**Asparaginase-Associated Pancreatic Injury is Related to Pancreatic Levels of the Endogenous Counteracting Enzyme Asparagine Synthetase and Intra-Pancreatic Nutrient Stress**

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**Background:** The anti-leukemic drug asparaginase causes pancreatitis in about 10% of users, and the mechanisms underlying this painful complication are not known. Asparaginase primarily depletes circulating asparagine (Asn), and the endogenously expressed enzyme, asparagine synthetase (ASNS), replenishes Asn. In this study, we explored the role of ASNS in the pancreas as a mechanism for preventing pancreatic damage due to asparaginase.

**Methods and Results:** We began by determining the expression pattern of ASNS in the pancreas. Compared to several other organs, ASNS was most highly expressed in both the human and mouse pancreas, and, within the pancreas, ASNS was primarily present in the acinar cells. High baseline pancreatic ASNS levels were associated with higher baseline activation of PERK signaling in the pancreas, and inhibition of PERK in acinar cells lessened ASNS expression. Asparaginase exposure, but not the common pancreatitis triggers, uniquely upregulated ASNS expression, indicating that the increase is mediated by nutrient stress. Knockdown of ASNS in the 266-6 acinar cells provoked acinar cell injury and worsened the damage observed with asparaginase, while forced ASNS overexpression protected against asparaginase-induced injury. We generated inducible ASNS knockouts and found that, within two weeks of inducing ASNS deletion in adult mice, there was spontaneous pancreatic injury. The mechanism for this *in vivo* effect appears to be through Asn depletion, since an Asn-deficient diet, along with asparaginase exposure, led to a similar change. We are in the process of evaluating the effect of rescuing Asn loss due to ASNS deficiency.

**Conclusion:** ASNS is highly expressed in the pancreatic acinar cells through heightened basal activation of PERK, and ASNS appears to be crucial to maintaining acinar cell integrity. The implications are that ASNS is especially hardwired in the pancreas to protect against both baseline perturbations and with Asn depletion due to asparaginase.