

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 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 was 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 implication in the epidemiology of the disease. Antimicrobial susceptibility of *Shigella flexneri* strains isolated from diarrhoea cases were determined to assess if there is any significant increase in resistance level of the isolates to antimicrobial agents. Also to find out the relatedness amongst the multiresistant strains of *Shigella flexneri* various molecular typing methods such as DNA plasmid profile and pulsed field gel electrophoresis analysis was carried out.

All *Shigella flexneri* isolates were tested for their antimicrobial susceptibility patterns to evaluate the possible mechanism of quinolone resistance. During the study period *S. flexneri* was the most prevalent serogroup and *S. flexneri* serotype 2a was the predominant serotype among the strains isolated. All *S. flexneri* were found to be multiple antibiotic resistant, few strains of *S. flexneri* type 2a were resistant to fluoroquinolone and the MIC of these strains were >256, 4-8, 10-16, and 12-16 µg/ml for nalidixic acid, ciprofloxacin, norfloxacin, and ofloxacin respectively. Few strains were also found resistant against gatifloxacin also. However, all were found susceptible against azithromycin and ceftriaxone. All the tested strains uniformly harbored *ipaH*, *ial*, *Shigella* enterotoxin 1 genes. Digestion of chromosomal DNA with the restriction endonuclease XbaI produced clearly resolvable restriction endonuclease analysis (REA) pattern after PFGE. Different pattern was identified amongst the isolated strains.

6.2 Porin Induced Polarization of Peritoneal Macrophage and Activation of T cells for Th1/Th2 Response

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The cytokines analyzed in the present study were chosen for their critical role in determining macrophage (M Φ) polarization. Analysis of relative fluorescence intensity demonstrated that porin induced 14% of the CD11b cells to express TNF- α and 15% cells to express IL-12, the cytokines known for triggering M1 type response. IFN- γ , known for its Th1 bias, was found to augment the intracellular expression of IL-12 by 1.5-fold (Fig. 6.2.1).

No significant increase in IL-10 positive cells could be found in response to porin. In order to demonstrate that the porin-mediated expressions of TNF- α and IL-12 are primarily TLR2 dependent, the M Φ were preincubated with neutralizing anti-TLR2 Ab. The data show that CD11b cells expressing TNF- α and IL-12 over untreated control were reduced by 40% and 52% respectively by anti-TLR2 Ab (Fig.6.2.2).

The release of the two cytokines was studied by ELISA. Porin-treated M Φ (0.2 million cells) released 10.8 pg of TNF- α and 9.46 pg of IL-12 over untreated control. Preincubation of M Φ with neutralizing anti-TLR2 Ab prior to porin treatment released 4.5 pg of TNF- α and 3.6 pg of IL-12 over untreated cells, showing 57% and 61% inhibition of the two cytokines respectively (Fig.6.2.3A). Similarly, pretreatment with NF- κ B translocating inhibitor showed 81% drop in release for TNF- α and 51% for IL-12 (Fig.6.2.3B). The data underline the importance of both TLR2 and NF- κ B in regulating porin-induced TNF- α and IL-12 expression.

Porin (10 μ g/million cells)-charged M Φ were cocultured with CD3⁺ CD4⁺ T cells purified by pan CD4⁺ T cell isolation kit and MACS system (Miltenyi Biotec) for 4 days for studying the up-regulation of the T cell activation molecules, and 7 days for chemokine receptor or cytokine expression.

We found that the CD3⁺ CD4⁺ T cells expressed the early activation molecule, CD69, 4-fold, the CD80-CD86 ligand, CD28, 2-fold and the CD40 ligand CD154 (CD40L), 2.5-fold higher than the cells cocultured with M Φ in absence of the immunogen.

Among the panel of chemokine receptors that determine Th polarization, CCR5, the receptor for CCL3, CCL4 and CCL5, was found to be 2-fold higher than that of control. CXCR3 that determines Th1 type response besides CCR5, was found to be marginally expressed. On the contrary, CCR3 that is associated with Th2 response was found to be absent.

