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Cilia as mechanosensors and a cause of disease

Cilia are present on virtually all cells in the human body, and biologists have long known that humans experience their environment through cilia. Light, odorant, and sound perception depend on these microtubule-filled, complex organelles present on cells in primary sensory tissues. One of the first diseases attributed to ciliary dysfunction, Kartagener's Syndrome, was associated with the triad of sinusitis, bronchitis, and situs inversus. The January 2005 issue of *Laboratory Investigation* featured an article by **Zhang *et al*¹** (p. 45), in which loss of polaris (*Tg737*), an intraflagellar transport (IFT) protein required for ciliogenesis, in the appropriate murine background, caused developmental abnormalities in both the exocrine and endocrine pancreas. This included a diabetogenic phenotype, with impaired glucose tolerance and insulin secretion. The current issue of this journal presents a comprehensive review of the biological role of cilia, above and beyond their obvious role in fluid motility. **Pan *et al*²** (p. 452) now detail how discoveries on the mechanism of assembly of cilia (flagella) in the lowly, biflagellated, eukaryotic green alga *Chlamydomonas* have triggered a renaissance of interest in these organelles, particularly with regard to their mechanosensory roles. *Chlamydomonas* researchers have elucidated the machinery for transporting the protein components of cilia and flagella in all ciliated/flagellated eukaryotic cells between their site of synthesis in the cell body and their site of assembly at the tip of the flagellum (ie, IFT). Remarkably, disruption of *IFT* genes in mice leads to polycystic kidney disease (PKD), and the proteins specifically mutated in PKD are present on the sensory cilia of the nematode worm, *C. elegans*. There is thus a direct connection between PKD and cilia. Pan *et al* then go on to a parallel discussion of the biology of ciliary proteins and their human gene mutations being identified in PKD, the related disorder of nephronophthisis, and the rare human disorder, Bardet–Biedl Syndrome (BBS), in which patients exhibit phenotypes of common human diseases, including obesity and increased incidence of hypertension and diabetes. The authors advance the premise that the primary cilia present on cells throughout the body, even those on brain neurons, may be essential for known and as yet undiscovered cilium-generated signaling functions. The known specific disorders arising from mutations in ciliary proteins point to their central role in embryological

development and adult life. Unraveling such mysteries as to how defects in the basal bodies of cilia could lead to an obesity disorder offers the prospect of making progress into understanding disorders with profound health consequences in North America.

References

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Nephrotoxicity from apical uptake of fatty acid binding protein

Renal proximal tubule epithelial cells have among the highest metabolic rates in the body. Support of such metabolism requires substantial metabolic fuel. In addition to glucose, free fatty acids are a valid source of metabolic energy for oxidative metabolism. Fatty acid delivery to the kidney is via the circulation. Albumin is thought to be the primary serum protein for delivery of free fatty acids to the kidney. However, fatty acids also bind to fatty acid binding protein (FABP) released from the liver (hence the designation L-FABP). This is a member of the FABP family, 14–15 kDa proteins involved in the binding of free fatty acids in the intracellular transport of free fatty acids from the plasma membrane to sites of oxidation and esterification, and to the nucleus for gene regulation. FABPs also bind a number of other potentially toxic biologically active ligands, including eicosanoids, bilirubin, bile acids, and xenobiotics. Entry of FABP into the circulation promotes delivery of a variety of ligands to other tissues. Unfortunately, among them free fatty acids are toxic to the kidney in nephrotic conditions. This appears to be due to increased glomerular filtration of serum proteins, thereby exposing proximal tubule cells to ligands in the apical space. As patients with liver damage have elevated serum levels of L-FABP, it is possible that L-FABP may play a pathological role in their own right in nephrotic conditions. Thus, there is value in elucidating the mechanism by which L-FABP and its bound free fatty acids are taken up by renal epithelia.

In this issue, **Oyama *et al*¹** (p. 522) examined the cellular and subcellular localization of L-FABP in the rat kidney. Immunohistochemical studies

localized L-FABP to the lysosomes of proximal tubule epithelial cells. In rats with carbon tetrachloride (CCl₄)-induced acute liver injury, high levels of L-FABP in the circulation and in the kidney were detected, which were of hepatic origin. Remarkably, uptake of L-FABP into renal epithelia was from the apical domain, confirming glomerular filtration of this protein into the urinary space prior to proximal tubular re-uptake. Quartz-crystal microbalance analysis revealed that L-FABP bound to megalin, a multi-ligand endocytotic receptor on proximal tubular epithelial cells that plays a key role in endosome-to-lysosome trafficking. *In vitro* studies demonstrated that megalin mediated uptake and catabolism of L-FABP. These results implicate apical endocytotic uptake of L-FABP and its bound ligands, with subsequent trafficking to lysosomes for degradation and release of the ligands. This constitutes a novel renal uptake pathway for L-FABP, and lends insight into how this carrier of hydrophobic molecules can be an agent for nephrotoxicity.

