulence-associated plasmid, and none were labeled in minicells which no longer harbored this plasmid. The similarity of the plasmid encoded polypeptides in the invasive strains of *S. flexneri*, *S. sonnei* and *E. coli* was thus found to be consistent with the DNA sequence homology found in the virulence plasmids of these organisms. This paper suggests that identification of plasmid restriction fragments which encode the invasive phenotype determinants may allow cloning and amplification of these determinants in avirulent recipients which may well serve as potential live vaccine candidates.

Hale TL, Formal SB. Cytotoxicity of *Shigella dysenteriae* 1 for cultured mammalian cells. Am J Clin Nutr 1980 Nov;33(11):2485-90

A sensitive, quantitative assay of cytotoxicity was used to evaluate the kinetics of cytolysis in toxin-sensitive (HeLa cells) and toxin-resistant (Henle 407) cell lines exposed to an invasive toxigenic strain of *Shigella dysenteriae* 1 (3818T), a noninvasive, toxigenic strain 38180 and hypotoxigenic strain 725. Cytolysis of HeLa and Henle 407 cells exposed to these strains was measured by release of (^{3}H) uridine from prelabeled monolayers. A latent period of 8 h or more was required for lysis of HeLa cells exposed to noninvasive, toxigenic strain or to partially purified Shiga toxin. Protein synthesis was inhibited during this period. In contrast Henle 407 cells that were exposed to strain 38180 or to exogenous Shiga toxin were unaffected. When either Henle 407 or HeLa cells were infected with invasive toxigenic strains, rapid lysis ensued. Quantitative micro-assay of cytosol toxicity showed that Shiga toxin was produced intracellularly by strain 3818T. The data indicate that intracellular Shiga toxin does play a role in cytolysis of infected mammalian cells.

Hale TL, Formal SB. Protein synthesis in HeLa or Henle 407 cells infected with *Shigella dysenteriae* 1, *Shigella flexneri* 2a, or *Salmonella typhimurium* W118. Infect Immun 1981 Apr;32(1):137-44

The effect on protein synthesis in HeLa and Henle 407 cells due to invasion by three bacterial species i.e. *Shigella dysenteriae* 1, *S. flexneri* and *Salmonella typhimurium* which differ in invasive potential and in *in vitro* toxin production. Protein synthesis was studied by incorporation of ^{14}C leucine into protein. The two cell lines differed in susceptibility to the effects of exogenously applied Shiga cytotoxin. All invasive *Shigella* strains (which synthesize this toxin to a greater or lesser degree) were found to inhibit protein synthesis in both cell lines with equal efficiency. Leucine accumulation continued in these cells, but the labeled amino acid was preferentially incorporated into bacterial protein. *S. typhimurium* W118, which has not been shown to elaborate a Shiga-like toxin, had little effect on protein synthesis among the infected host cell. The Shiga toxin was found to be fully potent when released into the cytosol of Henle cells which are resistant to exogenous cytotoxin. Since toxin resistance in cultured mammalian cells has been equated with the absence (or masking) of a receptor on the plasma membrane, it is concluded that invasive *Shigellae* circumvent the requirement for a toxin receptor by multiplying intracellularly.

Hale TL, Bonventre PF. *Shigella* infection of Henle intestinal epithelial cells: role of the bacterium. Infect Immun 1979 Jun;24(3):879-86

This paper describes experiments designed to assess the role of *Shigella flexneri* 2a in the infection of Henle 407 cell monolayers which can be used as an *in vitro* assay system to quantitate *Shigella* infection. It was assumed that *Shigella* infection of an epithelial-like cell in culture is analogous, at least in its fundamental aspects, to infection of an epithelial cell of the colonic mucosa. Using the Henle 407 human intestinal epithelial cell line as host cells, a standardized experimental protocol which allowed quantitative measurement of infection was developed. Intracellular residence of infection organisms was confirmed by indirect fluorescent-antibody staining of unfixed and methanol-fixed (Henle 407) cells and by quantitative bacteriological culture of disrupted host cells after infection. The process of *Shigella* entry into cells was evaluated by chemical or physical modulation of the bacterium under controlled experimental conditions. *Shigellae* were subjected to mild heat, ultraviolet radiation, aminoglycoside antibiotics and immunoglobulins raised against *S. flexneri* 2a. The data show that specific heat-stable surface antigens unique to *S. flexneri* 2a are apparently not the sole factor responsible for the initiation of infection. Evidence is also presented suggesting that metabolic activity on the part of the infecting bacterium is a pre-requisite for entry into the host cell. Infection of cell cultures *in vitro* is useful since a relatively uniform population of cells can be infected under defined conditions which allows for selective modification of either the infectious agent or the host cell.

Hale TL, Morris RE, Bonventre PF. *Shigella* infection of Henle intestinal epithelial cells: role of the host cell. Infect Immun 1979 Jun;24(3):887-94

A set of *in vitro* experiments was designed to ascertain the role of the host cell (Henle 407 embryonic intestinal epithelial cell) in the initiation of *Shigella flexneri* infection. It was found that the entry of *S. flexneri* into cells was suppressed by reagents which inhibit uptake of particles by phagocytic cells. The compounds tested included cytochalasin B, dibutyryl- cyclic adenosine monophosphate (AMP), choleragen (*Vibrio cholerae* enterotoxin), iodoacetate, and dinitrophenol. Infection of Henle cell was inhibited by cytochalasin B at concentrations of 1.0 µg/ml or greater. Dibutyryl-cyclic AMP at concentrations of 1 mM and choleragen at 0.1 µg/ml caused significant suppression of infection. Iodoacetate or dinitrophenol, at 0.1 mM concentrations, inhibited internalization of virulent *Shigellae*, and a combination of these compounds inhibited infection at 0.01 mM concentrations. Preincubation of Henle cell monolayers with the combination of iodoacetate and dinitrophenol (0.05 mM) also inhibited infection markedly. The data suggest that infection of epithelial cells by *S. flexneri in vitro* is accomplished by an endocytic process induced by virulent bacteria. The process appears to be similar to uptake of particles by phagocytic cells. Ultrastructural analysis by transmission electron microscopy provided corroborative evidence of phagocytosis of *Shigellae* by Henle cells in that intracellular bacteria were often observed within membrane-limiting vacuoles resembling phagosomes. The endocytic event appears to be induced by factors provided by virulent and not avirulent *Shigellae*.

Hale TL see Kopecko DJ

Hale TL see Sansonetti PJ

Hendrix TR see Steinberg S

Hendrix TR see Steinberg SE

Hentges DJ see Osato MS

Hirschhorn K see Keusch GT

Hirschman SZ see Keusch GT

Hisatsune K see Okamura N

Hoberman HD see Dubos RJ

Holcombe J see Kopecko DJ

Honjo S see Ogawa H

Hornick R see Formal SB

Hornick RB see DuPont HL

Hornick RB see Keusch GT

Hornick RB see Levine MM

Howard JG, Whitby JL. The neurotoxin of *Shigella shigae*. Comparative study of the effects produced in various laboratory animals. Br J Exp Pathol 1956 Jun;37:272-8

Ikeuchi T see Osada Y

Imaizumi K see Ogawa H

Islam S see Koster F

Izhar M, Nuchamowitz Y, Mirelman D. Adherence of *Shigella flexneri* to guinea pig intestinal cells is mediated by a mucosal adhesin. Infect Immun 1982 Mar;35(3): 1110-8

The mechanism of adherence of non-pilated clinical isolates of *Shigella flexneri* to the intestinal mucosa of a number of animals was studied. Guinea pig colonic epithelial cells released by treating sections of colon with EDTA, dithiothreitol and citrate solutions avidly adhered *S. flexneri* bacteria. Adherence of *S. flexneri* to the guinea pig colonic cells was Ca^{2+} (1 mM) and time-dependent. The pH optimum was 6.2 and almost no attachment (<5%) was observed at low temperature (4°C). The average number of bacteria bound to colonic cells was 70 per cell, whereas attachment to cells isolated from the ileum region was six bacteria per cell. Adherence to guinea pig colonic cells was inhibited (50%) by several carbohydrates, such as 0.1% fucose or 0.5% glucose, as well as by a lipopolysaccharide preparation (10 µg/ml) isolated from *S. flexneri*. Fixation of the bacteria with glutaraldehyde or preincubation of the bacteria with lectins or proteolytic enzymes did not affect their adherence. Proteolytic digestions or fixation of the epithelial cells, as well as pretreatments with lipopolysaccharide or fucose solutions, abolished their ability to adhere bacteria. These results suggest that a carbohydrate-binding substance on the surface of guinea pig colonic epithelial cells is responsible for the attachment of the *Shigella* bacilli.

