Virulence of invasive *Salmonella* Typhimurium ST313 in animal infection models

Ellen Higginson, PhD
Center for Vaccine Development
University of Maryland, Baltimore
Background

• *Salmonella* Typhimurium ST313 strains are commonly isolated from the blood of febrile patients

• 50% of ST313 infections present without diarrhea

• ST313 strains differ genetically from ST19

• Show phenotypic differences in:
  • Motility
  • Biofilm formation
  • Macrophage survival
Do these genotypic and phenotypic differences reflect a difference in virulence?

• Are ST313 strains more invasive than ST19 strains?
• Do ST313 strains induce less diarrhea than ST19 strains?
Virulence in mice

- Determined the 50% lethal dose of strains using:
  - 3 ST313 strains and 3 ST19 strains, all isolated from blood cultures in Mali
  - BALB/c and CD-1 mouse strains
  - Adult and juvenile mice
- No significant difference in LD$_{50}$ between sequence types
Dissemination in mice

- Infected mice perorally with I77 (ST19) or D65 (ST313)
- Sampled spleen, liver and blood at different timepoints
- No difference in organ counts
- Significant difference in blood counts at 24 h post-challenge

P = 0.08

P < 0.001
Rhesus macaque infection model

- Rhesus macaque studies first carried out in 60s-70s (Kent *et al.*, 1966; Rout *et al.* 1974)
- *Salmonella* infected animals had diarrhea, and showed signs of severe intestinal inflammation
- We recently revived this model to investigate vaccine safety in SIV-positive and healthy rhesus macaques (Ault *et al.* 2013)
- Similar symptoms to humans (diarrhea, fever, lethargy, weight loss, intestinal inflammation)
• 6 Indian rhesus macaques (3/group) were challenged intragastrically with I77 (ST19) or D65 (ST313)

• Monitored for 3 weeks for signs of infection including:
  • Clinical signs (temperature, weight loss, WBC count)
  • Bacterial load in stool
  • Stool grade
  • Blood culture
  • Histopathological analysis of organs
  • Bacterial counts in organs
Rhesus macaque infection model

• 6 Indian rhesus macaques (3/group) were challenged intragastrically with I77 (ST19) or D65 (ST313)

• Monitored for 3 weeks for signs of infection including:
  • Clinical signs (temperature, weight loss, WBC count)
    • Bacterial load in stool
    • Stool grade
  • Blood culture
    • Histopathological analysis of organs
  • Bacterial counts in organs
RM infection – stool counts

- Stool counts were done to determine intestinal burden
- ST19 infected animals:
  - shed more bacteria at days 3 and 4 post-challenge
  - shed for a longer period than ST313 infected RM

** P < 0.01
Animals were scored each day for severity of diarrhea:
- 1 – mild
- 2 – moderate
- 3 – severe

ST19 infected RM had more severe diarrhea than ST313 infected RM.

No ST313 infected RM had moderate or severe diarrhea.
RM infection - histology

- Histology was done on organs at necropsy
- Higher levels of inflammation seen in the organs of ST19 infected rhesus macaques
Summary of RM data

• ST19 strain I77 induced significant levels of diarrhea in infected animals

• ST313 strain D65:
  • Caused less diarrhea
  • Showed decreased colonization of the intestines and reduced shedding
  • Induced less histopathology in organs (liver, colon, ileum and MLN)
Conclusions

• The mouse model is limited in its ability to model virulence of *Salmonella* Typhimurium ST313

• Rhesus macaques are a good model for *Salmonella* Typhimurium induced gastroenteritis

• As postulated from epidemiological studies, ST313 strains are less able to induce diarrhea in a RM model of infection

• Whether or not ST313 are inherently more invasive will require further analysis in the RM model using earlier timepoints
Acknowledgements

- Dr Girish Ramachandran
- Dr Aruna Panda
- Dr Sharon Tennant

Funding:
Center for Excellence in Translational Research - NIH

- Clinical Microbiology
  - Jasnehta Permala-Booth
  - Sunil Sen

- Comparative Medicine
  - Dr Louis DeTolla
  - Dr Eugene Ateh
  - Dr Michael Lipsky