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

F. Robertson3, V. Male2, A. Cuff2, L. Magill3, G. Wright1, B. Fuller3, B. Davidson3

1Department of Immunology, Edinburgh Napier University, Edinburgh 2Department of Infection and Transplantation 3Department of Surgery and Interventional Science, University College London, London, United Kingdom

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 Bl6 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, IFNγ, and TNFα were measured by intra-cellular staining and flow cytometry and was compared between the groups.

Results
IFNγ production by CD4+ T cells was significantly raised following IR injury (groups 1 vs 3, p=0.02) however RIPC did not significantly reduce IFNγ production by CD4+ T cells (groups 3 vs 4, p=0.57). Although TNFα production increased significantly in other cell types, TNFα 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
IFNγ 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.