Jacewicz M, Keusch GT. Pathogenesis of *Shigella* diarrhea. VIII. Evidence for a translocation step in the cytotoxic action of Shiga toxin. J Infect Dis 1983 Nov; 148(5):844-54

A variety of metabolic inhibitors were used to determine whether an energy-dependent translocation step is involved for entry of the Shiga toxin to reach its cytoplasmic target in HeLa cell model. Previous studies have shown that at 37°C the toxin inhibits protein synthesis in HeLa cells. But at 4°C the toxin binds well to the glycoprotein cell surface receptor but does not affect protein synthesis or result in cytotoxicity and subsequently can be largely removed by washing of the monolayers, suggesting an energy involving internalization system. Agents known to inhibit glucose metabolism, mitochondrial energy production, protein synthesis as well as drugs that alter the cytoskeletal system or the functional attributes of lysosomes, were employed to examine the nature of the events that follow the binding of the toxin to the cell surface receptor. Metabolic inhibitors reduced both cytotoxicity and binding of the toxin. The effect was most pronounced with oligomycin and potassium cyanide, inhibitors of oxidative phosphorylation, whereas inhibitors of glycolysis were least effective. Effects on cytotoxicity were partially reversed in the presence of the membrane permeabilizer dimethylsulfoxide. All agents tested except actinomycin D also diminished endocytosis. Various cytochalasins, colchicine, vinca alkaloids, chloroquine, and steroids also reduced the activity of the toxin. Because these diverse agents all have a mechanism via which endocytosis can be interrupted or fate of the endocytized molecules can be altered and all reduce the cytotoxicity of Shiga toxin in the sensitive HeLa-cell system, it is concluded that the toxin in internalized and probably processed within membrane-bounded vesicles in a fashion leading to its entry into the cytoplasm, where it inhibits ribosomal protein synthesis.

Jacewicz M see Keusch GT

Kabir S, Ali S, Akhtar Q. Ionic, hydrophobic, and hemagglutinating properties of *Shigella* species (letter). J Infect Dis 1985 Jan;151(1):194

The factors associated with the cell surface of *Shigella* species that may contribute to adhesion to the colonic mucosa were investigated. *Shigella* organisms *(Shigella boydii, S. flexneri, S. dysenteriae* and *S. sonnei)* regardless of their serotypes adhered strongly to the anion exchange matrix, DEAE-cellulose, suggesting the anionic nature of the cell. The bacterial strains were found to be weakly hydrophobic as most of them did not adhere to octyl Sepharose gels. The *Shigella* isolates did not agglutinate human (group O), chicken, and/or sheep erythrocytes. The authors postulate that divalent cations such as Ca^{++} may play a role in forming bridges between the anionic surface components of *Shigella* species and epithelial cells which are also anionic in nature.

Kamat RS see Bhogale SR

Kapfer C see Sansonetti PJ

Kent TH see Formal SB

Keren DF, McDonald RA, Scott PJ, Rosner AM, Strubel E. Effect of antigen form on local immunoglobulin A memory response of intestinal secretions to *Shigella flexneri*. Infect Immun 1985 Jan;47(1):123-8

Keren DF, Collins HH, Baron LS, Kopecko DJ, Formal SB. Intestinal immunoglobulin A responses in rabbits to a *Salmonella typhi* strain harboring a *Shigella sonnei* plasmid. Infect Immun 1982 Jul;37(1):387-9

Salmonella typhi 5076-IC, which contains a plasmid that encodes the form I antigen of *Shigella sonnei* and which expresses *S. typhi* 9 and 12 and *S. sonnei* form I antigens, was used to immunize rabbits via chronically isolated ileal loops. Intesti-

nal immunoglobulin A (IgA) activity was detected against *S. typhi*, *S. sonnei* form I, and *S. typhi* strain 5076-IC. The present study thus indicates that the genetically stable transconjugant *S. typhi* 5076-IC is effective in stimulating the local IgA response to both the parent *Salmonella* and the plasmid-borne *S. sonnei* form I antigens. Therefore, this bivalent oral vaccine offers a means to elicit local immunity to both the intestinal pathogens by peroral immunization.

Ketyi I, Vertenyi A, Pacsa S, Kocsis B. Enterotoxin production by *Shigella flexneri* type 2a, strain no. M42-43. Acta Microbiol Acad Sci Hung 1978;25(4):319-25

Enterotoxin produced by *Shigella flexneri* 2a, strain M42-43, is similar to the "Shiga-like" cytotoxic enterotoxin and shares common features with that of other *S. flexneri* strains. On the basis of molecular filtration and neutralization experiments it is suggested that the same molecule carries these biological characteristics. The authors suggest that the antigenic relationship between choleragen, *Escherichia coli* heat-labile toxin, *S. flexneri* heat-stable toxin and this cytotoxic enterotoxin from strain M42-43, and the biological relations of enterotoxins could be due to the fact that the phenomenon is governed by modulation of one *tox* gene.

Ketyi I, Malovics I, Vertenyi A, Kontrohr T, Pacsa S, Kuch B. Heat-stable enterotoxin produced by *Shigella flexneri*. Acta Microbiol Acad Sci Hung 1978;25(3): 165-71

In this paper the enterotoxigenic character of *Shigella flexneri* is described. Filtrates and ultrasonic extracts of *S. flexneri* showed positive in the rapid permeability factor (PF) test and also proved positive in suckling mice and ligated rabbit loop tests within 4 h. Delayed PF was not detected; the rabbit loop dilatation test read after 18 to 24 h, the mouse pad edema reaction, the test for elongation effect of Chinese hamster ovarian (CHO) cells were all negative. In the delayed PF test a strong "blanching" effect was observed. A filtrate of an enterotoxicity negative (Ent^-) *Escherichia coli* strain was positive only in the rapid PF test, while filtrate and ultrasonic extract prepared from an Ent^+ *E. coli* strain showed a positive reaction in all tests for enterotoxins (heat-stable and heat-labile) including the rapid PF test. Ultrasonic extracts of a *S. flexneri* and an Ent^- *E. coli* strain concentrated by freeze-drying were fractionated on a Sephadex G-100 column. *S. flexneri* fractions of 60-70 ml were positive for the following: rapid PF, dilatation capacity in suckling mice, and the balanching effect in the delayed PF test. No positive reaction was found in the delayed PF test and in CHO cell culture. Similar fractions of Ent^- *E. coli* carried substances responsible for the rapid PF and the blanching effect (but without suckling mice positivity). It is concluded that the enterotoxicity of *Shigella* strains may have a role in the clinical appearance of bacillary dysentery.

Ketyi I, Vertenyi A, Malovics I, Kontrohr T, Pacsa S. Unique features of heat-stable enterotoxin of *Shigella flexneri*. Acta Microbiol Acad Sci Hung 1978;25(3): 219-27

Keusch G, Jacewicz M, Pereira M. Alterations in surface determinants correlates with resistance of cloned HeLa cells to *Shigella* toxin. Clin Res 1981;29(2):533A

Keusch GT. Bacterial toxins as virulence factors: Shiga bacillus dysentery viewed as a toxinosis. Mt Sinai J Med NY 1976 Jan-Feb;44(1):33-41

Keusch GT, Papenhausen PR, Jacewicz M, Hirschhorn K. Comparison of *Shigella* (s) and cholera (c) toxin effects using lymphocytes (l) as target cells. Clin Res 1976 Oct;24(4):287A

Keusch GT, Jacewicz M. The pathogenesis of *Shigella* diarrhea. V. Relationship of Shiga enterotoxin, neurotoxin, and cytotoxin. J Infect Dis 1975 May;131 (Suppl):S33-9

In this report, the relationship between different biological activity of the *Shigella dysenteriae* 1 i.e.,enterotoxicity, neurotoxicity and cytotoxicity was studied. All three toxic activity were present in equivalent extent in a fresh enterotoxin preparation as well as in a well-studied 20 year old partially purified preparation of neurotoxin from the same organism. Multiple protein bands were present in each toxin studied. By Sephadex gel filtration chromatography, isoelectric focusing in a sucrose gradient and polyacrylamide gel electrophoresis, two separate HeLa cell fractions were obtained. The larger molecular weight fraction (MW 40,000; isoelectric at pH 7.2) was associated with both the neurotoxic and enterotoxic activity. The smaller molecular weight fraction (MW 20,000; isoelectric at pH 6.1) possessed the cytotoxic activity. These data suggest that Shiga enterotoxin and neurotoxin are closely related proteins and may even be identical. The authors hypothesize that the low molecular weight cytotoxin with pH of 6.1 may be a subunit of the larger toxin that is capable of acting directly on the HeLa cell.

Keusch GT, Grady GF, Mata LJ, McIver J. The pathogenesis of *Shigella* diarrhea. I. Enterotoxin production by *Shigella dysenteriae* 1. J Clin Invest 1972;51:1212-8

The enterotoxin produced by a strain of *Shigella dysenteriae* 1 isolated from a patient with dysentery in Guatemala was characterized. The toxin was produced in liquid broth cultures. Partial purification by ultrafiltration on graded polymeric and Sephadex G-150 suggested an approximate molecular weight of 55,000 - 60,000. The partially purified toxin had several properties: it was heat-labile, pronase sensitive, activated by alkaline pH, neurotoxic to mice and elicited fluid production in rabbit ileal loops; it however failed to cause increased vascular permeability in skin. When the activities of equal weights of identically prepared *Vibrio cholerae* and *S. dysenteriae* enterotoxins were compared in the rabbit ileum the latter caused a significantly smaller volume response with increased concentrations of potassium, chloride and protein. If these biological activities prove to be possessed by a single molecular species, it is suggested that it be renamed *Shigella* enterotoxin in recognition of the physiologically more relevant biological action.

