ELMO1 Regulates the Induction of Autophagy and Bacterial Clearance During *Salmonella* Infection

Arup Sarkar[1,2], Courtney G Lyons[2], Sarah Fox[2], Thaddeus Stappenbeck[3], Lars Eckmann[4], Soumita Das[2] and Peter B. Ernst[2]

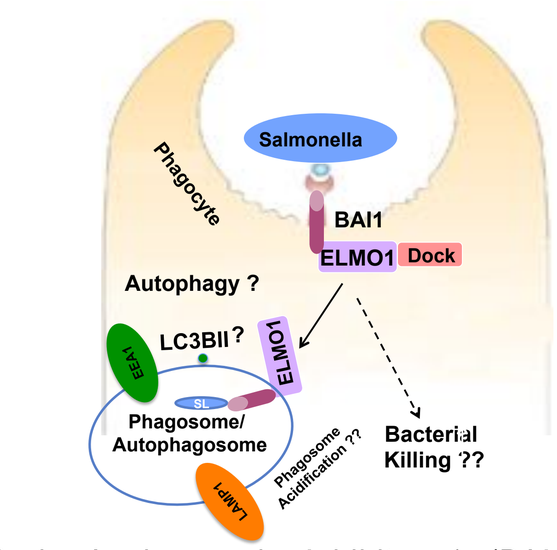
[1]Trident School of Biotech Sciences, TACT, Bhubaneswar, Odisha, India, [2]Division of Comparative Pathology & Medicine, Dept. of Pathology, University of California San Diego, San Diego, CA, USA, [3]Dept. of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA, [4]Dept. of Medicine, University of California San Diego, San Diego, CA, USA

A. ABSTRACT

Macrophages are specialized phagocytic cells involved in clearing invading pathogens by phagolysosomal and autophagic degradation. In an earlier report we showed that Brain Angiogenesis Inhibitor 1 (BAI1) recognizes bacterial lipopolysaccharide (LPS) and by binding to Engulfment and Cell Motility 1 (ELMO1), mediates the clearance of *Salmonella* in macrophages. Here we hypothesize that BAI1-ELMO1 pathway plays a crucial role in bacterial clearance by modulating host cell immune responses. *Salmonella* infection in J774 cells increases accumulation of an autophagic marker LC3B in an ELMO1 dependent manner. Silencing of ATG5 in ELMO1 knockdown cells confirms that ATG5 is essential for ELMO1-mediated LC3B regulation. This result indicates that ELMO1 regulates conventional autophagy. Subcellular fractionation shows that like BAI1, ELMO1 is also present in phagosomes. Furthermore, we show that the lysosomal environment of ELMO1 knockdown cells are more acidic and proteolytic in nature as compared to empty vector-transfected cells. In addition, we confirm that faster recruitment of the Early Endosomal Antigen 1 (EEA1) and the Lysosomal Marker LAMP1 occurs in ELMO1 knockdown cells. These results suggest that there is an interrelationship between autophagic regulation and clearance of pathogens through ELMO1 mediated events. Taken together, we conclude that ELMO1 is an important modulator for the clearance of enteric pathogens by controlling cellular autophagy.

