

## 9. Studies on other bacterial pathogens

### 9.1 Mode of action of *Yersinia enterocolitica* heat stable enterotoxin (YSTa) in rat intestinal epithelial cell.

Investigator: M.K. Chakrabarti

The principal objective of this study was to evaluate the mechanism of action of heat stable enterotoxin secreted by *Yersinia enterocolitica*. *Yersinia enterocolitica* heat stable enterotoxin (YSTa) was purified from the culture filtrate using ammonium sulfate precipitation, DEAE Sephacel and Sephacryl S-100 HR column chromatography. Fractions were tested for enterotoxicity by suckling mice assay. It was found that purified YSTa raised  $[Ca^{2+}]_i$  in a dose dependent manner and the optimal level of  $[Ca^{2+}]_i$  was achieved by incubating cells with 10ng YSTa. We reported earlier that Y-STa stimulated phospholipase C activity. It was also found that Y-STa induced rise in intracellular calcium level by calcium influx from extracellular environment as well as  $IP_3$  mediated calcium mobilization from intracellular calcium store. In further support of the involvement of  $IP_3$  mediated calcium mobilization in the mechanism of action of Y-STa evidence we have directly measured the intracellular  $IP_3$  level and found that Y-STa increased the intracellular  $IP_3$  level (Toxicon, 45(3), 361-367, 2005). Moreover, it was found that PLC- $\gamma$  isoform might have a direct role in calcium influx across the plasma membrane.

During the reported period an attempt has been made to evaluate the involvement of nuclear calcium signaling in the mechanism of action of Y-STa. Calcium imaging with Time Series Confocal Microscopy shows that Y-STa stimulates both the nuclear and cytosolic calcium level in rat intestinal epithelial cells where rise in nuclear calcium precedes the cytosolic events. Western blot analysis reveals higher density of  $IP_3$  receptor ( $IP_3R$ ) type II in nuclear membrane compared to cytosol, which may be the cause of early rise of nuclear calcium level. Moreover, immunofluorescence study in Laser Scanning Confocal Microscope with anti-Protein kinase C- $\alpha$  (PKC- $\alpha$ ) antibody shows that nuclear PKC- $\alpha$  translocates earlier from nuclear interior to nuclear envelope in comparison to the translocation of cytosolic PKC- $\alpha$  to plasma membrane. Inhibition of PKC- $\alpha$  translocation by chelation of nuclear and cytosolic calcium with BAPTA (intracellular calcium chelator) has suggested that nuclear and cytosolic PKC- $\alpha$  translocation are calcium dependent. So, we propose for the first time that Y-STa regulates the nuclear and cytosolic calcium signals in a distinct temporal manner in rat intestinal epithelial cells.