Laboratory Investigation (2004) 84, 663–664

www.laboratoryinvestigation.org

INSIDE LAB INVEST

Laboratory Investigation (2004) **84,** 663–664. doi:10.1038/labinvest.3700100

Don't swallow, breathe deep: isolating cells from our interior surfaces

The pulmonary alveolar surface is covered by Type I and Type II alveolar epithelial cells that are morphologically and functionally distinct. Type II cells have been extensively studied; their functions include surfactant release, ion transport, and serving as a cellular proliferative and differentiation compartment for generation of Type I cells. In contrast, very little is known regarding the function of the Type I cells *per se*, partially due to the difficulty of their primary isolation and culture. In this issue, the study by **Chen *et al***[1] (p. 727) reports an improved and reproducible method for the isolation of highly pure type I and type II cells by immunomagnetic selection, taking advantage of the expression of the apical membrane protein T1α on Type I cells. The isolated cells recapitulate their *in vivo* phenotype, remain functional, and are suitable for culture. The protocol also could be used for isolation of Type II cells from hyperoxia-injured lungs. Thus, this paper describes an important enhancement for investigating the cell biology of both Type I and Type II alveolar epithelial cells. This should be useful for gene expression profiling, study of lung injury and repair, and further investigation of ion transport, surfactant generation, and barrier function.

In a separate paper in this issue, **León and Roy**[2] (p. 804) report improved isolation of human small bowel intraepithelial lymphocytes. These cells reside in the intraepithelial space, at the frequency of about 1 intraepithelial lymphocyte per 10 absorptive enterocytes. They are important effector cells of the mucosal immune system, with key roles in immune surveillance against infections and other antigens, and in maintaining oral tolerance through immunomodulation. Their study as an isolated cell population has been made difficult by the challenge of purifying these cells. The usual methods involve disaggregation of an isolated intestinal epithelial layer, density gradient separation, and lastly magnetic bead separation that selects for the lymphcoytes. In this new method, the authors take advantage of the fact that enterocytes are more fragile. The stress of disaggregation induces them to express an early marker of impending apoptosis: phosphatidylserine, which is normally intracellular. By using annexin-V-coated magnetic beads, which have high affiinity for phosphatidylserine, the enterocytes can be removed. A higher yield of isolated intraepithelial lymphocytes can be obtained, >95% pure and 99% viable. Most importantly, these lymphocytes are untouched by the selection algorithm, and hence are more amenable to *in vitro* functional and molecular studies.

These two reports may thus enhance our ability to use isolated cell populations *in vitro* to better understand the environmental- and immune-mediated diseases of our lungs and small intestine.

References

1 Chen J, Chen Z, Narasaraju T, *et al*. Isolation of highly pure alveolar epithelial type I and type II cells from rat lungs. Lab Invest 2004;84: 727–735.
2 León F, Roy G. Isolation of human small bowel intraepithelial lymphocytes by annexin V-coated magnetic beads. Lab Invest 2004;84: 804–809.

Multiplex diagnosis of genetic disorders: surface plasmon resonance and biosensor technology

Detection of genetic mutations became immensely easier with the advent of polymerase chain reaction (PCR)-based technologies. Use of common primers that fall on either side of the region of interest can permit ready detection of deletions or insertions in the genetic sequence, while use of primers that specifically bind to the region of interest permits identification of known point mutations. Biosensor technology offers a different opportunity for detecting genetic mutations, whereby a PCR reaction is conducted to encompass the entire genomic region of interest that might contain mutations. The PCR product is then immobilized on a biosensor chip. Short single-stranded PCR probes for the specific genetic mutations of interest can then be run over the chip individually, and binding to the immobilized long-strand genomic PCR product assessed through surface plasmon resonance (SPR). Each PCR probe can be rinsed off to regenerate the biosensor chip. The cycle time for each PCR probe application is of the order of 10 min, and hence this is a rapid turnaround technology for diagnostic purposes. This technology has been used successfully to detect the cystic fibrosis ΔF508 and W1282X mutations, and for detection of the beta°39 mutation of the human beta-globin gene in beta-thalassemia. In this issue, **Feriotto *et al***[1] (p. 796) demonstrate that this approach can be used to detect the point mutations of beta-thalassemia in a multiplex manner. Considering that clinical beta-thalassemia is frequently the result of a compound heterozygote genotype, with a number of allelic possibilities, the capability of rapidly identifying multiple genetic mutations is quite attractive. Feriotto *et al* present

the methodology for detecting the following mutations in the human beta-globin gene: beta°39, beta°IVSI-1, beta + IVSI-6, and beta + IVSI-110; each diagnostic PCR probe is 11–12 bases in length. Discrimination between normal human subjects, heterozygous and homozygous patients is readily achieved for all four mutations. This is a novel advance for parallel diagnosis of multiple mutations. The method is real-time, and hence may be of considerable value for antenatal diagnosis of genetic diseases. In addition to wild-type and homozygous conditions, heterozygotes and compound heterozygotes for mutations can readily be detected. This particular report was for detection of the mutations of beta-thalassemia; one can envision broad applications for this technique.