B. BACKGROUND

BAI1-ELMO1 Mediated Phagocytosis

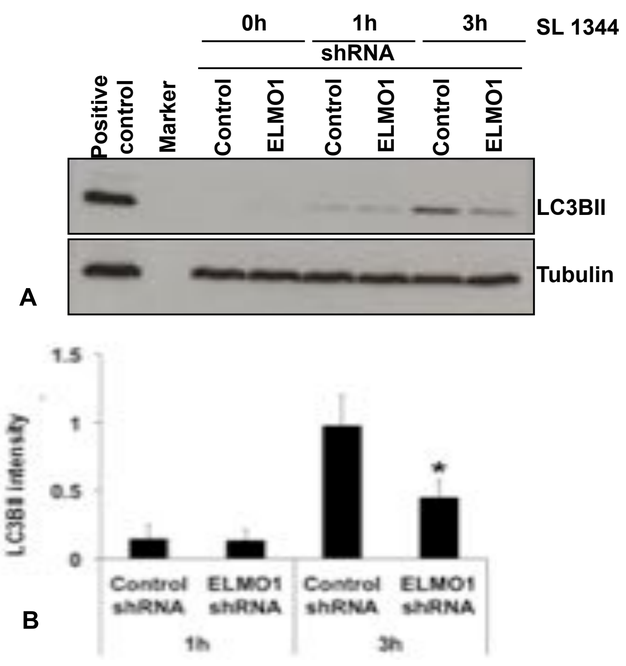


- Brain Angiogenesis Inhibitor 1 (BAI1) is a phagocytic receptor that binds apoptotic cells as well as Gram negative bacteria and engulfs the target through ELMO1 (Engulfment and Cell Motility 1) mediated signaling.
- Macroautophagy clears pathogens as well as apoptotic cells to maintain tissue homeostasis.

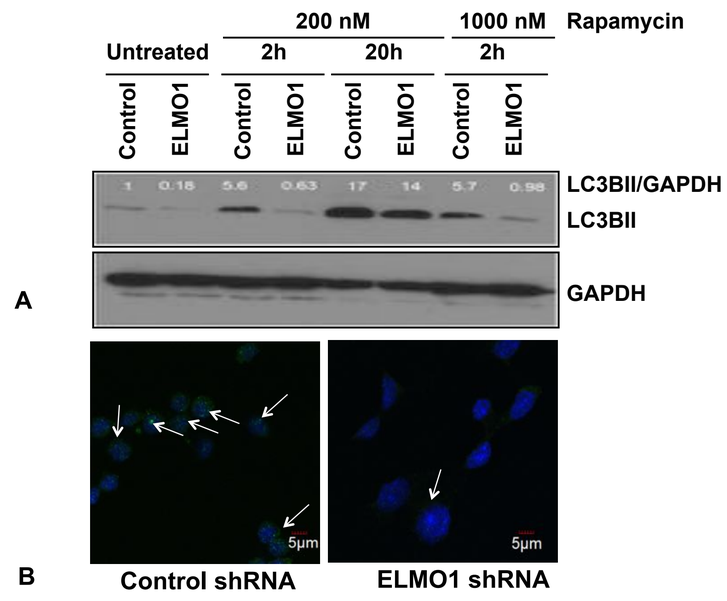
C. AIM OF THE STUDY

To investigate the role of the BAI1/ELMO1 engulfment pathway in the induction of autophagy and role in clearance of pathogens during *Salmonella* infection.