Keusch GT, Jacewicz M, Levine MM, Hornick RB, Kochwa S. Pathogenesis of *Shigella* diarrhea:serum anticytotoxin antibody response produced by toxigenic and non-toxigenic *Shigella dysenteriae* 1. J Clin Invest 1976 Jan;57:194-202

The serum anticytotoxin immune response during natural and experimentally induced Shiga bacillus dysentery in man was investigated. Natural infection resulted in the rapid appearance of toxin neutralizing antibody, which disappeared some time between 9 and 18 months after infection. Time-course of immunoglobulin production was investigated in sera obtained serially from 7 to 50 days after infection from experimentally infected human volunteers. Although the serum antibody response of the toxin was similar to that observed for O-polysaccharide, its biological activity was destroyed by heat and proteolytic enzymes. Neutralizing antibody was present only in the IgM fraction isolated by sucrose density gradient ultracentrifugation. This was confirmed by the use of solid-phase immunoglobulin affinity chromatography and there was no evidence of a shift in the immunoglobulin class of antibody from IgM to IgG. A laboratory mutant derived from wild-type *Shigella dysenteriae* 1, which does not produce readily detectable toxin *in vitro* was also

found to elicit a serum IgM anticytotoxin antibody response in volunteers developing clinical illness after oral challenge with the organism. Biologically active cytotoxin was recovered when this mutant organism was grown in liquid media with controlled iron concentration. The mutant cytotoxin was heat-labile, neutralized by antiwild-type cytotoxin antibody, and was separable by isoelectric focusing into two fractions with pH 7.2 and 6.1 like the wild-type toxin. It is concluded that although epithelial cell invasion assumes importance in the pathogenesis of shigellosis, it is premature to conclude that it is the sole virulence characteristic of the genus.

Keusch GT, Jacewicz M. Pathogenesis of *Shigella* diarrhea. VII. Evidence for a cell membrane toxin receptor involving β1 → 4-linked *N*-acetyl-D-glucosamine oligomers. J Exp Med 1977;146:535-46

To determine whether or not mammalian cells possess a membrane receptor for Shiga cytotoxin and also to characterize the nature of this receptor, the bindings of the toxin to HeLa cells and to isolated rat liver cell membranes were studied. The investigation involved an indirect consumption assay of toxicity from the medium or by determination of cytotoxicity to the HeLa cell monolayer. Both liver cell membranes and HeLa cells removed toxicity from the medium during incubation, in contrast to Wl-38 and Y-1 mouse adrenal tumor cells, both of which neither bound to the toxin nor were affected by it. Toxin uptake was directly related to concentration of membranes added, time and temperature, and indirectly related to the ionic strength of the buffer used.

Three basic approaches were used to characterize the membrane receptors. These included (i) enzymatic destruction of the receptor, (ii) competitive inhibition of toxin binding with a variety of sugars, oligosaccharides and glycoproteins and (iii) specific receptor blockade by using lectins with known binding specificities. The receptors were destroyed by proteolytic enzymes, lysozyme and phospholipases (which markedly altered the gross appearance of the membrane preparation), but not by a variety of other enzymes. Of 28 carbohydrate and glycoprotein haptens studied, including cholera toxin and ganglioside, only the chitin oligosaccharide lysozyme substrate, per *N*-acetylated chitotriose, chitotetrose, and chitopentose were effective competitive inhibitors. The triose, N,N',N" triacetyl chitotriose was found to exert maximum inhibitory effect on three lectins studied as possible receptor blockers i.e., phytohemagglutinin, concanavalin A, and wheat germ agglutinin; only the latter, which is known to possess specific binding affinity for N,N',N" triacetyl chitotriose, was able to block toxin uptake by both HeLa cells and rat liver cell membranes. These three distinct lines of evidence point to the fact that mammalian cells do indeed possess a toxin receptor with involvement of oligomeric β1 → 4-linked *N*-acetyl glucosamine in the receptor. This receptor was found to be clearly distinct from the cholera toxin receptor GM_1 ganglioside.

Keusch GT, Jacewicz M. The pathogenesis of *Shigella* diarrhea. VI. Toxin and antitoxin in *Shigella flexneri* and *Shigella sonnei* infections in humans. J Infect Dis 1977 Apr;135(4):552-6

In addition to invasiveness, the possible role of toxin production as a virulence mechanism in *Shigella flexneri* and *S. sonnei* was investigated. Two strains of *S. flexneri* and one of *S. sonnei* were studied for toxin production *in vitro*. All of the three strains produced a cell-free HeLa cell cytotoxin that showed marked similarity to that produced by *S. dysenteriae* 1. Each toxin eluted in two distinct peaks on chromatography with Sephadex G-150, was destroyed by heating

at 90°C for 30 min, and was neutralized by *S. dysenteriae* 1 antitoxin. Patients with infections due to *S. flexneri* and *S. sonnei* developed antibody that neutralized *S. dysenteriae* 1 toxin *in vitro*. Antibody activity in three of the seven positive sera was associated only with the IgM fraction as separated by sucrose density gradient ultracentrifugation. The time course of antibody response resembled that found in infections due to *S. dysenteriae* 1 in which an IgM antitoxin antibody has also been described. Since three species of *Shigella* are found to be toxigenic, it is possible that bacterial toxin may play a role, along with bacterial invasion, in the pathogenesis of infections due to *S. flexneri, S. sonnei* and *S. dysenteriae* 1.

Keusch GT, Jacewicz M, Hirschman SZ. Quantitative microassay in cell culture for enterotoxin of *Shigella dysenteriae* 1. J Infect Dis 1972 May;125(5):539-41

A quantitative microassay for *Shigella dysenteriae* enterotoxin, cytotoxic to HeLa cell monolayers, was developed. This toxin caused rapid detachment of cells from glass surfaces. The number of cells that detached during overnight incubation was directly related to the quantity of toxin present in the medium. The assay system involves enumeration of the number of cells that remain fixed to glass surface after exposure to the toxin. The technique is 1000-fold more sensitive than titration in the rabbit ileum (capable also of detecting subnanogram quantities of toxin), and more reproducible being also less expensive. This paper suggests that the HeLa cell system may be adaptable to other cytotoxic bacterial products, such as the exotoxin of *Corynebacterium diphtheriae*.

Keusch GT. Receptor mediated endocytosis of *Shigella* cytoxin. In: Middlebrook J, Kohn L, eds. Receptor mediated binding and internalization of toxins and hormones. New York: Academic Press, 1981:95-105

Keusch GT, Jacewicz M. Serum enterotoxin-neutralizing antibody in human shigellosis. Nature (New Biol) 1973;241:31-2

Keusch GT, Mata LJ, Grady GF. *Shigella* enterotoxin: isolation and characterization. Clin Res 1970 Apr;18(2):442

Keusch GT. *Shigella* infections. Clin Gastroenterol 1979 Sep;8(3):645-62

Keusch GT, Donohue-Rolfe A, Jacewicz M. *Shigella* toxin(s): description and role in diarrhea and dysentery. Pharmacol Ther 1982;15:403-38

Keusch GT see Donowitz M

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Khavkin TN see Vino-Yasenetsky MV

Kim R, Corwin LM. Factors affecting virulence of *Shigella flexneri:* avirulent strain with altered metabolism of succinate, fumarate, and malate. Infect Immun 1973 Apr;7(4):625-30

This paper reports that a spontaneous avirulent mutant of *Shigella flexneri* 2a exhibited altered metabolism of tricarboxylic acid (TCA) cycle acids, but the effect was found to be strongly dependent on growth conditions. Succinate,

fumarate and malate were oxidized much slower by the mutant cells harvested from stationary - phase cultures grown with vigorous shaking than the wild-type cells. Cell-free extracts from such cells showed similar differences in oxidative ability. These differences were less pronounced when cells were harvested from the exponential phase of growth and were nonexistent when the cells were grown without shaking. There was no difference between wild-type cells and cells from exponential phase cultures in oxidative capability of TCA cycle acids, when these acids were used as carbon sources. Stationary phase cells grown on succinate lost succinoxidase activity, but wild-type cells retained more than twice the activity of mutant cells. The effect of growth conditions on oxidative differences between the two strains was similar to the differences in succinate uptake observed under similar conditions.

Kim R, Corwin LM. Mutation in *Shigella flexneri* resulting in loss of ability to penetrate HeLa cells and loss of glycerol kinase activity. Infect Immun 1974 May; 9(5):916-23

This paper presents data to show that the mutated gene affecting glycerol utilization in strains of avirulent *Shigella flexneri* (related to glycerol kinase) is also associated with HeLa cell penetration. An avirulent mutant of *S. flexneri* 2a was obtained as a spontaneous opaque colonial variant of the virulent strain which grows as translucent colonies on meat extract agar. In addition to the loss of virulence and glycerol kinase activity, it showed several other altered characteristics: lowered ability to oxidize tricarboxylic acid cycle intermediates, increased electrophoretic mobility, and decreased sensitivity to sodium lauryl sulfate. Genetic analysis has revealed that the gene governing glycerol kinase activity in *Shigella* has a different chromosomal locus than that from *Escherichia coli*. Furthermore, transduction of the *Shigella* glycerol kinase gene (glp K) into the avirulent *Shigella* strain can restore the ability to penetrate HeLa cells, whereas the gene from *E. coli* cannot. About half of the glp K mutants lose this ability, and only about half of the revertants of an avirulent glp K mutant regain it. This indicates that more than one gene affects glycerol kinase activity in *Shigella*, only one of which is associated with penetration. Glycerol kinase activity is closely correlated with changes in electrophoretic mobility, but does not appear to have any relationship to sodium lauryl sulfate sensitivity nor to the oxidation of tricarboxylic acid cycle intermediates.