References

1 Feriotto G, Breveglieri G, Finotti A, *et al*. Real-time multiplex analysis of four beta-thalassemia mutations employing surface plasmon resonance and biosensor technology. Lab Invest 2004;84: 796–803.

Lipoprotein lipase in atherosclerosis: friend or foe?

Lipoprotein lipase (LPL) is an important enzyme for lipoprotein metabolism and has been shown to play a critical role in atherosclerosis. Generally, LPL secreted from the muscle and adipose tissues is considered antiatherogenic because it can accelerate the hepatic removal of atherogenic remnant lipoproteins and thus lower plasma levels of cholesterol. However, it is not known whether increased LPL activity itself is antiatherogenic, or whether its antiatherogenic effect is due to its lipid-lowering effect. In this issue, the paper by **Ichikawa *et al***[1] (p. 713) describes the use of transgenic rabbits expressing a human LPL transgene to determine the mechanism of the antiatherogenic effect of the enzyme. With equally high hypercholesterolemia, the transgenic rabbits developed greater aortic atherosclerosis than control rabbits. The transgenic rabbits had higher levels of highly atherogenic, small-sized LDLs, whereas control rabbits had higher levels of the less atherogenic large-sized remnant lipoproteins. This study provides the first evidence that LPL exerts a dual function in terms of its atherogenicity: it is antiatherogenic through enhancement of receptor-mediated remnant lipoprotein catabolism; and proatherogenic via the generation of a large quantity of small-sized LDLs.

References

1 Ichikawa T, Kitajima S, Liang J, *et al*. Overexpression of lipoprotein lipase in transgenic rabbits leads to increased small dense LDL in plasma and promotes atherosclerosis. Lab Invest 2004;84:715–726.

Through the lens clearly: Rho GTPases and ocular development

The vertebrate lens is a highly organized structure with epithelial cells overlying interior fiber cells. Normal development and growth of the lens requires differentiation of epithelial cells into fiber cells, a process that requires extensive remodeling of the cellular cytoskeleton. The intracellular signaling pathways governing this reorganization appear to involve Rho GTPases (Rho and Rac). Inhibition of these GTPases during development affects lens transparency and cytoskeletal organization. In a previous publication in this journal, **Rao *et al***[1] presented a transgenic mouse model in which impaired lens-specific Rho GTPase function results in a severe ocular phenotype, including cataract and microphthalmia. In this current issue, the same group now examines in detail the morphological consequences of Rho GTPase dysfunction, and the alterations in expressed genes.[2] Fiber cells of the transgenic mouse lens were swollen and disorganized, with stunted growth and accumulation of large vacuoles. The fiber cells exhibited decreased immunostaining for key cytoskeleton and plasma membrane proteins despite normal protein levels by western blot analysis (F-actin, β-catenin, aquaporin-0, connexin-50). Gene expression analysis of wild-type *vs* transgenic mice revealed 44 differentially expressed genes relating to formation of extracellular matrix and basement membrane, transport and trafficking, cell survival and death, and protein degradation; genes pertaining to the cytoskeleton were not differentially expressed. In other cell types, inhibition of Rho GTPases leads to impaired actin polymerization, stress fiber formation, and formation of focal adhesions and adherens junctions. Rho GTPase activity is thought to control actin cytoskeletal organization and hence cell-to-cell interactions. The current study clearly demonstrates the profound effects of such inhibition on lens morphology, with evidence of markedly altered cellular function as well. The outcome is impaired lens organization and reduced lens transparency. At the very least, this study has value for understanding ocular development. One can hope that continued work on this model may provide insights as well into the formation of cataracts.

References

1 Rao PV, Wawrousek E, Tamm ER, *et al*. Rho GTPase inactivation impairs lens growth and integrity. Lab Invest 2002; 82:231–239.
2 Maddala R, Deng P-F, Costello JM, *et al*. Impaired cytoskeletal organization and membrane integrity in lens fibers of a Rho GTPase functional knockout transgenic mouse. Lab Invest 2004;84:679–692.