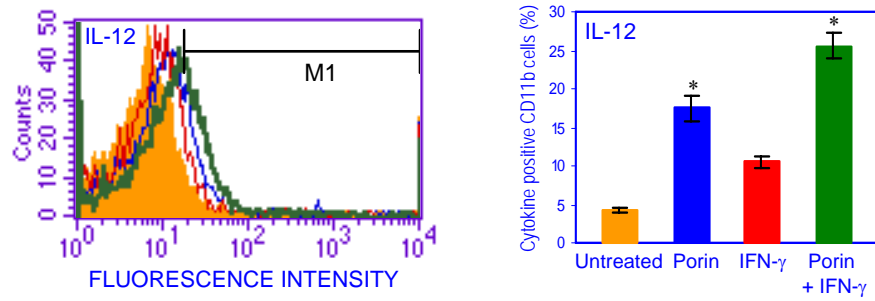


Fig. 6.2.1

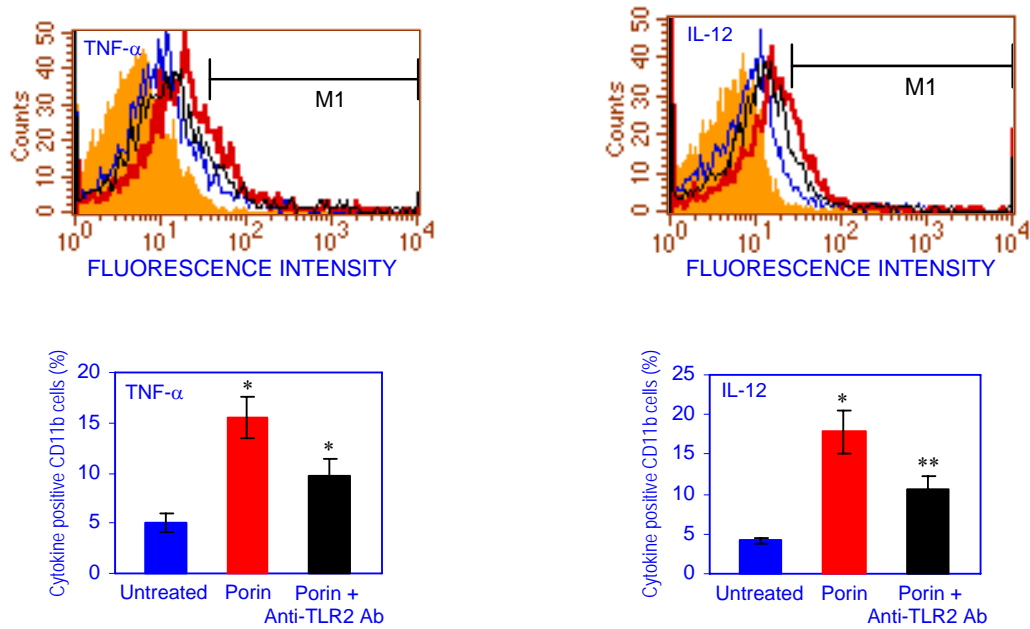
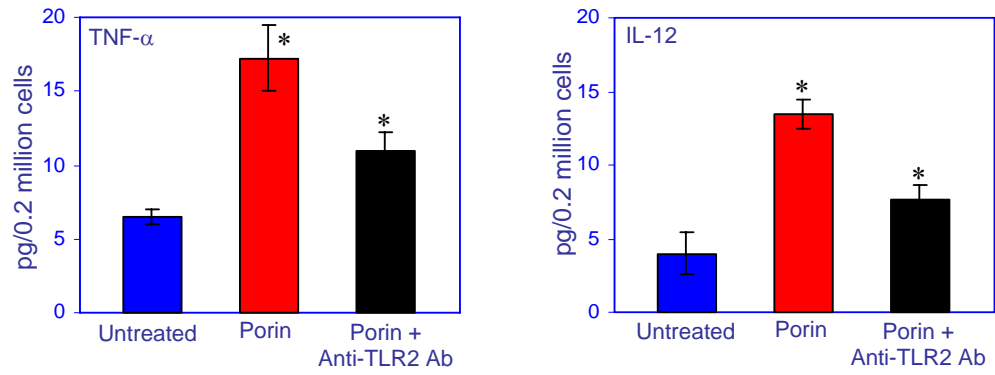
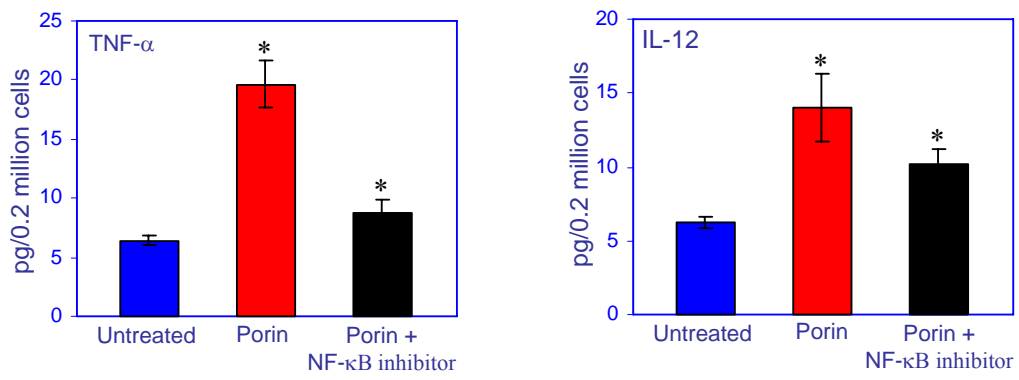


Fig. 6.2.2

A**B****Fig. 6.2.3**