Kinsey MD, Formal SB, Dammin GJ, Giannella RA. Fluid and electrolyte transport in rhesus monkeys challenged intracecally with *Shigella flexneri* 2a. Infect Immun 1976 Aug;14 (2):368-71

To define the relationship between invasion and inflammation of the colon and the occurrence of jejunal transport abnormalities, studies were conducted on water and electrolyte transport, histology, and bacteriology in rhesus monkeys, infected by introducing *Shigella flexneri* 2a directly into the cecum. In contrast with the pattern of disease seen after oral administration, cecal inoculation resulted in clinical disease in 64% of animals, of which 94% manifested dysentery alone being rarely preceded by mild diarrhoea. Histologically, invasion and inflammation was limited to the colon. When compared with controls, secretion of water and sodium was found to occur in the colon of infected monkeys, whereas transport was normal in the jejunum and ileum. The data indicate that severe dysentery can result from cecal injection of *Shigellae*, and colonic secretion in dysentery may infrequently result in mild diarrhoea. It is also suggested that the occurrence of severe watery diarrhoea may require jejunal secretion resulting from an undefined interaction between jejunal mucosa and organisms during transit through the small intestine.

Koch PK, Olitzki L. The action of dysentery toxins on different laboratory animals. Exp Med Surg 1946;4:54-68

Koch PK see Olitzki L

Kochwa S see Keusch GT

Kocsis B see Ketyi I

Kondo N see Okamura S

Kontrohr T see Ketyi I

Kopecko DJ, Baron LS, Hale TL, Formal SB, Noon K. Cloning the plasmid-mediated form I O-antigenic determinants of *Shigella sonnei*. Abst Ann Meeting Am Soc Microbiol 1983:60

Kopecko DJ, Washington O, Formal SB. Genetic and physical evidence for plasmid control of *Shigella sonnei* form I cell surface antigen. Infect Immun 1980 Jul; 29(1):207-14

The role of plasmid(s) in the expression of form I antigen of *Shigella sonnei* was investigated. Virulent *S. sonnei* synthesizes a surface antigen termed form I (displaying smooth colonial appearance on agar medium) which appears to be one of several requirements needed for this host to invade epithelial cells. Upon re-streaking on agar media, form I cells readily and irreversibly generate form II cells (rough appearing colonies) that lack the form I antigen and are avirulent. Plasmid deoxyribonucleic acid of form I and form II cells of 4 different *S. sonnei* strains isolated from widely different geographical locations (Japan, England and the United States) was analyzed by agarose gel electrophoresis. A large plasmid (120 megadaltons in 3 of the strains), present in form I cells, was always absent from form II variants. No attempt was successful in transferring conjugally only this large plasmid from form I to genetically marked form II cells. However, a composite molecule that apparently formed by recombination between the large form I plasmid and a self-transmissible plasmid, was found to transfer the form I trait. The transconjugant *S. sonnei* strains acquiring the form I antigen could retransfer this trait to *S. sonnei*, *S. flexneri* or *Salmonella typhi*. These findings demonstrate that *S. sonnei* form I antigen synthesis is mediated by a large plasmid which is also lost spontaneously at a relatively high frequency. Despite this observed instability of the virulent form, *S. sonnei* continues to be the major cause of shigellosis in the United States.

Kopecko DJ, Sansonetti PJ, Baron LS, Formal SB. Invasive bacterial pathogens of the intestine: *Shigella* virulence plasmids and potential vaccine approaches. In: Levy SB, Clowes RC, Koenig EL, eds. Molecular biology, pathogenicity and ecology of bacterial plasmids. New York: Plenum, 1981:111-21

Kopecko DJ, Holcombe J, Formal SB. Molecular characterization of plasmids from virulent and spontaneously occurring avirulent colonial variants of *Shigella flexneri*. Infect Immun 1979 May;24(2):580-2

Avirulent opaque (O-type) colonial variants of *Shigella flexneri* were studied to determine whether loss of virulence is associated with alteration in plasmid content. Studies have revealed that the spontaneous transition of the virulent translucent (T-type) form of *S. flexneri* to the avirulent form is not accompanied by any detectable change in molecular size or form of the four plasmid species

found in the virulent parent form. It is possible that the high frequency of colonial transition involves a specialized genetic recombination system.

Kopecko DJ, Formal SB. Plasmids and virulence of enteric and other bacterial pathogens (editorial). Ann Intern Med 1984 Aug;101(2):260-2

Kopecko DJ see Formal SB

Kopecko DJ see Keren DF

Kopecko DJ see Sansonetti PJ

Koster F, Levin J, Walker L, Tung KSK, Gilman RH, Rahaman MM, Majid MA, Islam S, Williams RC. Hemolytic-uremic syndrome after shigellosis: relation to endotoxemia and circulating immune complexes. N Engl J Med 1978 Apr 27;298(17):927-33

Kuch B see Ketyi I

LaBrec EH, Schneider H, Magnani TJ, Formal SB. Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery. J Bacteriol 1964 Nov; 88(5):1503-8

The present report summarizes experiments in which a parent strain of *Shigella flexneri* 2a and a colonial mutant derived from it was studied in various *in vivo* and *in vitro* models. From the results of this work, a concept of the pathogenesis of bacillary dysentery is developed and as a corollary, an insight into some properties which render a dysentery bacillus pathogenic is presented.

Both the strains were studied in three animal models and were found to be equally virulent for mice when living cells suspended in hog gastric mucin were injected by the intraperitoneal route. Feeding the parent strain to starved guinea pigs, followed by the intraperitoneal injection of opium, resulted in the formation of ulcerative lesions in the intestinal tract leading to the death of these animals, but the colonial variant failed to cause these effects. When administered orally to rhesus monkeys, the parent strain produced diarrhoeal symptoms and intestinal lesions; the variant caused neither symptoms nor pathology in this species. Serological and growth studies conducted both *in vivo* and *in vitro* did not assist in defining the characteristics present in the parent strain and those absent in the colonial mutant. The virulent parent strain possessed invasive ability to penetrate the bowel epithelium and to enter the lamina propria, to infect and multiply within HeLa cells, and to penetrate epithelial cells of the guinea pig cornea. The avirulent strain possessed none of these abilities. It is suggested that epithelial cell penetration and at least limited survival in the lamina propria are the necessary attributes for pathogenicity of dysentery bacilli and are the characteristics which set them apart from nonpathogenic *Escherichia coli* strains and avirulent *Shigella*.

LaBrec EH see Formal SB

Laveck GD see O'Brien AD

Leibowitz J see Olitzki L

Leppla SH see Brown JE

Levin J see Koster F

Levine MM, DuPont HL, Formal SB, Hornick RB, Takeuchi A, Gangarosa EJ, Snyder MJ, Libonati JP. Pathogenesis of *Shigella dysenteriae* 1 (Shiga) dysentery. J Infect Dis 1973 Mar;127(3):261-70

The potential role of invasiveness and enterotoxigenicity in pathogenesis of *Shigella dysenteriae* 1 was investigated. Two fully virulent (invasive toxigenic) and two modified mutant (noninvasive toxigenic, and invasive nontoxigenic) Shiga strains were defined in animal and *in vitro* models. The virulent strains produced the disease in volunteers in doses as low as 10^1 organisms. Although large number of Shiga organisms were excreted in stool during illness, presence of free toxin in small intestinal samples could not be demonstrated. Proctoscopy and biopsy revealed clinical colitis of the large bowel. Noninvasive toxigenic strain (10^6 - 10^{10} organisms) were well tolerated by 85 of the 86 volunteers. One volunteer had dysentery after the organism had reverted to an invasive form. In contrast, invasive nontoxigenic strains caused shigellosis in monkeys and volunteers. Thus the pathogenicity of *S. dysenteriae* 1 was directly related to its invasiveness. The role of Shiga toxin in human disease needs to be studied further.

