ImmunoTools *special* Award 2014

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In situ hypothermic perfusion during right-sided liver resection

Liver surgery is often the only curative option for patients diagnosed with a primary or secondary hepatic tumor. Although liver resection is currently performed with an acceptable mortality rate, severe postoperative complications still occur in about 30% of patients that undergo a major liver resection (i.e., resection of ≥3 out of 8 anatomical liver segments). Because the liver is a highly vascularized organ, transection of the hepatic parenchyma often coincides with a considerable amount of blood loss and the consequent need for blood transfusion, both important predictors of postoperative outcomes. Major liver resection is therefore often performed under vascular inflow occlusion (VIO), a surgical technique that entails clamping of the afferent blood vessels (i.e., portal vein and hepatic artery proper) in order to reduce blood loss during parenchymal transection. However, while VIO is an effective means to curtail blood loss during such operations, it also induces an inevitable side effect in the form of hepatic ischemia-reperfusion (IR) injury.

Hepatic IR injury, which results from the temporary deprivation and subsequent restoration of blood flow, clinically manifests itself as liver damage with a consequent reduction in liver function that, in most severe cases, culminates in acute liver failure. Although these clinical effects of IR injury generally do not emerge until 6-24 hours after the operation, the underlying pathological processes are triggered as a result of the ischemic insult. During ischemia, the lack of oxygen halts oxidative phosphorylation in mitochondria, which, in combination with on-going metabolic processes, gradually depletes cellular energy (ATP) stores. In addition, large amount of reactive oxygen and nitrogen species (ROS/RNS) are produced in the mitochondria during early reperfusion. ROS/RNS are potent inducers of mitochondrial permeability transition that, in combination with ATP depletion, culminates in hepatocyte necrosis and hence the release of damage-associated molecular patterns (DAMPs) into the extracellular space. Hepatocyte-derived DAMPs activate Kupffer cells, the liver's resident macrophages, thereby eliciting the

generation of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8. These cytokines subsequently attract neutrophils to the liver, which produce large amounts of detrimental ROS/RNS and proteases. These inflammatory processes ultimately result in the massive hepatocyte necrosis that characterizes IR injury and which, most importantly, significantly hampers regeneration of the liver remnant. Accordingly, interventions that alleviate IR injury are warranted and, since hypothermia is an established means to preserve organ grafts in the transplantation setting, its *in situ* application is also thought to be effective in protecting the liver during VIO.

Therefore, we are currently investigating a new method for *in situ* hypothermic perfusion (IHP) in patients as a possible means to reduce IR injury following right-sided major liver resection performed under VIO (Reiniers *et al.*, J Am Coll Surg 2013, Epub ahead of print). During the operation, participants of this study are randomized between an intervention (i.e., VIO with IHP) and a control group (i.e., VIO alone). When participants are allocated to the IHP group, their liver is cooled to a temperature of 28°C during parenchymal transection by flushing the organ with a chilled crystalloid perfusion solution (i.e., lactated Ringer's solution). Blood and liver tissue samples are obtained from all participants at different time points before and after the surgery so as to determine the putative protective effect of IHP and to elucidate its working mechanism.

The ImmunoTools Award would allow us to investigate the effect of IHP on the inflammatory response that underlies IR injury. Specifically, the human IL-6, IL-8, and TNF-α ELISA sets will enable us to determine the levels of these cytokines in plasma following VIO with or without IHP. Data obtained with these assays could contribute to our understanding of the mechanisms that underlie hepatocellular protection by IHP, since these cytokines are important factors in the pathophysiology of IR injury. If we were to be granted the ImmunoTools Award we would like to receive our 15 reagents in the form of five human IL-6, IL-8, and TNF-α ELISA sets, which will allow us to analyse our complete study cohort for these cytokines.

ImmunoTools *special* AWARD for **Megan Reiniers** includes 18 reagents

recombinant human cytokines rh TNF-alpha

human IL-4 ELISA-set, human IL-6 ELISA-set, human IL-8 ELISA-set, human IL-12p40 ELISA-set, human TNF-alpha ELISA-set,

recombinant mouse cytokines rm G-CSF, rm GM-CSF, rm GRO-b/CXCL2, rm IFN-gamma, rm IL-1beta, rm IL-6, rm IL-10, rm IL-17A, rm IP-10/CXCL10, rm MCP1/CCL2, rm RANTES/CCL5, and rm TNF-alpha

DETAILS