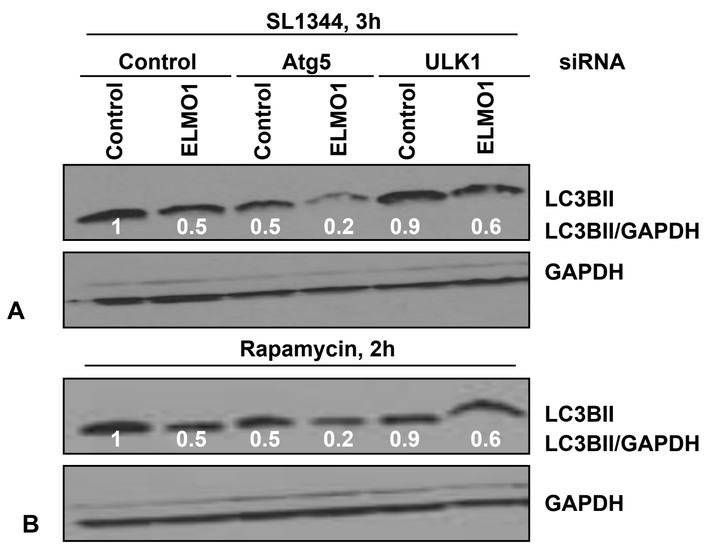
D. RESULTS



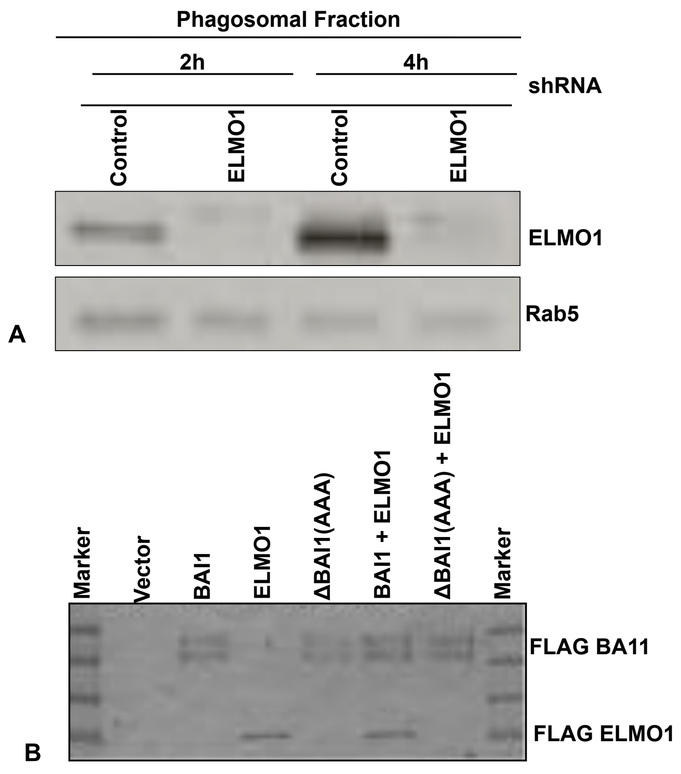
Infection With *Salmonella* induces autophagy in J774 cells in an ELMO1 dependent Manner. (A) Control and ELMO1 shRNA cells were incubated with *Salmonella* (SL1344) for different time points. After the indicated incubation period, cells were washed to remove unphagocytosed bacteria. Cells were then lysed and Western blot analysis was performed using LC3B antibody. Results revealed a significant increase in LC3BII level in control J774 cells compared to ELMO1 shRNA cells at 3 h of time point. (B) The densitometric analysis was shown by the ratio of LC3BII/ β-Actin from 3 independent experiments.



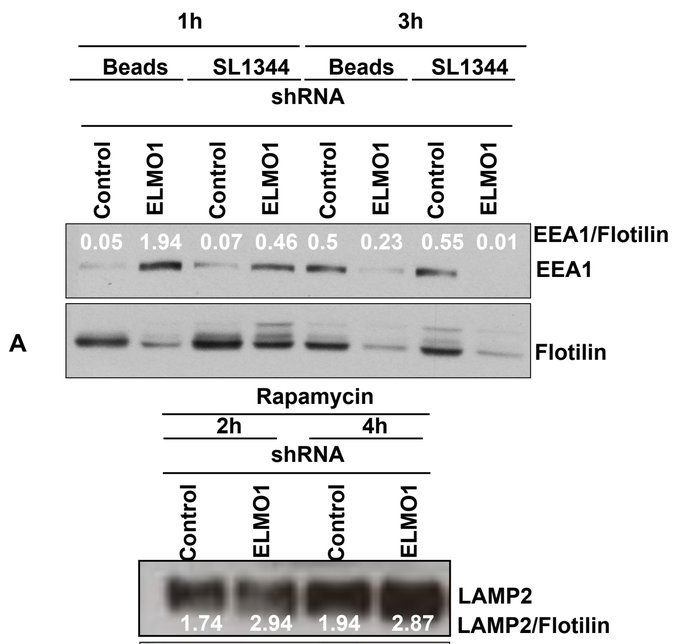
Rapamycin, the mTOR inhibitor induces autophagy in J774 cells in an ELMO1 dependent Manner. (A) Control and ELMO1 shRNA (J774) cells were treated with mTOR inhibitior rapamycin (using two different concentrations of 200 nM or 1000 nM) for indicated time points. Cells were lysed and used for Western blot with LC3B antibody. Control J774 cells showed increased expression of LC3BII form after rapamycin treatment compared to ELMO1 shRNA cells. (B) The same samples from (A) on coverslips were used for confocal microscopy. After 2 h of rapamycin treatment coverslips were fixed, stained with anti-LC3B antibody and Alexa Fluor 488 secondary antibody. Control shRNA cells showed increased number of puncta formation compared to the ELMO1 shRNA cells.