Levine MM, Woodward WE, Formal SB, Gemski P, Jr, DuPont HL, Hornick RB, Snyder MJ. Studies with a new generation of oral attenuated *Shigella* vaccine; *Escherichia coli* bearing surface antigens of *Shigella flexneri*. J Infect Dis 1977 Oct;136(4):577-82

This paper describes the reactogenicity, immunogenicity, shedding pattern and efficacy of an oral vaccine consisting of *Escherichia coli* bearing surface antigens of *Shigella flexneri* 2a. In an attempt to develop a safe, proliferating, oral attenuated vaccine against shigellosis, genes that control synthesis of group and type-specific somatic antigens of *S. flexneri* 2a were transferred via conjugation to a recipient strain of *E. coli*. The resultant hybrid (*E. coli* expressing *Shigella* surface antigens) vaccine strain, PGA1 42-1-15, believed to have complete (smooth) lipopolysaccharide, was given to volunteers in two vaccination-challenge studies. The vaccine was well tolerated and gave evidence of intestinal proliferation. In trial no. 1, volunteers given two doses of vaccine, a month apart, were challenged after eight weeks with 10^4 virulent *S. flexneri* 2a. Attack rates were comparable in vaccinees (50%) and controls (40%). In trial no. 2, subjects were given three weekly doses of vaccine and were challenged four weeks later with a small inoculum (10^2) of *S. flexneri* 2a. Again, attack rates among vaccinees (47%) and controls (39%) were similar. It is unclear why this theoretically ideal, live *Shigella* vaccine failed to protect against *S. flexneri* 2a. It is speculated that presence of certain additional factors may be needed in this vaccine to elicit an effective immune response.

Levine MM see Keusch GT

Libonati JP see DuPont HL

Libonati J see Formal SB

Libonati JP see Levine MM

Licheva TA see Petrovskaia VG

Life CA see Formal SB

Lines DR see Brown KJ

Lines RB see Brown KJ

Lycheva TA see Nastichkin IA

McDonald RA see Keren DF

McIver J, Grady GF, Keusch GT. Production and characterization of exotoxin(s) of *Shigella dysenteriae* type 1. J Infect Dis 1975 May;131(5):559-66

Exotoxin of *Shigella dysenteriae* 1 was purified and characterized by production in a semicontinuous fermenter system. Enterotoxicity, neurotoxicity and cytotoxicity of the exotoxin were assayed by: rabbit ileal loop test, mouse lethality after parenteral injection and HeLa cell toxicity, respectively. The toxin, though highly active, was found to be a minor component of the crude preparation of culture filtrate when disc electrophoresis was carried out. Purification of filtrate toxin by isoelectric focusing in polyacrylamide gel revealed two toxic moieties. One was resolvable as a single band with an isoelectric point (pI) of 7.25, a molecular weight of 72,000 and all three types of biologic activity. The second moiety which was isoelectric at pH 6.0 contained two subcomponents and further contrasted with the toxin band isolated at pI 7.25 by being more cytotoxic though devoid of enteroneurotoxin activity.

McIver J see Flores J

McIver J see Keusch GT

Macri BP see Boroff DA

Madonna GS, Allen RC. *Shigella sonnei* phase I and phase II: susceptibility to direct serum lysis and opsonic requirements necessary for stimulation of leukocyte redox metabolism and killing. Infect Immun 1981 Apr;32(1):153-9

This paper describes the differences in serum opsonic requirements necessary for phagocytosis and killing of virulent phase I and avirulent phase II bacteria by polymorphonuclear leukocytes (PMNL). The synthesis of the lipopolysaccharide O-specific repeat polymer by *Shigella sonnei* phase I is a clearly defined bacterial virulence factor necessary for penetrating epithelial cells; *S. sonnei* phase II does not synthesize this antigen and is uniformly avirulent. Using normal and immune serum, the opsonic requirement relative to differences in gross lipopolysaccharide structure, was investigated by quantification and comparison of PMNL metabolism and PMNL-mediated microbial action to phase I and phase II organisms. The stimulation of PMNL O_2-redox metabolism, as required for oxidative killing, was quantified by a chemiluminescent technique. Serum and serum-phagocytic killing assays were used to evaluate the susceptibility to direct serum or serum PMNL-mediated killing. For optimal opsonification of *S. sonnei* phase I, both heat-stable and heat-labile humoral factors were required i.e., humoral recognition, as assayed by the rate and extent of PMNL activation, was effected by phase I specific immunoglobulins plus the classical pathway of complement. *S. sonnei* phase II was susceptible to direct complement-mediated serum killing. Opsonification of the phase II microbe, as measured by PMNL-associated chemiluminescence, was effected by complement in the absence of immune antibody. The results of this study indicate that lipopolysaccharide O-specific repeat polymer expression determines the susceptibility to direct serum bacteriolysis and the opsonification requirements necessary for PMNL-mediated microbicidal action.

Magnani TJ see LaBrec EH

Majid MA see Koster F

Malovics I see Ketyi I

Marakusha BI, Petrovskaia VG. Mapping of mutations in genes of flexner *Shigellae* controlling the synthesis of certain ribosomal proteins and study of the effect

of these mutations on bacterial virulence . Zh Mikrobiol Epidemiol Immunobiol 1980;(12):25-31

Martin JL see Mathias JR

Masek K, Smetana R, Raskova H. Depletion of catecholamines by *Shigella shigae* toxin in the mouse brain. Biochem Pharmacol 1961;8:8-9

Mata LJ see Keusch GT

Mathias JR, Carlson GM, Martin JL, Shields RP, Formal S. *Shigella dysenteriae* 1 enterotoxin: proposed role in pathogenesis of shigellosis. Am J Physiol 1980 Nov; 329(5):G382-6

Maurelli AT, Curtiss R, III. Bacteriophage Mu *d* 1 (Ap^r lac) generates *vir-lac* operon fusions in *Shigella flexneri* 2a. Infect Immun 1984 Sep;45(3):642-8

Maurelli AT, Blackmon B, Curtiss R, III. Temperature-dependent expression of virulence genes in *Shigella* species. Infect Immun 1984 Jan;43(1):195-201

The effect of growth temperature on the virulence of *Shigella* spp. was investigated. Virulence was assessed by *in vitro* infection of Henle intestinal epithelial cells in tissue culture and Sereny test. The expression of virulence in *Shigella* species was found to be dependent on the temperature at which the bacteria are grown. When grown at 37°C strains of *Shigella flexneri* 2a, *S. sonnei* and *S. dysenteriae* 1 were fully virulent. When grown at 30°C, these bacterial strains were found to be avirulent. They could neither penetrate Henle cells nor produce conjunctivitis in guinea pigs. Strains grown at 33°C were partially invasive in the Henle cell assay, whereas strains grown at 35°C were as invasive as strains grown at 37°C. The temperature-induced loss of virulence was completely reversed by shifting the growth temperature from 30° to 37°C. The percentage of Henle cells invaded by bacteria increased with increasing time of growth at 37°C. Restoration of invasiveness after growth at 30°C required protein synthesis. When *Shigellae* were grown at 30°C and shifted to 37°C for 2 h in presence of chloramphenicol, the bacteria remained noninvasive. Similar treatment of a culture grown at 37°C did not remove its virulence. The plasmid profile of these *Shigella* strains grown at 30° and 37°C were found to be identical, thus indicating phenotypical avirulence at low temperature, excluding a temperature-dependent curing of the virulence plasmid as an explanation for the loss of virulence after growth at 30°C. These results suggest that expression of one or more genes required for virulence of *Shigella* spp. are subject to regulation by growth temperature.

Mirelman D see Izhar M

Mise K see Ogawa H

Miwatani T see Takeda Y

Morgan RS see Bridgewater FAJ

Morris RE see Hale TL

Mulczyk M see Adamus G

Murakami M see Okamura N

Nagai T see Okamura N

Nakamura A see Ogawa H

Nakaya R see Ogawa H

Nakaya R see Okamura N

Nastichkin IA, Lycheva TA, Petrovskaia VG. (Influence of the transfer of F' plasmids of different length on the virulence of *Shigella sonnei*). Zh Mikrobiol Epidemiol Immunobiol 1983 Sep;(9):63

This paper describes the influence of F' plasmids of different length, carrying the genetically characterized *Escherichia coli* K-12 chromosomal regions on the virulence of *Shigella sonnei*. The study revealed that in contrast to *S. flexneri* a recessive gene is present in the area of the lactose operon in *S. sonnei*, which causes keratoconjunctivitis. This was proved by the transfer of F' plasmid of different length. The above gene was functionally independent on the influence of the F factor as such as the transconjugants which received the so-called "intermediate" plasmid carrying the *E. coli* K-12 chromosomal genes from *lac* I to *tsx* retained their virulence. The location of the gene(s) responsible for evoking keratoconjunctivitis was found to be to the left of the gene *lac* I.

Noon K see Kopecko DJ

Nuchamowitz Y see Izhar M

O'Brien AD, Thompson MR, Gemski P, Doctor BP, Formal SB. Biological properties of *Shigella flexneri* 2a toxin and its serological relationship to *Shigella dysenteriae* 1 toxin. Infect Immun 1977 Mar;15(3):796-98

A toxin extracted from heat-inactivated, alkaline-treated *Shigella flexneri* 2a showed biological properties similar to those of *S. dysenteriae* 1 toxin. The *flexneri* 2a toxin was lethal to mice, enterotoxic for ileal loops of rabbits and cytotoxic for HeLa cells. Although crude Shiga extracts were found to be toxic, cell extracts of *S. flexneri* exhibited all three toxic activities only after partial purification. Specific toxin activity of crude Shiga extracts was significantly greater than that of partially purified *S. flexneri* 2a toxin. A serological relationship between *S. flexneri* 2a and *S. dysenteriae* 1 toxin was shown by cross neutralization tests. Since *S. flexneri* and *S. dysenteriae* produce related toxins, it could be assumed that both toxins play a similar role in shigellosis.

