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In vitro models of AKI reveal cell injury specific cytokine responses

Dr. Giulio Podda[1], Dr. Mark Dockrell[1]
[1]*South West Thames Institute for Renal Research, London, United Kingdom*

Background: Acute Kidney Injury (AKI) is a common life threatening condition(1). The nature and severity of the renal injury determine the progression from AKI to CKD.
The Proximal tubule epithelial cell (PTEC) is one of the main targets of AKI and the precise outcome of the injury therefore may depend on the PTEC response(2-3).
The role of TGF-β in renal fibrosis is well known but there is increasing evidence for a role of novel cytokines such as IL-18 and IL-15. However there is no significant data about their expression in PTEC in early AKI. We established three different in vitro model of AKI (septic, Ischaemia-reperfusion injury(IRI) and aminoglycoside toxicity) with the aim of observing the immediate cellular response and subsequently test the hypothesis that IL-15 and IL-18 are involved in determining the outcomes of AKI.

Methods: Primary human PTEC expressing GGT were cultured on collagen IV and cellular fibronectin. Aspects of IRI were reproduced by treatment with NaN3 for 60 min followed by incubation in 17 mMol D-Glucose. For aminoglycoside toxicity cells were incubated with 1 mMol gentamicin. Lipopolysacharides (LPS) treatment was used as the septic model. Cells were treated for 18 and 48 hours followed by RNA extraction and their media collected. IL-18, IL-15 and TGF-β expression were measured by Real-time QPCR. We analysed secreted NGAL and NAG as markers of degree of injury.

Result: Significantly higher NAG activity was detected in NaN3, LPS and gentamicin models of AKI compared to control (p=0.0014, 0.0125 and 0.0028), with no difference between the models. All NGAL results were below the level of detection. Results from the LPS treatment at 18hr tended to be variable with no obvious pattern despite consistent NAG results. There was no significant change in TGF-β expression in any of the models.
In the collagen samples, at 18 hr, both gentamicin and NaN3 reduced the expression of IL-18. At 48 hours IL-18 in gerntamicin treated cells had returned to basal levels but remained suppressed with NaN3 treatment. The expression of IL-18 was significantly increased with LPS. When cells were grown on fibronectin IL-18 expression at 18 hr was similar to that seen in cells on collagen (above), however at 48 hours there was a tendency for a rapid increase with NaN3 and conversely no changes with gentamicin. The IL-18 response to LPS was similar under both conditions.
Regarding IL-15, in the collagen samples, NaN3 induced no change at 18 hr but significant elevation at 48 hr. Whereas gentamicin caused suppression of IL-15 at 18 hr through to 48hr. In cells grown on fibronectin both gentamicin and NaN3 suppressed IL-15 expression at 18 and 48 hours. LPS induced a transient increase in IL-15.

Conclusion: Our pilot data indicates that the cytokine responses of renal epithelial cells to AKI of equal severity varies significantly depending on the nature of the injury. The relative expression of key opposing mediators such as IL-15 and IL-18 may influence the nature of the renal recovery.