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EDITORIAL

In focus in HCB

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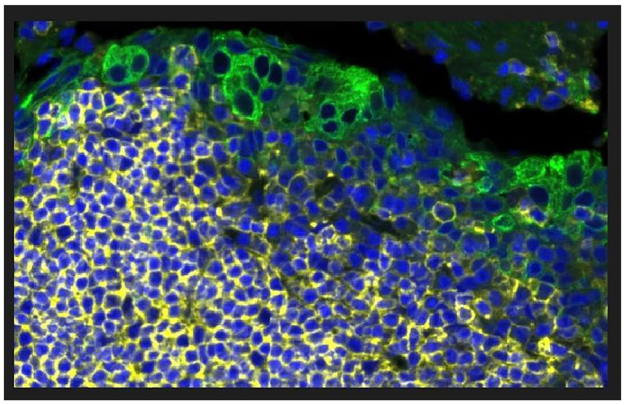

In this month's editorial, we will highlight two manuscripts describing (1) a newly developed electron microscopy method to determine the precise membrane distributions of the glycerophospholipids phosphatidylserine and phosphatidylethanolamine in the obligate intracellular parasite *Toxoplasma gondii* and (2) the relationship between placental tissue 8-hydoxyguanine immunostaining (indicative of cellular oxidative stress) and fetal size at birth, placental histology, fetal sex, and other pregnancy characteristics. We wish you enjoyable reading of these highlights, as well as this month's entire issue!

A virulent asymmetry of membrane phospholipids in *Toxoplasma gondii*

Since its invention by Steere (1957) and Moor et al. (1961), freeze-fracture electron microscopy has proven to be a crucial technique for ultrastructural studies of biological and model membranes (see Meier and Beckmann 2018 for a recent review). The combination of the freeze-fracture technique with immunogold labeling (Fujimoto 1997; Fujimoto et al. 1996; Fujita et al. 2009) provided a quantum leap for the investigation of membrane integral proteins and lipids at nanoscale resolution. In their present study, Konishi et al. (2023) have investigated and quantified the distribution of phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn) in the cytoplasmic and exoplasmic membrane leaflets and of GM3 ganglioside as a membrane raft marker in *T. gondii*, including strains of different virulence levels. The rationale for performing this work was the known importance of these phospholipids for the growth of the rapidly replicating tachyzoite stage of *T. gondii,* and lack of information of their distribution in the plasma membrane and the inner membrane complex of tachyzoites. For quick-freeze freeze-fracture replica labeling of PtdSer, recombinant GST-tagged C2 domain of mouse MFG-E8 was used, and subsequently detected with rabbit anti-GST antibody and 10 nm gold-labeled anti-rabbit IgG. PtdEtn was labeled with biotinylated duramycin followed by mouse anti-biotin antibody and 10 nm gold-labeled anti-mouse IgG, and GM3 ganglioside by anti-GM3 antibody and 10 nm gold-labeled anti-mouse IgG. Of note, the authors validated both the specificity and quantifiability of their technique applying recombinant GST-tagged C2 domain of mouse MFG-E8 using liposomes containing different phospholipids. Thus, in *T. gondii* tachyzoites, they could detect PtdSer in the cytoplasmic and exoplasmic leaflet of both the plasma membrane and the middle membrane of the inner membrane complex, but practically nothing in the cytoplasmic leaflet of the inner membrane of the inner membrane complex. Since the middle and inner membranes are continuous, this

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was interpreted to indicate a restricted movement of PtdSer between the two membranes. As previously demonstrated for mammalian cells and budding yeast, and confirmed in the present study, the labeling intensity for PtdSer was higher in the cytoplasmic membrane leaflets of tachyzoites. However, and in contrast to fibroblasts and budding yeast, the labeling density for PtdEtn was higher in the cytoplasmic leaflet of the tachyzoite plasma membrane. Additional differences in labeling intensities for PtdSer and PtdEtn existed for the cytoplasmic and exoplasmic leaflets of the middle and inner membranes. Interestingly, striking differences related to differences in virulence of the tachyzoites could be observed. The expression of PtdSer and PtdEtn as well as of GM3 in the luminal (exoplasmic) leaflet of the inner membranes was significantly higher in the more virulent RH strain compared to the less virulent PLK strain. The authors concluded that their findings suggest a correlation between the expression levels of both PtdSer and PtdEtn and the number of lipid rafts in the inner membranes on one side and the virulence-related motility of *T. gondii* tachyzoites on the other.

Cellular oxidative stress and fetal growth restriction

Fetal growth restriction, associated with many adverse perinatal outcomes, is a complication of pregnancy in which a fetus does not attain its predicted growth potential (ACOG Practice Bulletin 2021). The causation of growth restriction may be classified as maternal, fetal, and placental, though the underlying responsible pathways are common to the three and related to suboptimal uterine-placental perfusion and fetal nutrition (ACOG Practice Bulletin 2021). Issues with malperfusion of the placenta can often be accompanied by increases in cellular oxidative stress, which can be assessed by immunohistochemical techniques for the detection of 8-oxyguanine (8-oxo-G) residues (Nakae et al. 2005). Xodo et al. (2023) have semi-quantitatively assessed the 8-oxo-G immunostaining profile as an indication of cellular damage through oxidative stress on tissue microarray slides from a group of placental tissue samples chosen to represent the following different fetal growth patterns at birth: (1) FGR, fetal growth restriction; (2) SGA, small for gestational age; (3) AGA, appropriate for gestational age; (4) LGA, large for gestational age. Comparing the different fetal growth groups, influence of fetal sex status, and placental histology, a selection of their results demonstrated (1) the overall percentage of syncytiotrophoblasts with positively stained nuclei was greater in LGA compared to late FRG placental samples; (2) the 8-oxo-G immunostaining intensity in the cytoplasm of syncytiotrophoblasts was lower in SGA and LGA, compared to AGA placental samples; (3) a sex-specific pattern of 8-oxo-G immunostaining, highlighted by increased expression in the placental syncytiotrophoblasts, stromal and endothelial cells in AGA males compared to females; (4) histological differences in late fetal growth restriction placentae in which lesions resulting from maternal vascular malperfusion manisfested as accelerated villous maturation in females and avascular villi in males; (5) high-intensity staining of 8-oxo-G in the cytoplasm of placental syncytiotrophoblasts from newborn males correlated with thrombi occurring in the chorionic plate and villi, while similar high-intensity immunostaining in placental endothelial and stromal cells from females correlated with higher values of birthweight. Interestingly, no relationship was observed between higher levels of oxidative stress in placentae and decreased fetal growth. Taken together, the results of this very comprehensive study indicate that oxidative stress patterns in male and female placentae suggest that fetal growth is regulated differently between the two sexes and may not be the sole factor responsible for the pathophysiology accompanying fetal smallness at term.

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