Cell-free DNA release during programmed cell death in ischemia reperfusion injury

Transplantation is invariably associated with acute allograft injury caused by ischemia reperfusion injury (IRI). This injury causes cells of the allograft to undergo various forms of programmed cell death including apoptosis and necroptosis. During programmed cell death, immunogenic molecules are released from cells, one of which is cell-free DNA (cfDNA). We hypothesize that cfDNA is released by microvascular endothelial cells (MVECs) during programmed cell death of IRI and that cfDNA acts as both a biomarker for cellular injury as well as a biologically active molecule capable of amplifying inflammation and organ injury.

Our results indicate that cfDNA is released by MVECs under both apoptotic and necroptotic conditions *in vitro*, as well as during IRI in an *in vivo* mouse model. We have also shown that cfDNA release is ameliorated by blocking necroptosis *in vivo* with the use of RIPK3$^{-/-}$ mice that are incapable of undergoing necroptosis. Lastly, we have shown that cfDNA is capable of activating immune cells, showing that NK cell activation markers are upregulated when purified NK cells are subjected to cfDNA *in vitro*.

Our results indicate that cfDNA is a potential biomarker of allograft injury in a renal transplant setting. Donor-derived cfDNA from blood or urine may give rise to novel non-invasive tests to diagnose graft damage. cfDNA also appears to exacerbate inflammation by activating immune cells to produce pro-inflammatory cytokines which further escalates inflammation. It may be prudent to inhibit the release of cfDNA in a transplant scenario, a goal our lab is currently working towards.