INSIDE LAB INVEST

Laboratory Investigation (2005) **85**, 1183–1186.
doi:10.1038/labinvest.3700336

EPEC: pecking away at the tight junction

Enteropathogenic *Escherichia coli* (EPEC) is an important human gastrointestinal pathogen that causes significant pediatric morbidity and mortality in developing countries. Current understanding of EPEC infection suggests that this noninvasive organism causes disease after attaching to colonic epithelium, to produce the classic attaching and effacing lesions. Although typical enterotoxins are not secreted, EPEC does inject bacterial products into epithelial cells via a type III secretion system. These injected proteins initiate multiple signal transduction cascades that result in alterations in ion transport and reduced intestinal barrier function. These in turn are thought to combine to cause the watery diarrhea that is typical of EPEC infection. Studies of EPEC pathogenesis have primarily been performed *in vitro* using cultured cells due to the lack of *in vivo* models. *In vitro* studies have shown that the *E. coli* secreted protein F (EspF), which is injected via the type III secretion system, is required for EPEC-induced barrier dysfunction. These observations have not, however, been validated *in vivo*.

Savkovic *et al*¹ recently described a mouse model of EPEC infection. In this issue of *Lab Invest*, Shifflett *et al*² (p. 1308) report studies using this *in vivo* model to define the role of EspF in EPEC disease pathogenesis. They first demonstrate ileal and colonic barrier dysfunction within 1 day of colonization with wild-type EPEC. Since the tight junction is the primary determinant of intestinal epithelial barrier function,³ the authors continue by analyzing the distribution of occludin, a transmembrane protein localized to the tight junction. The data show that occludin removal from the tight junction into cytoplasmic vesicles correlates with barrier dysfunction. In contrast, animals infected for 1 day with Δ espF EPEC were similar to uninfected control mice; neither barrier function nor occludin localization was altered. Surprisingly, both barrier dysfunction and occludin distribution were altered 5 days after Δ espF EPEC infection. At this later time point of infection, mice infected with Δ espF EPEC were indistinguishable from those infected with wild-type EPEC. In light of this unexpected observation, the authors theorized that non-bacterial factors, such as cytokine-induced inflammatory processes, may have caused the structural and functional tight junction disruption observed with longer durations of infection. They consequently

assessed expression of tumor necrosis factor α (TNF α), which is associated with *in vitro* and *in vivo* intestinal epithelial barrier dysfunction.³ Mucosal TNF α levels were elevated 5 days, but not 1 day, postinfection in both Δ espF- and wild-type EPEC-infected mice.

This study therefore emphasizes the importance of an animal model of EPEC infection. *In vitro* systems, although critical for our understanding of the nature of bacterial disease, cannot fully recapitulate the conversation that occurs between pathogens and mucosal surfaces. This study both supports *in vitro* conclusions regarding EspF function in early stages of EPEC infection and also suggests that host inflammatory responses elicited by this organism are involved in chronic infection. Thus, in addition to informing us about the complexities of EPEC pathogenesis *in vivo*, this study underscores the power of combining *in vitro* and *in vivo* studies.

Erika A Sullivan

References

- 1 Savkovic SD, Villanueva J, Turner JR, *et al*. Mouse model of enteropathogenic *Escherichia coli* infection. *Infect Immun* 2005;73:1161–1170.
- 2 Shifflett DE, Clayburgh DR, Koutsouris A, *et al*. Enteropathogenic *E. coli* disrupts tight junction barrier function and structure *in vivo*. *Lab Invest* 2005;85:1308–1324.
- 3 Clayburgh DR, Shen L, Turner JR. A porous defense: the leaky epithelial barrier in intestinal disease. *Lab Invest* 2004;84:282–291.

Islands in the storm: pancreatic fibrosis and islet preservation in chronic pancreatitis

Obliteration of functional tissue and deposition of extracellular matrix in the parenchyma of solid organs is a central theme in the development of end-stage organ failure. In the case of chronic liver disease, activation of normally quiescent hepatic stellate cells leads to their proliferation and acquisition of a myofibroblast phenotype, with deposition of collagen and other extracellular matrix components in the subsinusoidal space of Disse. The pancreas also contains stellate cells, and increasing attention is being given to the role that these cells play in the development of chronic pancreatitis. A major breakthrough in elucidating the biology of hepatic stellate cells was the establishment of a stable *in vitro* cell line.

In the current issue of *Lab Invest*, **Jesnowski et al**¹ (p. 1276) now report immortalization and characterization of a human pancreatic stellate cell line, RLT-PSC. Primary pancreatic stellate cells were isolated by an outgrowth method, and were immortalized by transfection with SV40 large T antigen and human telomerase (hTERT). RLT-PSC cells retained the phenotype of activated pancreatic stellate cells, as demonstrated by their expression of α -smooth muscle actin (α SMA), vimentin, desmin, and glial fibrillary acidic protein. Functionally, TGF β 1 treatment upregulated the cellular expression of α SMA, collagen type I, fibronectin, and TGF β 1 (thereby establishing an autocrine loop). Importantly, the RLT-PSC could be deactivated by incubation on Matrigel in the presence of *N*-acetyl cysteine. This was manifest by decreased expression of the proteins mentioned immediately above, a decrease in proliferation, and reacquisition of a vitamin-A storing phenotype. Hence, this report provides a valuable cell source for further studies of pancreatic fibrosis, its role in the evolution of pancreatic disease, and the potential for therapeutic benefit by its inhibition.