O'Brien AD, Laveck GD. Immunochemical and cytotoxic activities of *Shigella dysenteriae* 1 (Shiga) and Shiga-like toxins. Infect Immun 1982 Mar;35(3):1151-4

The basis for the reduced toxic activity of Shiga-like toxins produced by *Shigella flexneri* in comparison to Shiga toxin produced by *S. dysenteriae* 1 was examined. Differences in Shiga toxin production by different *S. dysenteriae* 1 strains were also investigated. Toxins in culture supernatants and bacterial lysates of *S. dysenteriae* 1 and *S. flexneri* were quantitated by a cytotoxicity assay and a newly developed radioimmunoassay. Cytotoxin titers paralled toxin antigen levels. So variation in cytotoxicity among *Shigellae* probably reflects differences in toxin

yield rather than specific activity (cytotoxicity per microgram of toxin antigen). A smooth and invasive strain of *S. dysenteriae* 1 produced significantly more toxin antigen than did a rough and noninvasive strain of the same species. It is suggested that explanation for strain-dependent differences in toxin yields may be obtained from studies with isogenic pairs of *S. dysenteriae* 1.

Ogawa H, Nakamura A, Nakaya R, Mise K, Honjo S, Takasaka M, Fujiwara T, Imaizumi K. Virulence and epithelial cell invasiveness of dysentery bacilli. Jpn J Med Sci Biol 1967 Aug;20:315-28

Ogawa H see Osada Y

Okamoto K see Takeda Y

Okamura N, Nagai T, Nakaya R, Kondo S, Murakami M, Hisatsune K. HeLa cell invasiveness and O antigen of *Shigella flexneri* as separate and prerequisite attributes of virulence to evoke keratoconjunctivitis in guinea pigs. Infect Immun 1983 Feb; 39(2):505-13

The role of O antigen of *Shigella flexneri* in HeLa cell invasion and in invoking keratoconjunctivitis in guinea pigs was investigated. Many rough mutants from isogenic smooth virulent and avirulent smooth strains of *S. flexneri* were isolated and grouped into several sensitivity patterns to lipopolysaccharide phages. Many of the rough mutants isolated from a virulent smooth strain were capable of penetrating tissue culture cells but incapable of producing a positive Sereny test. No rough mutant obtained from smooth avirulent strains were found to be capable of penetrating HeLa cells. Sugar composition of lipopolysaccharide of some represtative strains was analysed chemically. No correlation between HeLa cell invasiveness and chemotypes of lipopolysaccharide was found, indicating little significance of oligosaccharides (of the rough core), as well as O antigens, in the ability of *S. flexneri* to penetrate HeLa cells. When the O antigen gene from a smooth avirulent *Shigella* HFr strain was transferred to invasive rough strains, most of the transconjugants that expressed O antigens regained the ability to produce a positive Sereny test. To find the approximate locus (or loci) on the genome of an invasive rough strain necessary for the ability to penetrate HeLa cells, intergeneric conjugation between rough *S. flexneri* and *Escherichia coli* K-12 Hfr strain was employed. It was found that two chromosomal loci, the *rha* and *lac-gal* regions, controlled the ability to penetrate HeLa cells. The results suggest that O antigens and the ability to penetrate tissue culture cells are independent and are prerequisite attributes of virulence in *S. flexneri* in evoking keratoconjunctivitis in guinea pigs. It is also demonstrated that at least two chromosomal genes are necessary to acquire the ability to penetrate HeLa cells.

Okamura N, Nakaya R. Rough mutant of *Shigella flexneri* 2a that penetrates tissue culture cells but does not evoke keratoconjunctivitis in guinea pigs. Infect Immun 1977 Jul;17(1):4-8

This paper describes the significance of O antigen in the invasive process of shigellosis. A rough mutant (5503-01) produced smooth opaque colonies, whereas its parent strain (5503), a virulent strain of *Shigella flexneri* 2a, produced characteristic green-gold translucent colonies. Characterization of 5503-01 by agglutination tests, rhamnose content (indicator for the presence of the O-repeat unit of lipopolysaccharide) and sensitivity spectra to "rough-specific" phages revealed that it had lost the specific somatic antigens. The 5503-01 strain penetrated HeLa or L cells and multiplied within the cytoplasm but could not evoke

keratoconjunctivitis in guinea pigs. The properties of this strain were remarkably stable against serial passages and preservation for a long period. The ability of the rough mutant having ability to penetrate tissue culture cells but not evoke keratoconjunctivitis in guinea pigs suggests that specific O antigen is not of significance in the early stage of the invasive process in shigellosis. Such rough mutants could be useful experimental material for the study of invasive mechanisms of *Shigella* bacilli.

Olitzki L, Leibowitz J, Berman M. Further investigations on chemistry, toxicity and other biological properties of different fractions of dysentery bacteria. Br J Exp Pathol 1937 Aug;18:305-16

Olitzki L, Bendersky J, Koch PK. Studies on the toxins of *Shigella dysenteriae* (Shiga). J Immunol 1943;46:71-82

Olitzki L see Koch PK

Olsnes S, Eiklid K. Isolation and characterization of *Shigella shigae* cytotoxin. J Biol Chem 1980 Jan 10;255(1):284-9

Shigella shigae cytotoxin obtained from two different sources: a crude 26-year-old preparation and a pressure dialysed culture medium, was purified and characterized. The purification steps involved repeated chromatography at low salt concentration on acid treated chitin column and elution with 1M NaCl. The cytotoxin after the initial passage was labeled with ^{125}I. The labeled partially purified toxin obtained after the second passage through the column was mixed with unlabeled rabbit hemoglobin as a carrier and then further purified by chromatography on DE 52 column followed by sucrose gradient centrifugation. When characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the purified toxin was found to consist of two bands having molecular weights of 30,500 and 11,000. This indicates that the intact toxin consists of one heavy chain and four to five copies of a light chain. In the isoelectric focusing experiment, *Shigella* toxin was recovered from a broad zone between pH 5.8 and pH 7.5, probably due to charge heterogeneities in the large and small chains.

Most of the cell lines tested were completely resistant even to high concentrations of *Shigella* toxin. Vero cells and one strain of HeLa cells were very sensitive: 2.5 pg/ml of pure toxin induced 50% inhibition of protein synthesis overnight in HeLa cells. The resistance of the other cell lines to the toxin is in accordance with previous reports of lack of binding sites for *Shigella* toxin in some cells. The highly potent cytocidal effect of this toxin on sensitive cells suggests that it is essential for the necrosis in the colon epithelium.

Olsnes S see Eiklid K

Olsnes S see Reisbig R

Osada Y, Une T, Ikeuchi T, Ogawa H. Divalent cation stimulation of cell infectivity of *Shigella flexneri* 2a. Jpn J Microbiol 1975;19:163-6

Osada Y, Ogawa H. Phagocytosis stimulation by an extracellular product of virulent *Shigella flexneri* 2a. Microbiol Immunol 1977;21(1):49-55

Osada Y, Ogawa H. A possible role of glycolipids in epithelial cell penetration by virulent *Shigella flexneri* 2a. Microbiol Immunol 1977;21(7):405-10

Osato MS, Brawner TA, Hentges DJ. *In vitro* inhibition of DNA, RNA, and protein syntheses by *Shigella dysenteriae* type 1 enterotoxin. Am J Clin Nutr 1979 Jan; 32:268

A protein enterotoxin isolated from culture filtrates of *Shigella dysenteriae* 1 by Sephadex G-100 fractionation, followed by dialysis was examined to determine its molecular basis of action. Rabbit ileal loop positive samples were further studied to determine their effects upon deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis. Partially confluent monolayers (60 to 70%) of human intestinal epithelial cells pretreated with enterotoxin for a 2 h period were exposed to ^{3}H-labeled precursors. In all experiments appropriate positive controls (daunomycin, 10 μg/ml; actinomycin D, 1 μg/ml; cycloheximide, 10 μg/ml) and negative controls (normal, untreated), and heat-inactivated (100°C for 20 min) enterotoxin preparations were included as samples. Marked decrease in the capacity to incorporate the labeled precursors into DNA, RNA, and protein was exhibited by cell monolayers treated with the enterotoxin preparations as compared to the normal, untreated controls. Similarly, all positive controls depressed the incorporation of labeled precursors into cellular constituents, but to a much greater extent. Heat-inactivated enterotoxin depressed the incorporation of labeled precursors in all of the systems examined but to only a minimum extent. This may be attributed to the presence of residual endotoxic lipopolysaccharide in the samples (1 μg/ml concentrations). The results indicate that, on a temporal basis, both protein and RNA synthesis are the systems affected earliest by the enterotoxin treatment followed later by an inhibition of cellular DNA synthesis.