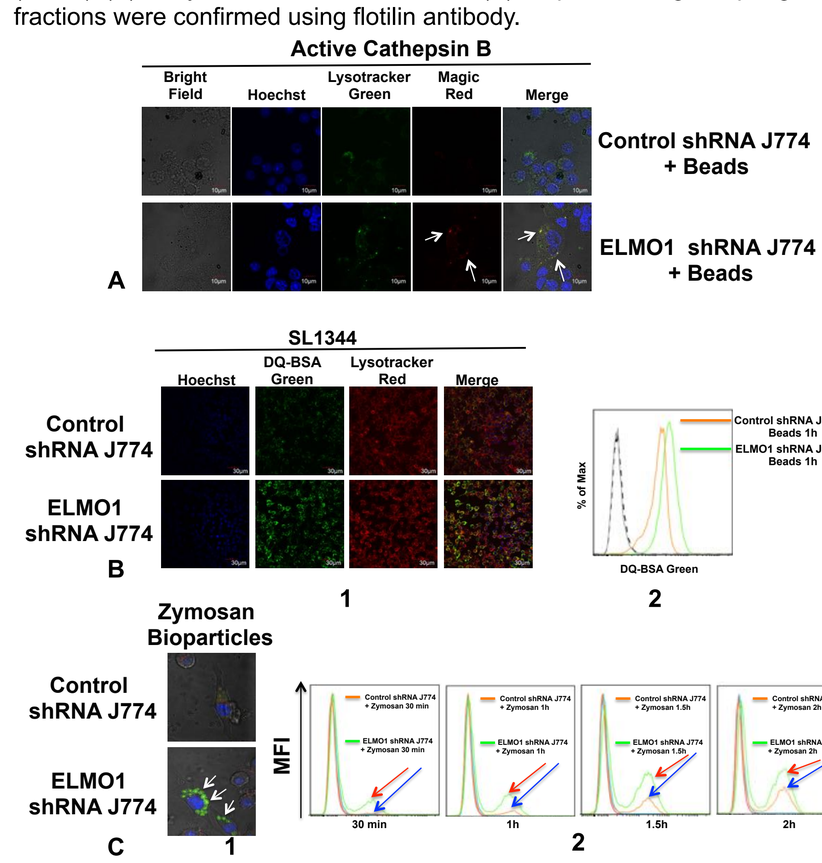
Effect of Atg5 and ULK1 knockdown on LC3BII expression in Control and ELMO1 shRNA cells after *Salmonella* Infection or challenged with Rapamycin. Control and ELMO1 shRNA cells were knockdown for Atg5 or ULK1 using siRNA and then incubated with *Salmonella* (SL1344) (A) or Rapamycin (200nM) (B) for indicated time points. Cells were harvested, lysed and western blot was performed using LC3B antibody. Results revealed decrease in expression of LC3BII when Atg5 and ULK1 was knocked down compared to untreated controls suggest the importance of Atg5 and ULK1 dependent LC3B expression



