

## **The effect of Remote Ischaemic Preconditioning prior to hepatic ischaemia reperfusion injury on CD4+ T cell cytokine production.**

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The lack of Ischaemia Reperfusion (IR) injury susceptibility in mice lacking CD4+ T cells identifies them as a key driver of this pathological process. Remote Ischaemic Preconditioning (RIPC) has been shown to ameliorate liver warm IR injury. The mechanism remains unclear. Whether RIPC alters CD4+ T cell cytokine function in the early period following IR injury remains to be elucidated.

### **Methods**

An established mouse liver lobar warm IR model was used with limb RIPC using 24 male B16 mice which were divided into 4 groups.

1: sham laparotomy (3 hours)

2: 3 cycles of 5 mins of RIPC (left femoral pedicle) followed by sham laparotomy (3 hours)

3: 45 minute warm hepatic IR injury (left and middle lobes) followed by 2 hours reperfusion

4: 3 cycles of 5 mins of RIPC (left femoral pedicle) followed by 45 minute warm hepatic IR injury (left and middle lobes) followed by 2 hours reperfusion

At the end of the experiment the animals were terminated, the livers were immediately harvested and intrahepatic lymphocytes were isolated and cultured in Brefeldin A for 4 hours prior to analysis by flow cytometry. CD4 T cell production of the pro inflammatory cytokines IL-6, IL-17A, IFN $\gamma$ , and TNF $\alpha$  were measured by intra-cellular staining and flow cytometry and was compared between the groups.

### **Results**

IFN $\gamma$  production by CD4+ T cells was significantly raised following IR injury (groups 1 vs 3,  $p=0.02$ ) however RIPC did not significantly reduce IFN $\gamma$  production by CD4+ T cells (groups 3 vs 4,  $p=0.57$ ). Although TNF $\alpha$  production increased significantly in other cell types, TNF $\alpha$  production by CD4+ T cells was not significantly raised following IR injury (groups 1 vs 3,  $p=0.2$ ). IL-6 and IL-17A production was minimal in all groups.

### **Conclusions**

IFN $\gamma$  is the primary cytokine released by CD4+ T cells following IR injury. RIPC does not alter CD4+ T cells cytokine production following hepatic IR injury.