Pacsa S see Ketyi I

Papenhausen PR see Keusch GT

Penner A, Bernheim AI. Studied in the pathogenesis of experimental dysentery intoxication: production of lesions by introduction of toxin into the cerebral ventricles. J Exp Med 1960 Jan;111(1):145-53

Pereira M see Keusch GT

Petrovskaia VG, Licheva TA. A provisional chromosome map of *Shigella* and the regions related to pathogenicity. Acta Microbiol Acad Sci Hung 1982;29(1):41-53

Petrovskaia VG see Bondarenko VM

Petrovskaia VG see Marakusha BI

Petrovskaia VG see Nastichkin IA

Pierce C see Dubos RJ

Powell C see Formal SB

Prizont R. Degradation of intestinal glycoproteins by pathogenic *Shigella flexneri*. Infect Immun 1982 May;36(2):615-20

This report deals with the glycosidases identified in a pathogenic strain of *Shigella flexneri* and their ability to degrade intestinal glycoproteins. Intestinal mucin glycoproteins were examined for their ability to sustain growth of pathogenic *Shigella*. Inoculation of germ-free cecal mucin glycoproteins with *S. flexneri* 4b resulted at 48 h in a 940-fold increase in the enteropathogen concentration. Investigation *in vitro* of enzymatic degradation by the pathogen led to the identification of a blood group B-degrading glycosidase produced by the bacteria. In *in vivo* experiments, fecal supernatants of mice monocontaminated with *S. flexneri* 4b contained an α-galactosidase active against the ρ-nitrophenyl-glycoside. This fecal α-galactosidase peaked 5 days after *Shigella* contamination, showing 2,8 ± 1.4 mU of enzyme activity per mg of protein. Contaminated fecal supernatants similarly destroyed the blood group B reactivity of cecal mucin glycoproteins. These data suggest that *S. flexneri* 4b could proliferate within ileocolonic environment by enzymatically degrading mucin glycoprotein sugars.

The author postulates that glycosidases, such as those identified in the present investigation, may not only promote colonization, but even mediate *Shigella* penetration into the intestinal mucosa by uncovering receptor sites from the glycoprotein sugar.

Prizont R, Reed WP. Possible role of colonic content in the mucosal association of pathogenic *Shigella*. Infect Immun 1980 Sep;29(3):1197-9

The association of *Shigella flexneri* to cecal membranes was studied by incubating the organism with cecal slices of germ-free mice. These slices were initially incubated with stool supernatants from germ-free, *Shigella*-monocontaminated, and normal animals. Slices were also incubated with a mixture of normal and *Shigella*-monocontaminated stool supernatants to resemble as closely as possible with *in vivo* cecal conditions that might exist during *Shigella* infection. *Shigellae* associated with slices were also evaluated by fluorescent anti-*Shigella* antibody. Histological examination of several pretreated slices revealed some features of autolysis. Quantitation of *Shigellae* in homogenates of treated slices revealed an increase of organisms only in those slices exposed to contaminated stool supernatants. It is suggested that the surrounding colonic content's nature also needs to be considered when examining the colonic association of *Shigella*.

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Reisbig R, Olsnes S, Eiklid K. The cytotoxic activity of *Shigella* toxin. Evidence for catalytic inactivation of the 60 S ribosomal subunit. J Biol Chem 1981 Aug 25;256(16):8739-44

The *in vivo* and *in vitro* activity of *Shigella shigae* toxin and its A chain was studied to determine its molecular basis of action by identifying the intra-

cellular target. *Shigella* toxin added to toxin-sensitive HeLa S_3 cells caused a rapid decrease in protein synthesis, but incorporation of labeled uridine into ribonucleic acid continued for several hours indicating that inhibition of protein synthesis was the primary activity of the toxin. This inhibition appears to be at the level of peptide chain elongation. An inhibitory effect on cell-free protein synthesis in rabbit reticulocyte lysate was exhibited by toxin pretreated first with trypsin and then with dithiothreitol and 8 M urea or 1% sodium dodecyl sulfate. Ribosomes treated with toxin or its A_1 fragment had lost most of their ability to polymerize (^{14}C) phenylalanine in a poly (U)-dependent cell-free system. The smallest active subunit in cell-free system was the A_1 fragment. It was more active than the intact A chain and the whole toxin which had been treated with trypsin, urea and dithiothreitol. Salt-washed ribosomes in simple buffered solutions were inactivated at a rate of at least 40 ribosomes/(min) (A_1 fragment). Addition of antitoxin immediately stopped further inactivation, but it did not reactivate the inactivated ribosomes.

60 S ribosomal subunits from toxin-treated ribosomes had a marked reduction in ability to support polyphenylalanine synthesis, whereas 40 S subunits from toxin-treated ribosomes retained their activity. Toxin-treated ribosomes retained their ability to incorporate (^3H) puromycin into growing peptide chains, indicating that the peptide bond formation is not the function inhibited. The polysome profile of intoxicated cells was found to be similar to that of control cells. The profile of polysomes treated with toxin *in vitro* was also unaltered indicating that *Shigella* toxin inhibits protein synthesis by the same mechanism *in vivo* and *in vitro*. The cytotoxic test system for *Shigella* toxin was improved and the stability of the toxin to various pH values, temperature and chemicals was also studied.

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Rout WR, Formal SB, Giannella RA, Dammin GJ. Pathophysiology of *Shigella* diarrhea in the rhesus monkey: intestinal transport, morphological, and bacteriological studies. Gastroenterology 1975 Feb;68(2):270-8

This paper describes the changes in fluid and electrolyte transport occurring in both the small intestine and the colon in *Shigella* diarrhoea and the relationship of these changes to alterations in intraluminal *Shigella* concentrations and in intestinal morphology. The occurrence of watery diarrhoea in shigellosis suggests involvement of the small bowel. Therefore, jejunal, ileal, and colonic water and electrolyte transport were studied in *Shigella flexneri* 2a-infected monkeys. Infected animals fell into three groups: dysentery alone, diarrhoea alone, or diarrhoea and dysentery. In controls, net water, sodium, and chloride absorption were seen in the jejunum, ileum, and colon. All infected animals demonstrated diminished colonic absorption or net colonic secretion. In monkeys with dysentery alone, this was the only transport defect observed. In contrast, animals with diarrhoea, either alone or in combination with dysentery, exhibited net jejunal secretion. Ileal transport was normal in all animals. A severe colitis with intramucosal *Shigellae* was seen in all symptomatic animals. In the jejunum or ileum, however, morphological changes were minimal and bacterial invasion was not seen. Therefore unlike the "toxigenic diarrhoeas" caused by *Vibrio cholerae*

Rowe B see Day NP

Rowson KEK see Bridgewater FAU

Rubinstein E see Goldenman

Sansonetti PJ, Hale TL, Dammin GJ, Kapfer C, Collins HH,Jr, Formal SB. Alterations in the pathogenicity of *Escherichia coli* K-12 after transfer of plasmid and chromosomal genes from *Shigella flexneri*. Infect Immun 1983 Mar;39(3):1392-1402

The role of chromosomal genes of *Shigella flexneri* in addition to its 140-megadalton (Mdal) invasive plasmid in conferring full pathogenicity was investigated. The 140-Mdal virulence plasmid (pWR110) was transferred to *Escherichia coli* K-12. Segments of *S. flexneri* chromosomal material were then transferred to plasmid bearing K-12 strains. The virulence of these transconjugants/hybrids was assessed by the HeLa cell model, rabbit ileal loop test and the Sereny test. K-12 strains harboring pWR110 only invaded HeLa cells but produced minimal lesions in the rabbit ileal mucosa and was negative in the Sereny test. Plasmid containing K-12 hybrids which had incorporated various *Shigella* chromosomal regions gave differential reactions in the rabbit ileal loop test and in the Sereny test. Analysis of these transconjugants indicated that three regions were linked with virulent phenotypes. Either of the *his* region (when the genes responsible for O-antigen synthesis were cotransferred) or the *kap* locus linked to the lac-gel region was sufficient to allow invasion of the rabbit ileal mucosa. The third region, *Shigella* chromosomal segment linked to the *arg* and *mtl* loci was necessary for fluid production in the rabbit ileal loop and for producing a positive Sereny test. These derivatives of an *E. coli* K-12 strain, constructed by stepwise conjugal transfer of a large plasmid and three chromosomal segments from *S. flexneri*, appeared to contain the necessary determinants for full pathogenicity in a variety of laboratory models. The present work adds confirmatory data to the concept that a multiplicity of genes, functioning in concert or in sequence is necessary to confer full invasiveness in *S. flexneri*.