One of the curious features of chronic pancreatitis is the relative preservation of functional islets, in the midst of fibrous obliteration of the exocrine pancreas. The mechanisms by which islets survive in an adverse environment initially infiltrated by inflammatory cells, followed by extensive deposition of fibrous tissue, are essentially unknown. In this issue, **Hasel et al**² (p. 1263) examined the hypothesis that antiapoptotic signaling in islet cells enables their survival in the midst of inflammation and fibrosis. The authors have previously shown that islets express the death-inducing ligand CD95L (FasL) but do not express its receptor CD95 (Fas). This privileged status is in contradistinction to exocrine epithelial cells, which neoexpress CD95 (Fas) along with HLA-DR, rendering them susceptible to FasL-induced apoptosis. Moreover, while islet cells in normal pancreas do not express TRAIL-R (tumor necrosis factor(TNF)-related apoptosis-inducing ligand receptors), there is a strong induction of the TRAIL-R4 in chronic pancreatitis. Curiously, TRAIL-R4 seems to be a decoy receptor for TRAIL, since this receptor lacks a cytoplasmic death domain. Similar to the FasL story, it is exocrine epithelial cells that neoexpress the death signal-transducing receptors TRAIL-R1 and -R2 (in addition to TRAIL-R4). Hence, exocrine pancreatic cells but not islet cells are susceptible to FasL- and TRAIL-induced apoptosis in chronic pancreatitis. Notably, pancreatic stellate cells (PSCs) are the major source of TRAIL production in chronic pancreatitis; inflammatory cells provide the FasL. In the current manuscript, the authors explored a potential alternative role for TRAIL-R4 in islet cells: induction antiapoptotic signaling pathways.

Human insulinoma cells treated simultaneously with TGF β , TRAIL, and IFN γ exhibited increased expression of TRAIL-R4. In turn, expression of NF κ B subunit mRNA was upregulated, as was expression of its antiapoptotic target gene, survivin. In addition, among the multitude of genes regulated by NF κ B are antiapoptotic genes such as those for inhibitor of apoptosis proteins (IAPs). IAPs prevent cell death by binding to and inhibiting active caspases. The above treatment led to the expression of cIAP1 by the insulinoma cells. Human pancreatic tissue was then examined for these proteins. The NF κ B-regulated proteins, survivin, and cIAP1 were found to be expressed in chronic pancreatitis, with highest levels in islets surrounded by fibrosis. In contrast, normal pancreatic tissue—both exocrine and endocrine—was largely devoid of expression of NF κ B constituents, survivin, and cIAP1. The authors conclude that islets not only evade immune attack by not exposing the functional death receptors Fas and TRAIL-R1 and -R2 (unlike their exocrine neighbors) but also by expressing TRAIL-R4, which upregulates NF κ B expression and its target genes survivin and cIAP1, thereby protecting the islets from apoptosis. These findings suggest that sustained enhanced expression of NF κ B subunits and, consequently, IAPs serve to preserve islet endocrine cells in the adverse microenvironment of chronic pancreatitis. Ironically, TRAIL production by pancreatic stellate cells thus promotes the destruction of the exocrine pancreas, while preserving the endocrine pancreas. Future insights into how the islet cell reprogramming occurs may promote efforts to diminish destruction of the exocrine pancreas in chronic pancreatitis. One can also wonder whether such insights might enhance efforts to prevent islet cell destruction in type I diabetes.

References

- 1 Jesnowski R, Fürst D, Ringel J, et al. Immortalization of pancreatic stellate cells as an *in vitro* model of pancreatic fibrosis: Deactivation is induced by Matrigel and *N*-acetylcysteine. *Lab Invest* 2005;85:1276–1291.
- 2 Hasel C, Bhanot UK, Sträter J, et al. Parenchymal regression in chronic pancreatitis spares islets reprogrammed for expression of NF κ B and IAPs. *Lab Invest* 2005;85:1263–1275.

Targeting HIF induction to rescue hypoxia-induced tubulointerstitial injury

Chronic hypoxia is a common primary consequence of glomerular disease due to capillary obstruction or microvascular insufficiency. This results in tubular atrophy and extracellular matrix deposition, which aggravates hypoxia and further amplifies renal

injury. Administration of angiogenic factors such as VEGF has shown beneficial results; however, the new capillaries produced under this treatment were of limited value due to significant leakage.

In a study published in this issue of *Lab Invest*, **Tanaka et al**¹ (p. 1292) have targeted the hypoxia-inducible factor (HIF) in the remnant kidney model of renal injury. In this model of systemic hypertension, hypoxia is present at very early stages, and the current study demonstrates that it plays a significant role in pathogenesis. HIF is a heterodimer, which controls transcription of a number of genes involved in angiogenesis, including VEGF. There are two HIF isoforms, with HIF α 1 expressed in glomerular and tubular epithelial cells, and HIF α 2 expressed in glomerular and tubular endothelial cells and fibroblasts. In hypoxic conditions, the α -subunit escapes prolyl-hydroxylation, which normally targets this protein for proteosomal degradation, allowing dimerization with the β -subunit and transcriptional activation. Cobalt prevents prolyl-hydroxylation and therefore acts as an HIF inducer. The authors showed that cobalt treatment significantly protected the remnant kidneys from tubulointerstitial injury. This protective effect was associated with HIF induction, and with the preservation of the peritubular capillary network through an increased proliferation of glomerular and peritubular endothelial cells. Importantly, the newly formed capillaries did not show any evidence of leakage.

The role of HIF was further clarified by a series of elegant *in vitro* experiments showing that HIF1 produced by epithelial cells indirectly promotes endothelial cell proliferation and that HIF2 induces capillary network formation.

This report should be a catalyst for further studies of the role of HIF in renal angiogenesis as it has been made clear that targeting this transcription factor that controls the expression of the hypoxia-induced angiogenesis pathway is a promising venue. The identification of a compound that would prevent prolyl-hydroxylation without the toxicity of cobalt would be a step in that direction.

Reference

- 1 Tanaka T, Kojima I, Ohse T, et al. Cobalt promotes angiogenesis via hypoxia-inducible factor and protects ischemic tubulointerstitium in the remnant kidney model. *Lab Invest* 2005;85:1292–1307.

A tale of thickening vs tethering: what really makes skin tight?

The quest for accurate animal models of clinically complex human diseases is arduous and often elusive. One such disorder is scleroderma, or systemic sclerosis (SSc), characterized by pathological alterations in vessels, the immune system,

and the extracellular matrix. Abnormal collagen accumulation is a key clinical feature that most animal models of SSc seek to recapitulate. SSc fibroblasts persistently overexpress genes responsible for procollagen and collagen production. While visceral lesions may produce significant morbidity and even mortality, skin is the most visible, accessible, and easily studied target for the fibrosing process that typifies SSc. Accordingly, many approaches have sought to identify SSc models in which fundamental pathways that influence transcriptionally activated dermal fibroblasts may be explored and understood.

Possibly the best studied model for SSc is one caused by a spontaneous mutation, namely the tight skin (Tsk1) mouse.¹ Mice carrying the *Tsk* mutation harbor a genomic duplication within the fibrillin-1 (*Fbn 1*) gene, resulting in a larger than normal in-frame *Fbn 1* transcript and resulting in the formation of oversized microfibrils. As the *Fbn 1* gene has also been implicated in the pathogenesis of SSc, the Tsk1 mouse has generated considerable interest in recent years. While homozygous mice die *in utero*, heterozygous Tsk1 mice have been considered to have fibrosis affecting skin, heart tissue, and tendon, as well as pulmonary emphysema. However, unlike SSc in humans, they lack vascular lesions or evidence of immune cell tissue infiltration. The dermal fibrosis that affects the skin has been regarded to be highly reproducible with full penetrance in the heterozygous animal. However, in this issue, **Baxter et al**² (p. 1199) have re-examined the current dogma with somewhat different results and conclusions. By investigating the Tsk1 mouse specifically in the context of its potential utility in evaluating antifibrotic therapeutic agents, they could not confirm a significant difference in dermal thickness or collagen content when Tsk1 mice were compared to wild-type controls. This was further supported by expression profiling, which indicated few changes in gene expression and no evidence of upregulation of the signaling axis that involves TGF β , a key cytokine implicated in SSc pathogenesis. While the animals did show increased pinch thickness at the scruff of the neck, this was considered more likely to be attributable to abnormal tethering of skin to underlying muscle layers than to actual dermal thickening. Baxter et al thus conclude that the tight skin mouse model is not suitable for testing the efficacy of antifibrotic agents on dermal thickness.

Why does this study by Baxter et al differ from previous ones that supported the Tsk1 model for studies of cutaneous pathology relevant to SSc? In their discussion, the authors address some of the discrepancies and offer possible explanations. Notwithstanding, this study is likely to generate additional critical re-examination of the Tsk1 mouse strain as a model for SSc. Research is the process of constant reappraisal based on evolving knowledge and technologies, and seemingly conflicting

data sets are a necessary part of the process that ultimately leads to the identification of fundamental biological truths. The study by Baxter *et al* also underscores the importance of recognizing the potential limits of animal models that, while still of great experimental value, seldom fully recapitulate the nuances of the distinctive pathologies inherent to most human diseases.

George F Murphy, MD

References

- 1 Pablos JL, Everett ET, Norris JS. The tight skin mouse: an animal model of systemic sclerosis. *Clin Exp Rheum* 2004;22(Suppl 33):S81–S85.
- 2 Baxter RM, Crowell TP, McCrann ME, *et al*. Analysis of the tight skin (Tsk1/+) mouse as a model for testing antifibrotic agents. *Lab Invest* 2005;85:1199–1209.