In focus in HCB

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In this issue of the journal, we highlight four Original Articles describing (1) the expression of fatty acid-binding proteins (FABPs) in septoclasts from tibae of FABP5^{-/-} mice; (2) the immunohistochemical analysis and chemical coding of enteroendocrine cells in dilated and non-dilated portions of *T. cruzi*-induced megacolon; (3) a modified and improved Golgi-Cox metal impregnation protocol combined with synchrotron radiation micro-computed tomography for the non-destructive 3D visualization of mouse spinal cord neuron networks; and (4) the role of cerebral perivascular macrophages in the production of type I collagen leading to fibrosis in a rat hypertensive model. As always, we do hope you enjoy these brief monthly manuscript synopses.

A tango for one: FABP4 as a substitute for FABP5 in cartilage septoclasts

Pre- and postnatal bone growth in the epiphyseal growth plate of long bones occurs by endochondral ossification. The calcified and uncalcified matrices of the growth plate contain chondrocytes, osteoclasts and septoclasts (Amizuka et al. 2012; Mackie et al. 2011; Schenk et al. 1967). The latter cell type is situated at the chondro-osseous junction of the growth plate and produces proteinases (Lee et al. 1995) and MMP-3 (Nakamura et al. 2004). Septoclasts also contain fatty acid-binding proteins (FABP), which appear to be important for their energy metabolism (Bando et al. 2014), proliferative activity, and morphology (Bando et al. 2017). In FABP5-deficient mice, no obvious tissue

abnormalities could be observed (Owada et al. 2002). In their previous work, Bando et al. (2017) were unable to detect overt histological abnormalities in the growth plate of FABP5-deficient mice. Since it has been reported that FABP5 deficiency could be functionally compensated by another FABP subtype (Owada et al. 2002), Bando et al. (2021) have now investigated by immunohistochemistry the presence of FABP3, -4, -5, and -7 in septoclasts in mouse tibiae. They were able to detect FABP4 in septoclasts of FABP5-deficient mice and found that the number of FABP4-positive septoclasts was increased about 2.5-fold as compared to wild-type mice (Fig. 1). In contrast to septoclasts of wild-type tibiae, the FABP4-positive septoclasts of FABP5-deficient mice showed nuclear staining for PPAR γ . Hence, the authors suggest that in septoclasts of FABP5-deficient mice both FABP4 and PPAR γ are induced to functionally compensate for the deficiency of FABP5 and PPAR β/δ . In more general terms, they conclude that both

Fig. 1 FABP4-immunoreactive cells in the chondro-osseous junction of the tibia of wild-type (**a–f**) and of FABP5^{−/−} (**g–i**) mice. Note the increased number of FABP4-immunoreactive cells in FABP5^{−/−} mice (**g**). From Bando et al. (2021)

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FABP5 and FABP4 are important for the shape and function of septoclasts.

Enteroendocrine cells in megacolon of Chagas disease

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* and affects the heart and colon (Bern 2015). In the gastrointestinal chronic form, megacolon develops as result of an inflammatory invasion of the enteric nervous system by cytotoxic T-lymphocytes causing neuronal loss affecting both excitatory and inhibitory enteric motor neurons (Adad et al. 2001; de Silveira et al. 2007). Chronic inflammatory conditions of the colon have been shown to affect the enteroendocrine cell population (El-Salhy et al. 2012; De Jonge et al. 2003). In their present work, Martins et al. (2021) have applied immunohistochemistry to detect enteroendocrine cells positive for chromogranin A (CgA), glucagon-like peptide 1 (GLP-1), 5-hydroxytryptamine (5-HT), peptide YY (PYY) and somatostatin (SST) in the dilated and non-dilated portion of *T. cruzi*-induced megacolon and in non-infected individuals. They observed an increase in enteroendocrine cells expressing GLP-1 and SST in the dilated portion compared to the non-dilated portion of the same patients and in control colon (Fig. 2). In addition, the authors noticed that enteroendocrine cells in which 5-HT and PYY were co-expressed were common in control colon, but were rare in the non-dilated and absent in the dilated portion of chagasic megacolon. Furthermore, they were unable to detect neurotensin, motilin, glucose-dependent insulintropic peptide (GIP), CCK,

ghrelin, CCK-gastrin, sulphated CCK and non-sulphated CCK in dilated or non-dilated portions from patients with megacolon. The main conclusion drawn by the authors was that the denervation and associated chronic inflammation in the chronic gastrointestinal form of Chagas disease are accompanied by changes in the number and coding of enteroendocrine cells that could contribute to disorders of motility and defense in the chagasic megacolon.

Modified Golgi-Cox staining protocol for 3D imaging strikes a cord...