Sansonetti P, David M, Toucas M. (Correlation between the loss of plasmid DNA and the transition from virulent phase II to avirulent phase II in *Shigella sonnei*.) C R Acad Sci (D) (Paris) 1980;290:879-82

Sansonetti PJ, Kopecko DJ, Formal SB. Involvement of a plasmid in the invasive ability of *Shigella flexneri*. Infect Immun 1982 Mar;35(3):852-60

The contribution of plasmids to the virulence of *Shigella flexneri* was investigated. Although some strains carried additional plasmids, all invasive *S. flexneri* strains, irrespective of serotype, were found to harbor a large plasmid (140 megadaltons (Mdal) in size). Loss of virulence, i.e., inability to invade HeLa cell monolayers and producing a negative Sereny test correlated completely with the spontaneous loss of the 140-Mdal plasmid by strains of serotypes 1, 2 and 5. To monitor plasmid transfer, the 140-Mdal plasmid of strain M90T (serotype 5) was tagged with

the kanamycin resistance transposon Tn5. This tagged nontransferable plasmid was mobilized with three conjugative plasmids - R386 (incompatibility group F_1) R64drd 11 (incompatibility group Iα), and R16 (incompatibility group O) into avirulent derivatives of the heterologous serotypes I and 2 which had lost the 140-Mdal plasmid. Transconjugants of both serotypes which had received pWR110 regained virulence. The restored virulence in serotypes 1 and 2 by this *S. flexneri* serotype 5 plasmid show that the 140-Mdal plasmids are functionally interchangeable. These results directly demonstrate that this 140-Mdal plasmid of *S. flexneri* encodes or regulates some function(s) required for epithelial cell invasion.

Sansonetti PJ, d'Hauteville H, Formal SB, Toucas M. Plasmid-mediated invasiveness of <*Shigella*-like> *Escherichia coli*. Ann Microbiol 1982;132:351-5

Invasive *Escherichia coli* is a *Shigella*-like microorganism which cause a dysenteric syndrome through invasion of the human colonic epithelium. This paper describes the contribution of plasmid(s) in the virulence of invasive *E. coli*. All invasive *E. coli* strains, irrespective of serotype, were found to harbor a large plasmid ~140 megadaltons (Mdal). Spontaneous variants of serotypes O143 and O124 had lost this plasmid and had become avirulent as determined by inability to penetrate HeLa cells and to provoke keratoconjunctivitis in guinea pigs. A plasmid was still present in some of these strains, although consistently smaller than those observed in the virulent isolates (i.e., 100 Mdal and less). This suggests that deoxyribonucleic acid sequences involved in the invasive process had been deleted. Virulence of these strains were restored when pWR110, a Tn5-labelled virulence plasmid of *Shigella flexneri* was transferred to them. These results demonstrate for the first time that invasive *E. coli* strains, irrespective of their serotype, harbor a 140-Mdal plasmid which is necessary for epithelial cell penetration. This work also demonstrates that *S. flexneri* and invasive *E. coli* share a common extrachromosomal control of their ability to penetrate into cells and are closely related when compared to *S. sonnei*.

Sansonetti PJ, Kopecko DJ, Formal SB. *Shigella sonnei* plasmids: evidence that a large plasmid is necessary for virulence. Infect Immun 1981 Oct;34(1):75-83

This study attempts to prove that the large *Shigella sonnei* plasmid, necessary for form I antigen expression, is essential for virulence and to provide additional genetic characterization of this and other *S. sonnei* plasmids. Virulent form I *S. sonnei* strains contain a 120-megadalton (Mdal) plasmid that is absent in their form II derivatives, which are always avirulent and devoid of O side chains. A total of 165 biochemical and antibiotic traits were assessed, but no experimentally useful phenotype could be associated with this large form I plasmid. Therefore, the form I plasmids of several *S. sonnei* strains were tagged with the antibiotic resistance transposons Tn3, Tn5, or Tn10. Transposon-tagged form I plasmids were not self-transmissible, but could be mobilized by the plasmid R386. Form II *S. sonnei* transconjugants for the form I plasmid acquired both virulence and the ability to synthesize form I antigen, establishing that these properties are plasmid-mediated. Further studies indicate that this 120-Mdal form I plasmid is physically unstable in any of several host bacteria and suggest that it is a member of the FI incompatibility group. Physical and genetic analysis have revealed that all *S. sonnei* strains examined (i.e., >20 worldwide isolates) carry 2 small plasmids, of 3.2 and 3.9 Mdals, which were found to encode either colicin E1 production or resistance to streptomycin and sulfonamides, respectively.

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Silva RM, Toledo MRF, Trabulsi LR. Plasmid-mediated virulence in *Shigella* species. J Infect Dis 1982 Jul;146(1):99

To correlate the presence of plasmids with virulence properties, 58 strains of *Shigella* (27 virulent and 31 avirulent) were analysed for their plasmid profile. All of the virulent strains contained a high molecular weight plasmid (120-140 megadaltons) that were absent in the avirulent strains. Bacterial invasiveness was tested by causation of keratoconjunctivitis in guinea pigs. The present work thus gives additional support to the concept that plasmid-borne genes are involved in the virulence of all *Shigella* strains.

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Steinberg SE, Banwell JG, Yardley JH, Keusch GT, Hendrix TR. Comparison of secretory and histological effects of *Shigella* and cholera enterotoxins in rabbit jejunum. Gastroenterology 1975 Feb;68(2):309-17

To determine if mucosal damage is a pre-requisite for *Shigella* toxin-induced secretion, the actions of cholera toxins and that of *Shigella* toxins were compared. The maximum secretion rate of *Shigella* toxin as determined from the slope of the response curve was 0.0033 ml per cm per min with a correlation coefficient (r) of 0.99, whereas that of cholera toxin was 0.0035 ml per cm per min (r=0.98). In addition, cholera toxin-induced secretion was associated with depletion of goblet cell mucus, whereas no change was seen in association with the response to *Shigella* toxin. Other than goblet cell depletion, there were no histological differences between loops secreting in response to cholera toxin and to *Shigella* toxin. Finally, the secretory effects of the toxins are not additive. These studies suggest that, in spite of apparent differences in the patterns of secretory response to the two toxins, they may share a rate-limiting step in the secretory process.

Steinberg S, Banwell JG, Keusch GT, Hendrix TR. The response of the rabbit jejunum to *Shigella* enterotoxin. Gastroenterology 1972 Apr;62(4):816

The cytotoxic and enterotoxic properties of an exotoxin isolated from a strain of *Shigella dysenteriae* associated with epidemic diarrhoea in Central America were investigated. Using *Shigella* enterotoxin, the secretory response of *in vivo* rabbit jejunal loops has been studied by a recirculatory perfusion technique with phenolsulfonphthalein as a volume marker. *Shigella* enterotoxin in concentrations (1-500 µg/ml) was included in the isotonic equilibrium perfusion solution. Serial samples (0.5 ml) from reservoir (20 ml) were taken at 30-60 min intervals for 3.9 h periods. A control loop in each animal was perfused with isotonic solution alone. Another group of animals were perfused in a similar manner with cholera toxin. At levels 200 µg/ml *Shigella* enterotoxin induced consistent fluid secretion which was similar in composition to cholera toxin induced fluid. *Shigella* enterotoxin induced rates of secretion (+0.194 ml/cm/h) were similar to cholera toxin during the period of linear response. However, *Shigella* enterotoxin secretion differed from cholera toxin in the greater latency of response and shorter duration of effect. No response to *Shigella* enterotoxin was observed until 1 h and significant fluid secretion only developed after 2½ h exposure. In addition, *Shigella* enterotoxin secretion diminished after 5 h and had almost ceased by 7½ h whereas cholera toxin loops continued to secrete at a constant rate. Control loops in *Shigella* toxin and cholera toxin treated animals demonstrated slight net secretion (+0.09 ml/cm/h) different from animals perfused with isotonic fluid alone (-0.001 ml/cm/h). Net glucose absorption was similar from *Shigella* enterotoxin and control loops after 2 h exposure. Mucus discharge from goblet cells was noted after exposure to cholera toxin but not to *Shigella* enterotoxin. *Shigella* enterotoxin fluid secretion differed from that induced by cholera toxin in the latency of response and duration of effect suggesting that the two toxins may have involved different steps in the activation of secretion.

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This paper reports the existence of a toxin in a culture filtrate *Shigella dysenteriae* 1 that causes morphological changes in Chinese hamster ovary cells and is distinct from the neurotoxin reported by previous workers. The toxin was partially purified by successive column chromatography on diethylaminoethyl-cellulose, Bio-Gel A-5m, and hydroxylapatite. It was separated from neurotoxin activity by diethylaminoethyl-cellulose column chromatography and no lethal toxicity to mice was demonstrated in the partially purified preparation. Preliminary experiments have demonstrated that the toxin causes an increase in vascular permeability in the skin test in rabbits. It is concluded that *S. dysenteriae* 1 produces a cytotoxic toxin, in addition to a cytotoxic neurotoxin.

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Watanabe H, Timmis KN. A small plasmid in *Shigella dysenteriae* 1 specifies one or more functions essential for O antigen production and bacterial virulence. Infect Immun 1984 Jan;43(1):391-6

The role of plasmids in the virulence of *Shigella dysenteriae* 1 W30864, which contains at least five species was investigated. Five virulence-deficient derivatives of the strain were obtained by means of a standard plasmid curing procedure. Absence of a small 6-megadalton plasmid in one of the derivatives resulted in reduced invasiveness for HeLa cells and failure to produce the somatic antigen. Transposon tagging of the pHW400 plasmid to produce pHW401 and the transfer of this derivative into a variant of strain W30864 lacking pHW400 confirmed the conclusion that the pHW400 plasmid encodes one or more functions involved in O antigen (lipopolysaccharide) biosynthesis and bacterial virulence. A plasmid of similar size was detected in all of the other examined strains of *S. dysenteriae* 1.

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