Perinatal exposure to Interleukin-6 (IL-6): a model to study influence of developmental insult on susceptibility to chronic kidney disease (CKD)

Tarak Srivastava
Robert E. Garola
Varun Chandra Boinpelly
Jianping Zhou
Daniel P. Heruth

See next page for additional authors
Authors
Chronic kidney disease is characterized by inflammation and proteinuria, decreased GFR, glomerular immunostaining following reduction mature systemic inflammation is associated with increased tumor necrosis factor IL levels indicates injury to the glomerular filtration barrier which precedes the onset of proteinuria in vitro growth (p<0.05) of LC in an 8 hour dose dependent manner. Significant increase was noted by IL 6 (0.01 ng/mL) concentration with maximal effect at 1 ng/mL. Pre-treatment with anti-IL-6 antibody completely blocked the increase in Pa (p=0.05).

IL-6 crosses the placental barrier during fetal development.
- Systemic inflammation is associated with increased tumor necrosis factor-α, IL-1, IL-6, interferon-γ, etc. Each of these cytokines plays an important role in the inflammatory response. However, only IL-6 can cross the placental barrier and enter the fetal compartment.
- Mother-to-fetus transfer of IL-6 makes it a unique surrogate to study gestational inflammation.

IL-6 is a prototypic pleiotropic cytokine.
- The IL-6 receptor is a member of the Cys Leu cytokine receptor super family. It consists of two transmembrane glycoproteins (IL-6Rα and gp130). IL-6 first binds with IL-6Rα and the complex is presented to gp130 resulting in signal activation.
- IL-6 modulates signaling through the JAK-STAT, PI3K-Akt, Raf-MEK-ERK cascades. PI3K-Akt and ERK signaling are required for renal organogenesis and adult renal function.

DNA methylation revealed increased Sphingomyelocystine
- LC-MS showed increased 5-methylcytosine but not 5-hydroxymethylcytosine suggestive of increased differentiated state.
- Representative LC chromatograms of 5-methyl-2-deoxycytidine (5mC) and 5-hydroxymethyl-2-deoxycytidine (5hmC) imprint methylation in fetal kidney DNA.

IL-6 increased glomerular albumin permeability (P_a) of the glomerular filtration barrier
- Increased P_a indicates injury to the glomerular filtration barrier which precedes the onset of proteinuria in chronic kidney disease.
- Isolated rat glomeruli were treated with IL-6 and change in P_a was determined using video microscopy. IL-6 increased P_a in a dose-dependent manner. Significant increase was noted by IL-6 at 0.01 ng/mL.

IL-6 caused actin filament rearrangement in podocytes
- Immunolabeled mouse podocytes were incubated with IL-6 for 1hr and the actin filaments were stained with Rhodamine-conjugated phalloidin. Images were obtained using a Leica TCS SPE confocal system.
- IL-6 caused actin filament rearrangement leading to the formation of a cortical ring. The more conspicuous finding was a dose-dependent appearance of “actin dots”. Actin dots represent focal adhesions that suggest actin bundling next to the plasma membrane.

Western blotting showed IL-6 upregulated Jak2/Stat3 in both fetal kidney and podocyte

Results

Background
Chronic kidney disease and obesity is characterized by inflammatory pathophysiology.
- Chronic kidney disease is characterized by inflammation and proteinuria, decreased GFR, glomerular sclerosis and progressive loss of renal function.
- Obesity is associated with subclinical inflammation and increased levels of circulating CRP, and IL-6.

Maternal obesity and kidney outcome in their children in humans.
- Epidemiological studies have shown maternal obesity during gestation is associated with small-for-birth-weight infants in the newborn, susceptibility to CKD, and congenital anomalies of the kidney and urinary tract (CANTU).
- Serum IL-6 is elevated in pregnant obese women. Serum IL-6 levels are 1.5- to 2-fold higher in pregnant obese women compared to non-obese pregnant women.

Maternal systemic inflammation causes developmental changes and susceptibility to chronic kidney disease in the offspring.

Objectives
(a) To examine the effect of injection of IL-6 on kidney development during mid-gestation, similar to levels observed in pregnant obese women, as a specific molecular surrogate of gestational inflammation.
(b) To examine the effect of IL-6 on glomerular filtration barrier.

Methods
- Pregnant C57BL/6 mice received either normal saline or IL-6 (10 pg/g BW) intraperitoneally on alternate days from E12.5. Fetal kidneys (E20.5) were removed from uterine horns by dissection and fixed in 10% formalin.
- OCT or used to isolate DNA, RNA and protein lysate for the following studies:
  - Histomorphometry and immunostaining for podocin and podocalyxin was performed.
  - LC-MS analysis of hydrolyzed DNA was performed to evaluate for methylation changes.
  - qRT-PCR for cell cycle and apoptosis genes.
  - Western blotting for JAK-STAT signaling.

- We used in vitro albumin permeability assay and cultured mouse podocytes to study the effect of IL-6 on filtration barrier and actin cytoskeleton of the podocyte respectively.

Perinatal exposure to Interleukin-6 (IL-6): A model to study influence of developmental insult on susceptibility to chronic kidney disease (CKD)

1. Perinatal exposure to IL-6, a surrogate for maternal inflammation, results in accelerated maturation of the kidney resulting in low birth weight and small kidney.
2. IL-6 causes injury to the glomerular filtration barrier and podocyte.
3. IL-6 upregulates the Jak2/STAT3 pathway.

The IL-6 mouse model will allow us to study the effects of developmental programming of maternal obesity on the long-term consequences in adulthood of the progeny.

Summary & Conclusion

Acknowledgements
1. Kierzemski Family Charitable Trust
2. Patton Trust Grant from Kansas City Area Life Sciences