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Don't blame it all on lymphocytes: pancreatic phagocytes claim their share in the diabetogenic process

Autoimmune destruction of the insulin-producing β -cells is preceded by an inflammatory stage called insulinitis, in which mixed cell populations infiltrate the pancreatic islets. Using the NOD mouse as a model, the respective pathogenic roles of the CD4⁺, CD8⁺ T cells, or B cells have been largely demonstrated by gene targeting, either by eliminating these specific lymphocyte populations, or by restricting their repertoire to single clones with diabetogenic specificity. However, high numbers of macrophages and dendritic cells (DCs) have been observed around the islets preceding the lymphocyte infiltration. Moreover, the migration of macrophages into the islets along with lymphocytes is a marker of imminent β -cell destruction. Previous observations have been only correlative. Macrophages and/or DCs cannot be easily eliminated by gene targeting. Nevertheless, long-term depletion of macrophages results in protection from diabetes, establishing a functional link between these cells and β -cell destruction. In the study presented in this issue, **Nikolic, Geutskens *et al*** (p. 487) used clodronate-loaded liposomes to mediate a reversible depletion of macrophages and DC in 8-week-old NOD mice, at a stage when they are developing progressive peri-insulinitis. This treatment resulted in a significant

reduction of diabetes by 35 weeks of age. Within 1 week of clodronate exposure, monocytes, macrophages and DCs reappeared in the circulation and the spleen with unchanged surface marker expression and antigen-presenting function. In contrast, the pancreas was devoid of macrophages and DCs up to 28 days after the treatment. Remarkably, this disappearance of pancreatic phagocytes was paralleled by the clearance of pancreatic lymphocytic infiltrates. Peri-islet T cells reappeared at about 28 days post-treatment, while it took up to 49 days for B cells to reappear at this location. These results strongly suggest that macrophages and DCs play an essential role in the local recruitment and retention of lymphocytes in the pancreas. Furthermore, temporary abrogation of this recruitment/retention at the early stages of insulinitis is sufficient to have a long-lasting protective effect and maintain β -cell function. The role of macrophages and DCs in recruiting and maintaining lymphocytes in the spleen has already been documented, although the mechanisms by which it occurs are unclear. The study by Nikolic *et al* suggests the existence of factors produced by the pancreatic phagocytes that attract and maintain lymphocytes. The identification of these factors would evidently provide a promising target to keep the pancreas free from auto-destructive lymphocytes. Given the differential response of the splenic and pancreatic phagocytes to clodronate as documented in this study, it is possible that these lymphocyte-recruiting factors are unique to the pancreas, which would increase their potential as therapeutic targets.

References

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Verification of single-cell genetics

Comparative genomic hybridization (CGH) is a powerful technique that allows the detection of genetic changes at the chromosomal and subchromosomal levels. While methods have been developed for the unbiased amplification of DNA from a single cell for subsequent CGH, it has not been possible to verify the results because the cell is destroyed during the procedure. In this issue of *Lab Invest*, **Langer, Geigl *et al*** (p. 582) show that the genome of an individual cell can be analyzed by two different single-cell techniques applied sequentially to the same cell. The authors employed normal cells; cells known to have trisomies of chromosomes 13, 18 or 21, well-characterized tumor cell lines, leukemia samples, and tissue sections. In the first

step, interphase fluorescence *in situ* hybridization (FISH) was performed and evaluated. Cells of interest were selected and collected by laser microdissection. The DNA was then amplified by PCR and subjected to CGH analysis. The authors found that single cells could be analyzed by CGH with the same accuracy as DNA derived from cell pools or large cell numbers, and that previous FISH did not alter the expected CGH profile. Therefore, sequential application of two single-cell techniques can be used to increase the accuracy and cell specificity of

single-cell analysis, and may have great potential in the clinical diagnosis of genetic diseases, in tumor genetics, and in forensics.

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