Studies on *Shigella* Species

**Section highlights**

- **Mechanism of development of high resistance to quinolones** were evaluated among *Shigella* isolates. It was found that mutations in the gyrA and parC are responsible for this phenomenon.
- **A 57 kDa major antigenic OMP of *Shigella dysenteriae* 1** induces cell-mediated immune response in Shigellosis. The above antigen provoked IL-2 secretion which is required for activation and maturation of CD-4 cells.
- **Porin of *Shigella dysenteriae* induces peritoneal B-1 cells to produce IgA specific mucosal immune response.**

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**6. Studies on Shigella Species**

**6.1. Molecular characterization of multi-drug resistant *Shigella flexneri* in Kolkata**

*Investigator:*

S.K. Niyogi

Shigellosis is a major public health problem in developing countries. Increased incidence of antibiotic resistance in *Shigella* spp. constitute a major concern. High frequency of resistance of *Shigella flexneri* to many of the first line antimicrobial agents (multi drug resistant) have been reported in recent years from Kolkata. Most of the conventional typing methods are based on the phenotypic properties of the microorganisms and offer little strain discriminatory information. The objective of this study is to analyze clonal relationships among isolates of multi-drug resistant *Shigella flexneri* using different molecular typing methods to determine changes at the genetic level and to understand their implications in the epidemiology of the diseases. During the period under study a total of 589 stool samples from Dr. B.C. Roy Memorial Hospital for Children were screened for detection of *Shigella* spp. To isolate *Shigella* spp., stool samples were inoculated onto MacConkey, XLD, HEA and SS agar plates (Difco, USA) and the resulting colonies which exhibited characteristics of *Shigella* spp were identified by conventional biochemical methods. Subsequently serogroups and serotyping were identified by slide agglutination using commercially available poly and monovalent antisera (Denka Seiken Co., Japan).
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Antimicrobial susceptibility tests were performed by an agar diffusion disk method as advocated by the National Committee for Clinical Laboratory Standards. Mueller Hinton agar was obtained from Difco, Detroit, USA and antimicrobial disks were obtained from Difco (Detroit, USA). MIC of the strains against different antimicrobial agents were determined by E-test (AB BIODISK, Solna, Sweden) following manufacturers instructions. Out of 589 stool specimens, 43 (7.3%) were positive for Shigella spp. Among Shigella strains 27 (4.6%) were Shigella flexneri, 5 (0.8%) were Shigella dysenteriae, 10 (1.7%) were Shigella sonnei and 1 (0.1%) was Shigella boydii. All these isolates were tested for their antimicrobial susceptibility patterns. Results showed that S. flexneri strain NK 2788 was highly resistant to fluoroquinolones. Quinolones are clinically useful group of antibacterial agents, which mediated its antibacterial activity by inhibiting the enzymatic activity of DNA gyrase and topoisomerase IV. In many enteric bacteria, quinolone resistance occurs due to point mutation in the genes responsible for gyrA, gyrB or topoisomerase IV (parC, parE) and sometimes both. To evaluate the possible mechanism of quinolone resistance in S. flexneri strain NK 2788, gryA and parC genes were characterized. For this, PCR primers were designed form the conserved region of S. flexneri 5'-TAC ACG GGT CAA CAT TGA GG-3' and 5'-TAA A TG A TT GCC GCC GTC GG-3' 5'-GTA CGT GA T CA T GGA CCC TG-3' and 5'-TTC GGC TGGTCG ATT A TT AA T GC-3' for getting the Quinolone Resistant Determining Region (QRDR). PCR amplicons of both the gryA (648 bp) and parC (548 bp) generated against either sensitive or resistant strains of S. flexneri were purified from agarose gels and used directly for nucleotide sequencing in an automated machine ABI Prism 310 auto sequencer. Detailed comparative analysis of the nucleotide sequence of gyrA and parC were made. Alignment analysis through BLAST revealed that gyrA and parC genes in quinolone sensitive S. flexneri had no point mutations in the QRDR and was homologous to that of E. coli K12 strains. Interestingly, S. flexneri strain NK 2788 showed some degree of variations at the nucleotide level. Alignment analysis of the
predicted amino acid residues of gyrA revealed that alteration at position 83 (serine to leusine) and position 87 (aspartic acid to aspargine) exist in the strain NK 2788. Analysis of the parC locus of the strain NK 2788 also showed alteration at the position 80 (serine to isoleucine) as compared to its sensitive counterpart. Our study indicated that very high resistance to quinolones exhibited by the strain NK 2788 may be a result to mutations in the gyrA and parC. Studies are underway to evaluate the resistance of other antibiotics also.

