Expression of Antimicrobial Ribonucleases During Peritonitis

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Nationwide Children's
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Riley Hospital for Children
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Antimicrobial Peptides (AMPs)

- Evolutionarily conserved component of the innate immune system
- Cationic proteins in the range of 3-15 kDa
- Antimicrobial activity against Gram-positive, Gram-negative, and fungal organisms
  - direct bactericidal activity
  - chemotaxis of other leukocytes
  - activate toll-like receptors
  - opsonization of bacteria
  - deplete nutrients of bacteria
- Expressed at all mucosal surfaces

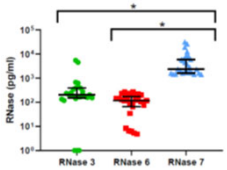
Peritoneal Dialysis

- Peritonitis
  - Most common reason for dialysis modality change
  - Frequent cause of hospitalization
  - Risk of mortality
- Do RNases play a role in the development of peritonitis?

Methods

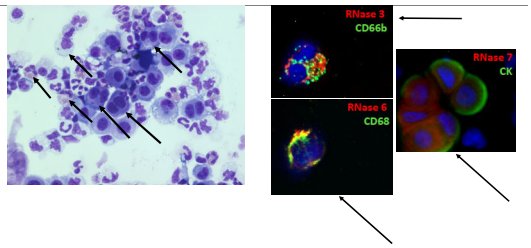
- 21 adults (age 41-82 years) receiving CAPD
- 6 children (age 3 mo-21 years) receiving CCPD
- Stable and without peritonitis for 1 month prior to sample collection
- Peritoneal Fluid Samples
  - Cell-free peritoneal effluent
  - Collected after ≥ 8 hour dwell

Protein Expression

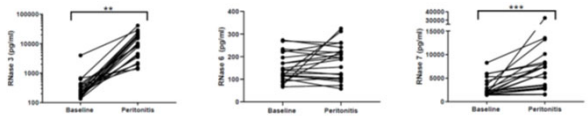


*Adjusted p<0.0001, Kruskal-Wallis Test with Dunn's correction for multiple comparison

RNases Localize to Cells in Peritoneal Effluent



RNase 3 & 7 Increase in Peritonitis



N = 22 patients (21 adult and 1 pediatric patient)

** p<0.0001, Wilcoxon matched-pairs signed rank test
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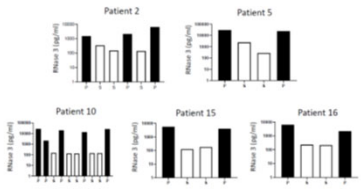
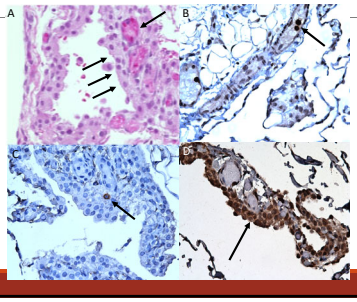
RNase 3 increases in repeated peritonitis episodes



Localization of RNases in Omentum (uninfected)

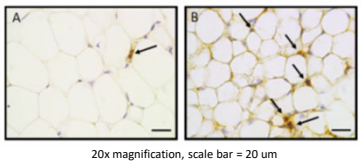


RNase 3

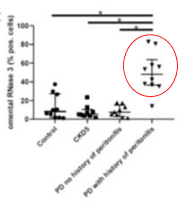
RNase 6

RNase 7

Impact of peritonitis on RNase 3 expression in omentum



20x magnification, scale bar = 20 um

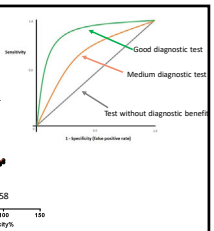


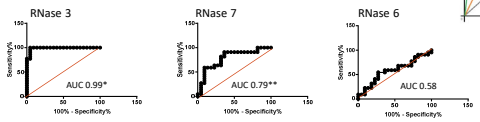
*Adjusted p<0.0001, Kruskal-Wallis Test with Dunn's correction for multiple comparison; 9-10 patients/group

Conclusions

- RNases (specifically RNase 3, 6, and 7) are present in peritoneal effluent
- RNases are localized to specific cells in the peritoneal effluent and omentum
- RNase 7 comes from mesothelial cells, implicating the peritoneal mesothelial cells involvement in innate immunity
- Omentum is a reservoir for RNase 3-positive cells, which increase after a peritonitis episode

Future Directions

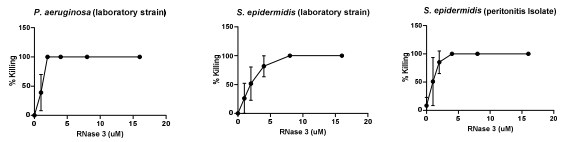




Receiver-operator curves were generated for each RNase and the area under the ROC curve (AUC) was determined. * $p < 0.0001$ and ** $p = 0.0012$.

- RNase 3 and 7 may be potential sensitive and early biomarkers for peritonitis
- In combination with other biomarkers, RNase expression maybe be helpful in identifying a true underlying cause of culture-negative peritonitis

Future Directions



Recombinant N-terminal RNase 3 peptide kills bacteria in a dose dependent manner. The average +/- SEM from 3 independent experiments is shown. In each experiment, the indicated concentration of peptide or PBS carrier is incubated with 12,500 CFU of the indicated bacteria for 90 minutes at 37C in a total volume of 25 ul, then plated on LB agar and cultured overnight. Colonies are enumerated the following day.

- Could AMPs like RNase 3 help prevent localized infections?

Future Directions

- Are prophylactic omentectomies increasing our pediatric patients' risk to develop peritonitis?
- Could the long-term exposure of RNase 3 to omentum lead to peritoneal membrane fibrosis?
  - Development of a peritonitis model in mice could help to better study this

Photo provided by Beth Smith, RN

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