1485

# Hepatic Nitric Oxide Generation as a Putative Mechanism for Failure of Intrahepatic Islet Cell Grafts

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PRIMARY nonfunction of islet allografts and chronic failure of intrahepatic islet autografts has been observed in large animals and humans, bringing into question the liver as the ideal site for islet transplantation. Our hypothesis is that inflammatory mediators released in the microenvironment at the transplant site might result in impaired islet engraftment. The L-arginine–dependent nitric oxide (NO) pathway, which is known to inhibit rodent islet primary function,[1] is a putative cytotoxic mediator.

## METHODS AND RESULTS

Human HC and human islets were isolated by collagenase digestion.[2,3] After 24 hours in culture, the tissue combinations of hepatocytes and islets were stimulated with or without a mixture of inflammatory cytokines and LPS (CM = IL-1, TNF-$\alpha$, IFN-$\gamma$, and LPS). In addition, the tissue combinations were exposed to $N^G$ monomethyl-L-arginine (NMA) a competitive inhibitor of the NO radical formation. Culture supernatants were tested 24 hours later for nitrite ($NO_2^-$) and nitrate ($NO_3^-$) as described.[2]

Human hepatocytes, islets, or the combination produced less than 5 $\mu$mol/L $NO_2^- + NO_3^-$ unless stimulated by CM. Even when stimulated by CM, human islets generated only low amounts of $NO_2^-$ and $NO_3^-$ (<8 $\mu$mol/L). However, HC cultured alone or with islets synthesize larger amounts of NO than HC or islets cultured alone (28 to 35 $\mu$mol/L). NMA inhibited $NO_2^-$ and $NO_3^-$ production. Thus, human liver cells can produce potentially islet-damaging amounts of nitric oxide. When we incubated exocrine pancreatic tissue with CM, only very small amounts of NO-generation was detected in supernatants, suggesting that the pancreas might be an alternative transplantation site for islets.

## REFERENCES

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Supported in part by the Juvenile Diabetes Foundation International, Grant No. 191142.

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0041-1345/92/$3.00/+0