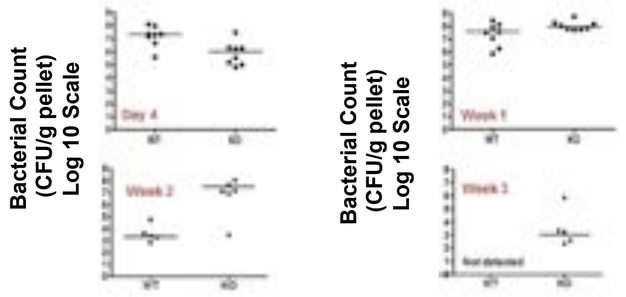
Both BAI1 and ELMO1 were Identified in the Phagosomal Fraction. (A) Control and ELMO1 shRNA cells were first incubated with polystyrene magnetic beads for indicated time points. Cells were harvested and carefully, the membranes were ruptured in ice cold PBS by simply passing the suspension multiple times through a needle and finally phagosomes were isolated with a magnet. Western blots were performed in the phagosomal fraction using ELMO1 antibody (upper panel) and for the phagosomal marker, Rab 5 (lower panel) (B) To identify the presence of BAI1 in the phagosomal fraction, HEK-293 cells were over expressed with FLAG tagged WT BAI, ELMO1 and with Mutant form of BAI1 singly or in combination. Phagosomal fractions were prepared as mentioned in (A) and the Western blot was done with FLAG antibody. The blot showed both BAI1 and ELMO1 were present in the phagosomal fraction however, ELMO1 was completely absent where ELMO1 was overexpressed with mutant form of BAI1.



ELMO1 regulates the recruitments of the endosome/lysosome markers. Control and ELMO1 shRNA J774 cells were incubated with polystyrene magnetic beads for different time points. Excess beads were first washed and phagosomal fractions were separated from cell lysates using magnetic separator, finally immunoblotting were performed for endosomal marker (EEA1) (A) or lysosomal marker LAMP2 (B). Equal loading for phagosomal fractions were confirmed using flotilin antibody.

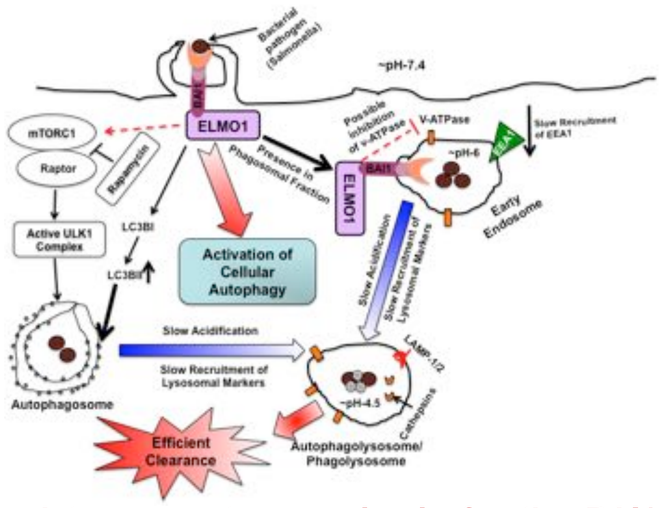


ELMO1 mediated regulation of endosomal proteolysis and phagolysosomal acidification. Control and ELMO1 shRNA J774 cells preloaded with either Lysotracker Green or Red for 2h were incubated with polystyrene bead (A), DQ-BSA (B) or pH rodo Zymosan bio-particles (C) for different time points, washed and subsequently cells were either incubated with magic Red cathepsin B (A) for 30 min and counterstained with hoechst for nucleus or simply left alone just counterstained with hoechst (B) and (C). Fluorescence were detected using confocal microscope, BD FACS calibur or spinning disc live cell microscope



The Role of ELMO1 on Bacterial killing. Control and ELMO1 knockout mice were infected with *Citrobacter spp.* and bacterial burden inside tissue were assessed sacrificing both wild type and infected mice on various time intervals. Graphic representation shows a comparative result of colony count per gram of tissue after various days of incubation actually points out the importance ELMO1 in the clearance of pathogens

E. SUMMARY AND CONCLUSIONS



These data suggest a novel role for the BAI1-ELMO1 engulfment pathway in the induction of autophagy and the clearance of pathogens