For visualizing neuronal networks, the Golgi metal impregnation stain has been the classical choice for neuroanatomists since its introduction by Camillo Golgi in 1873 (Golgi 1873). In efforts to improve the specificity and overall quality of the staining, the technique has been modified through the ages, starting with, and made famous by the neuroanatomist Ramon y Cajal (1909). Some of the modifications of the original staining method have also been given equally modified names, including the Golgi-Cox stain (Cox 1891). To this day, further modifications to this neuronal staining method to achieve optimal results under different experimental conditions and protocols continue to be reported (Narayan et al. 2020). In this light, Jiang et al. (2021) now provide an improved Golgi-Cox staining method optimized for 3D visualization of neurons in the mouse spinal cord using synchrotron radiation micro-computed tomography (SR μ CT). Their main modifications of the traditional Golgi-Cox

Fig. 2 Enteroendocrine cells in the colon of individuals with chagasic megacolon. Glucagon-like peptide (GLP-1) and somatostatin (SST) positive cells are significantly increased in the dilated portion as compared to non-dilated portion of chagasic megacolon. From Martins et al. (2021)

Fig. 3 The neuronal network in the ventral horn of the mouse spinal cord as visualized by a modified Golgi-Cox method (left) and its 3D-rendering by synchrotron radiation micro-computed tomography (right). From Jiang et al. (2021)

staining protocol included (1) perfusion of animals with artificial cerebrospinal fluid prior to removal of the spinal cord to remove blood cells from the vasculature; (2) increased rinsing cycles of tissue with distilled water to provide a clearer background; and (3) clearing of sections with methyl salicylate prior to application of a coverslip and imaging (the authors provide a very detailed protocol comparison in a supplementary figure). Their protocol modifications resulted in stained spinal cord sections with clearer background, a reduction in both staining artefacts and incomplete vasculature structures (Fig. 3). Combined with 3D imaging via SR μ CT, this enhanced Golgi-Cox metal impregnation staining method resulted in improved visualization of the thick neuronal network which is difficult to achieve with other 3D imaging methods such as multiphoton confocal microscopy. Moreover, the authors also provided quantitative analysis of their images, including soma density and length of axons. Finally, no X-ray damage of the sections from SR μ CT imaging was noted, making this technique a potentially useful non-invasive method for imaging thick neuronal tissue networks in health and disease.

Hypertensive cerebral vessels “call again...”

The role of macrophages in health and various disease models has been intensely studied (Shapouri-Moghaddam et al. 2018; Lapenna et al. 2018). As is well known, macrophages in general represent a heterogeneous cell type, and can assume various biological functions related to their tissue location (Wynn et al. 2013). A sub-type of macrophage closely associated with organ small vascular vessels has been identified and referred to as the “perivascular macrophage” (PVM) (Faraco et al. 2017). Akin to other macrophage sub-types, PVMs are believed to be involved in multiple physiologic functions, and perhaps to play a role in disease mechanisms such as Alzheimer’s Disease and diabetes (Faraco et al. 2017). Specifically, in brain, PVMs are located in the perivascular space (Virchow-Robin space), where they are believed to assist in the maintenance of tight junctions between endothelial cells and regulation of inflammation. (Faraco et al. 2017). Inagaki et al. (2021) have now used a rat model of hypertension (SHRSP/Izm) to investigate whether PVMs located adjacent to small cerebral vessels may be involved in the synthesis of collagens, leading to vascular fibrosis. Using multiple labeling and imaging techniques, including wide-field visualization immunohistochemistry, transmission electron microscopy, and combined immunohistochemistry and in situ hybridization they assessed the presence of collagen I antigen and mRNA in sections from the infundibulum region of the frontal brain. Their

Fig. 4 Electron micrograph of a cross-sectioned arteriole of a 12-week-old male hypertensive SHRSP/Izm rat. A perivascular macrophage filled with lysosomes is seen (a). At higher magnification, collagen fibers are present between a perivascular macrophage and smooth muscle cells (b). From Inagaki et al. (2021)

results showed (1) increased immunoreactivity for type I collagen around cerebral vessels in hypertensive animals at both 4 and 12 weeks of age compared to control animals; (2) increased presence of collagen fibers in the intercellular spaces near PVMs associated with small cerebral vessels in hypertensive animals (Fig. 4); and (3) colocalization of *Colla1* mRNA with the PVM-specific marker CD206 in cells associated with small perivascular vessels

from hypertensive animals. Taken together, their results strongly support the premise that PVMs located adjacent to cerebrovascular vessels in prolonged hypertensive animals produce abundant type I collagen leading to vascular fibrosis and associated atherosclerotic changes.

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