6.2. Antigenic recognition of *Shigella dysenteriae* outer membrane proteins using human convalescent sera and to evaluate their role in cell-mediated immune response in Shigellosis

Investigator :

A.K. Sinha.

The study was undertaken with the objectives mainly to recognition of major antigenic outer membrane proteins (OMPs) from *S. dysenteriae* 1 using acute and convalescent sera from human and to evaluate the T-cell function in cell-mediated immune (CMI) response and finally to demonstrate the protective role of such immunodominant components, if any, in animals model.

Previously, it has already been reported that up-regulation of IL-2 and its recovery was due to poor internalization of IL-2/IL-2R and re-expression of cytokines, as a result expressing of CD-25 (IL-2R) by the targeted cells for generating signals after immunization and re-stimulated with 57Kda antigenic fraction.
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The above major antigenic component might not inhibit the migration of the macrophages towards the activated response in turn it provoked IL-2 secretion which is required for the activation and maturation of CD-4 cells. Further in immunoblot analysis of the cell extracts from MNCs, it was noted the P70 of IP3 mediating receptors activated during T-cell signaling and phosphorylated by the PTKase through intracellular calcium ([Ca^{2+}]_i), which might help in generating IL-2 rather than IL-4. The above observations, suggested that signals for CD4 cells were up-regulated by inositol triphosphate (IP3)- mediated signals in immunized mice.

6.3. Up-regulation of mucosal immune response by porin of *Shigella dysenteriae* type 1: Study of Toll-like receptor, costimulatory molecule and immunoglobulin expression on murine B-1 cell

**Investigator:** T. Biswas

Keeping in view the significance of B-1 cells in regulating mucosal immunity, activation of B-1 cell by porin purified from *Shigella dysenteriae* type 1 was studied, since several immunopotentiating properties of porin makes the protein attractive to be studied as a possible vaccine candidate. Mice and human have...
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Phenotypically distinct populations of B cells, termed B-1 and B-2, that have been proposed to represent entirely separate B cell lineages. B-1 cells are further divided into B-1a that expresses CD5 and B-1b that express CD11b. Mucosal immunologists became interested in peritoneal cavity B-1 cells when it was shown that these cells reconstitute almost 50\% of intestinal IgA plasma cells. Results have shown mucosal IgA+ B cells in mice arose from two sources: peritoneal cavity (PerC) and Peyer’s patches (PP) B-1 cells. Our study showed that porin of \textit{S. dysenteriae} type 1 increased the mRNA levels for Toll-like receptor (TLR) 2 and TLR6 by 1.5- and 2.9-fold respectively, of peritoneal cavity B-1a and B-1b cells, implicating that coexpression of TLR2 and TLR6 is essential as a combinatorial repertoire for recognition of porin by the B-1 cells. Among the two key TLRs, TLR2 and TLR4, which are primarily responsible for recognizing majority of the bacterial products, TLR2 and not TLR4, participates in porin recognition. TLR2 got increased on both the B-1 cell populations whereas the TLR4 expression remained unaffected. Besides TLRs, mRNA for myeloid differentiating factor, an effector molecule associated with TLR-mediated response, was enhanced by 1.8-fold that suggests of its involvement in the activity of porin. Both of the B-1 cell populations expressed strongly the mRNA for NF-kB in presence of porin, that was 2.4-fold more than untreated control, conforming to the earlier finding that coexpression of TLR2 and TLR6, resulted in robust NF-kB activation for signaling. Porin treatment of B-1 cell populations of C57BL/6 mice, and C3H/HeJ mice in particular, selectively up-regulated the expression of the costimulatory molecules. CD80 expression got enhanced on the B-1a cells whereas CD86 got solely expressed on B-1b cells. B-1 cell “sister” populations might have evolved the mechanism of signaling via a specific costimulatory molecule so that these cells can divide its work load of responding to an antigen. Porin induced cell-surface expression of IgM and IgA on B-1 cell populations from C57BL/6 mice. At present we are studying to augment the porin mediated induction of IgA, the hallmark of mucosal immune response, by interleukin-6, known to participate in terminal differentiation of B cell and development of secretory IgA, on B-1a and B-1b cells.