The Function of NGAL-Positive Neutrophils in AKI

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Background

- Acute Kidney Injury (AKI) is abrupt loss of renal function that develops in under 48 hours, commonly due to ischemia.
- There is no cure for AKI, and should the damage be irreparable in both kidneys, renal replacement therapy (a kidney transplant) is necessary.
- Neutrophils are known to aid in the repair process by fighting off infection.¹
- Neutrophil Gelatinase-associated Lipocalin (NGAL) is sometimes expressed in neutrophils recruited to repair an injured kidney, and is known to sequester iron, thus limiting bacterial growth.²

Hypothesis:
NGAL-positive neutrophils will uptake iron, limiting bacterial growth and thus inflammation that would cause further damage to the kidney. They will be most prevalent during the initial stages of injury, then gradually begin leaving the site of injury. We also theorize that KIM-1 uptakes NGAL after it has sequestered iron, disposing of the harmful iron.

Specific Aims:
1. To determine the role of NGAL-positive neutrophils in the post-ischemic kidney.
2. To explore the prevalence of NGAL-positive neutrophils under many different conditions, including acute ischemia, chronic injury, and diabetes at various time points.
3. To explore the relationship between NGAL and KIM-1, and established biomarker of AKI, in the post-ischemic kidney.

Methods:

- Immunofluoroscopy
  - We stained 8 micron kidney sections, harvested at 24-, 48-, and 72-hours after ischemia-reperfusion injury. We then stained them for NGAL, Ly6G, and KIM-1. We looked at the staining data under the microscope at 40x resolution and counted the number of positive regions for the various proteins.

- Real Time qPCR
  - We used cDNA harvested from mice at 24-, 48-, and 72-hours after ischemia-repufusion injury and prepared a qPCR tray with the samples. We then observed the relative amount of gene expression calculated by comparing expression of KIM-1 and Interleukins 6, 8, 10, 17, 23 with the control being 18s, which marks all the RNA in the sample.

Data and Results:

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  - Non-Functional KIM-1
  - Functional KIM-1

  ![Images were captured on tissues harvested 24 hours post-ischemic injury. The tissue with non-functional KIM-1 (left) shows little colocalization of NGAL (green) and KIM-1 (red), whereas the tissue on the right, with functional KIM-1 protein, shows extensive colocalization of the two proteins.]

Future Directions and Applications:
- **Future Directions**
  - This study focused solely on the NGAL protein and a select number of related proteins. Going forward, more proteins in the post-ischemic kidney will be explored to help construct a more complete picture of the renal repair process.
- **Applications**
  - Genetic engineers can induce the up- and down-regulation of NGAL and its related proteins to promote fibrosis of the kidney after AKI, resulting in a functional kidney.

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Literature Cited: