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## Breaking down the blood–brain barrier

Regulation and maintenance of central nervous system (CNS) interstitial fluid homeostasis by the blood–brain barrier is largely the function of specialized cerebral capillary endothelial cells. In contrast to other vascular beds, the CNS capillary endothelium features continuous tight junctions and the absence of fenestrations or channels. Alterations in the blood–brain barrier occur commonly in diverse neurological conditions, including infectious and inflammatory processes, tumors, and even neurodegenerative diseases. Efforts to study the molecular pathobiology of disease-related blood–brain barrier alterations has been hampered by a paucity of well-characterized experimental models that could be manipulated to ask relevant mechanistic questions. In this issue, **Coisne *et al***[1] report a novel *in vitro* murine blood–brain barrier model, which may advance these efforts. Murine cerebral capillaries were isolated and capillary endothelial cells were cocultured with syngenic astrocytes, oligodendroglia and microglia. In this system, brain capillary endothelial cells (MBCECs) formed differentiated monolayers, which retained many blood–brain barrier characteristics such as low permeability to small tracer molecules, high trans-endothelial electrical resistance, expression of P-glycoprotein, and an appropriate cell border distribution of tight junction proteins (claudin-5, occludin-1, claudin-3, Jam-1). To validate this coculture system in a disease model, the effect of endotoxin exposure on the expression of cell surface adhesion molecules was examined. The investigators show that the important mediators of neuroinflammation ICAM-1 and VCAM-1 were upregulated by brain capillary endothelial cells when treated with endotoxin similar to the *in vivo* situation. This model provides a new tool for understanding leukocyte–brain endothelial cell interactions and blood brain–barrier patholobiology.

## Reference

1 Coisne C, Dehouck L, Faveeuw C, *et al*. Mouse syngenic *in vitro* blood–brain barrier model: a new tool to examine inflammatory events in cerebral endothelium. Lab Invest 2005;85: 734–746.

## Hepatic stellate cell expression of ADAMTS13: implications for TTP

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disorder featuring microangiopathic hemolytic anemia and thrombocytopenia as a result of microvascular platelet clumping. Although intravascular coagulation is not a major feature, microvascular thrombosis can nevertheless cause variable degrees of tissue ischemia and infarction. The cause of TTP has been traced to a deficiency in ADAMTS13 (von Willebrand Factor-cleaving protease), a circulating zinc metalloprotease that cleaves the hemostatic glycoprotein von Willebrand factor (VWF) in a shear-dependent fashion. This VWF-cleaving protease is essential for preventing platelet aggregation in the normal circulation, by cleaving the multimers of VWF released by endothelial cells. The human ADAMTS13 is a large protein, with 1427 amino-acid residues and possibly several splicing isoforms. The gene spans 37 kb on human chromosome 9q34, with 29 exons. Both the secreted pro-ADAMTS13 and ADAMTS13 are proteolytically active within the circulation. Clinical deficiency in ADAMTS13 may arise from genetic mutation, with up to 50 mutations now identified, or from the presence of circulating autoimmune antibodies. Liver disease and sepsis may also lead to decrements in circulating ADAMTS13 levels. While VWF is synthesized and secreted by endothelial cells throughout the body, the cellular source of ADAMTS13 has not previously been identified. The liver is the primary site of full-length ADAMTS13 mRNA expression, but this has been determined only at the whole organ level. Moreover, RT-PCR can detect the presence of ADAMTS13 transcripts in other tissues and in platelets, albeit at much lower levels. In this issue of *Lab Invest*, **Zhou *et al***[1] demonstrate that the most active source of ADAMTS13 is the hepatic stellate cell, a highly specialized cell lying in the space of Disse, beneath the sinusoidal endothelial layer of the hepatic sinusoids. Stellate cells have been the subject of intense study in regards to the evolution of fibrosis and cirrhosis. Under inflammatory and destructive conditions within the liver, stellate cells shed their normal function of lipid (and vitamin A) storage and become activated myofibroblasts. These activated cells proliferate, synthesize and secrete extracellular matrix proteins including collagen, and also secrete proinflammatory cytokines. As contractile cells, they also are capable of modulating sinusoidal blood flow through nitric oxide-sensitive mechanisms. Zhou *et al* first used *in situ* hybridization techniques to identify message within stellate cells,

confirmed ADAMTS13 expression with RT-PCR analysis of cell fractions enriched in hepatic stellate cells, and then demonstrated that such expression was not likely to come from contaminating cell types, especially Kupffer cells. They then cloned the mouse ADAMTS13 gene from primary hepatic stellate cells, and demonstrated its biochemical functionality and similarity to the human counterpart. The identification of this novel function for hepatic stellate cells gives substantive new opportunity for defining the regulation of ADAMTS13 transcription, translation and secretion in normal hemostasis and under pathological conditions, including TTP as well as other acquired prothrombotic states.

### Reference

1 Zhou W, Inada M, Lee T-P, *et al*. ADAMTS13 is expressed in hepatic stellate cells. Lab Invest 2005;85:780–788.

### LOX may provide a key

In this issue of *Lab Invest*, **Cuzzocrea *et al***[1] report that 5-lipoxygenase (LOX) knockout mice are protected from dinitrobenzene sulfonic acid-induced colitis relative to wild-type mice. This is an important observation because 5-lipoxygenase catalyzes the first step in leukotriene synthesis, the conversion of arachidonic acid to leukotriene A4. Given the central role of leukotrienes as inflammatory mediators, it is not surprising that neutrophil recruitment is blunted in 5-lipoxygenase knockout mice. Indeed, in other models of inflammatory diseases including experimental asthma, pneumonia, and encephalitis, 5-lipoxygenase knockout has been shown to reduce neutrophil infiltration. In the case of colitis, it is clear that leukotriene B4, which is synthesized by modification of leukotriene A4, is elevated in experimental trinitrobenzenesulfonic acid-induced colitis and that prostaglandin E2, which reduces colitis severity, also reduces mucosal leukotriene B4 levels. Moreover, 5-lipoxygenase inhibitors reduce mucosal leukotriene B4 levels as well as symptoms and histology damage in rodents and in patients with ulcerative colitis. Given the clinical implications of such therapy, it is important to understand the mechanisms by which 5-lipoxygenase inhibition, either pharmacological or genetic, ameliorates disease. Cuzzocrea *et al* have used both the lipoxygenase inhibitor zileuton and 5-lipoxygenase knockout mice to study events dependent on 5-lipoxygenase activity that lead to disease. They show that 5-lipoxygenase activity is necessary for both neutrophil migration *in vitro* and *in vivo* and suggest that the *in vivo* inhibition of neutrophil migration is partly mediated by blocking induction of P-selectin, E-selectin, ICAM-1, and VCAM-1 expression. **Cuzzocrea *et al***[2] have made similar observations in experimental pancreatitis. Thus, therapeutic inhibition of 5-lipoxygenase may be the key to minimizing damage caused by neutrophil infiltration in colitis and other diseases.

### References

1 Cuzzocrea S, Rossi A, Mazzon E, *et al*. 5′-Lipozygenase modulates colitis through the regulation of adhesion molecular expression and neutrophil migration. Lab Invest 2005;85: 808–822.

2 Cuzzocrea S, Rossi A, Serraino I, *et al*. 5-lipoxygenase knockout mice exhibit a resistance to acute pancreatitis induced by cerulein. Immunology 2003;110:120–130.