Journal of Controlled Release 229 (2016) 200

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Cover Story

Visualization of focal permeation sites within epithelial barriers

Epithelial tight junctions, as part of mucosal barriers, physically limit diffusion of larger solutes through the paracellular pathway. This epithelial barrier is important for understanding passage routes of macromolecules into internal compartments of the body. Delivery of macromolecular therapeutics via the oral route is most desirable. Development of effective penetration promoter systems has been active for enhancing the uptake by the paracellular pathway through epithelia. The epithelial barrier function is known to be pivotal for the pathogenesis of a number of diseases including autoimmune inflammatory bowel diseases (IBD) and various infectious diseases involving mucosae that are frequently culminating in sepsis. Currently, this barrier function is assessed by global barrier assays, such as the measurement of transepithelial resistance (TER) or the determination of flux rates of tracers across a barrier-forming tissue. Because these assays average tracer signals in time and space, the epithelial structure causing the barrier opening has not been functionally resolved. To overcome this limitation, Dr. Jan Richter and his colleagues present an easy-to-perform "sandwich assay" that allows accurate identification of paracellular passage loci of macromolecules [1].

The work by the Richter team represents a significant advancement in bringing forward our understanding of transport mechanisms for macromolecules to cross epithelia. Their assay is unique, in that it generates a positive signal on minimal background, thus allowing the detection of rare passage events. The authors show that avidin spontaneously binds to basolateral membranes of epithelial cells which allows for immobilization of biotin-tagged macromolecules that pass from the apical to the basal compartment. A second tag, a fluorophore, allows for detection by imaging. This setup enabled them to specifically label permeation sites, thus producing a specific and sensitive detection system. The method was proven to work with various epithelial cell lines and mucosal explants as well as with various tracers including dextrans of different sizes and labeled albumin. The technique was shown to allow for spatial (resolution in sub-μm range) and temporal analysis as well as size selectivity of barrier openings. Thus, it is possible to classify the transepithelial passage of macromolecules as either paracellular via bi- or tricellular junctions or via single cell defects (e.g., as a result of single cell apoptosis frequently found in various mucosal diseases). This ability is critical for evaluation of the distribution of such openings in settings including genetically manipulated epithelia or epithelia targeted with drug absorption enhancers. The method was also shown to be applicable to explanted mucosae, making it a promising tool for analysis of barrier function in various animal models. The modular composition of the assay allows researchers to use various types of macromolecules and to apply the assay for the retrieval of temporary barrier leaks.

By applying the sandwich assay the Richter team presents the first evidence for the existence of leaks that only appear transiently. The sandwich assay will help answer long-standing questions in epithelial physiology as to the mechanisms of the "leak pathway" [2], which are likely relevant to desired openings for drug delivery and undesired disease-associated leaks in epithelial barriers. The sites of macromolecule passage in colon mucosa was thought to be the surface colonocytes that have matured along the crypt-villous axis rather than cells deep down in the crypt that appear to seal the paracellular pathway more efficiently. This finding might be important to expose the lamina propria cells to the luminal antigen in diseases as IBD, where maturity of epithelial cells is shifted significantly and antigen exposure is believed to trigger disease activity [3].

The work by Richter and his coworkers introduces a new technique to the field of barrier research that will enable researchers to assign a barrier opening to the responsible structure in the epithelial layer. One of the most important properties of the sandwich assay is its ability to visualize the exact location of the failed barrier function that can be studied as a function of time or as the type of an epithelial system. Progress from merely global assessment of barrier function, e.g., TER, to spatial resolution of the barrier function is expected to accelerate further progress in various studies dealing with macromolecule transport.

References

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http://dx.doi.org/10.1016/j.matbio.2016.04.001