Bronchial endothelial cell phenotypes and the form:function relationship

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CULTURED SYSTEMS HAVE PROVEN highly useful for generating insight into normal and abnormal cell function, as illustrated by Brown et al. (1–3) in identification of mechanism(s) underlying familial hypercholesterolemia. In studies by Brown and coworkers, the isolation and culture of fibroblasts were made with relative ease, whereas other cell types are not readily accessible. Indeed, the generally poor accessibility of bronchial endothelial cells has hampered their in vitro purification. The study by Moldobaeva and Wagner, one of the current articles in focus (Ref. 11a, see p. L520 in this issue), reports successful isolation and culture of endothelial cells from the bronchial artery and microcirculation and ascribes the cell's origin to unique barrier regulatory properties.

To isolate bronchial artery endothelial cells (BAEC), the bronchoesophageal artery was perfused and incubated with collagenase. Dissociated cells were collected and plated on gelatin-coated culture dishes. After 4–5 days, colonies displaying the typical cobblestone morphology were transferred using cloning disks for expansion. To isolate bronchial microvascular endothelial cells, the mainstem bronchus was dissected, and its epithelium was removed. The microvessels to the cartilage were then dissected, incubated with collagenase, and filtered through a nylon mesh. Cells collected were grown on gelatin-coated dishes, and, after 4–5 days, were selected for expansion based on their typical cobblestone morphology. Both bronchial artery and microvascular cells displayed appropriate endothelial markers, including uptake of low-density lipoprotein, factor VIII/von Willebrand-associated antigen, and platelet-endothelial cell adhesion molecule-1 immunoreactivity, and were therefore judged to be representative of endothelia from the respective vascular sites.

It is now clear that not all endothelial cells are alike. For some time, it was evident that endothelial cells differed on the basis of their intercellular junctions and could be generally classified as “tight,” “continuous,” or “fenestrated.” In addition, high endothelial venules importantly regulate white blood cell recruitment (4, 6), and postcapillary venules form intercellular gaps in response to inflammatory agonists (18), both highly specific attributes of very specific cells. There is even evidence that endothelial cells immediately adjacent exhibit unique functions, some with so-called pacemaker activity that regulates cytosolic calcium concentrations (19). The cause of such disparate endothelial cell function between and within vascular beds is not fully understood but likely reflects environmental and epigenetic causes (16).

Moldobaeva and Wagner (11a) describe unique bronchial macro- and microvascular endothelial cell function in two aspects, including cell proliferation and barrier function. Their proliferation studies resolved that BAEC grow faster than their microvascular counterparts. The macrovascular cells grew equally well in any of six different media supplements. Remarkably, microvascular endothelial cells did not grow equally well in all media supplements; use of MCDB 131 essentially abolished growth. This medium was designed to optimize serum-free growth and has been successfully utilized to enhance conduit artery and microvascular endothelial cell proliferation (11, 14, 17). Absence of a growth response in bronchial microvascular endothelial cells using this medium provides important information regarding differences between these and BAEC and also regarding mechanisms controlling microvascular endothelial cell growth. It will be important to identify which component(s) of the MCDB 131 medium arrests microvascular endothelial cell growth. Similar observations have previously been extended to isolate multiple smooth muscle cell phenotypes from the pulmonary artery wall (7–9, 15).

Because microvascular endothelial cells were isolated from the bronchial mucosa, they were expected to possess greater permeability responses than BAEC, particularly in response to bradykinin. Basal dextran permeability was greater in microvascular cells than in macrovascular cells. Bradykinin transiently increased dextran (9.5 kDa) transfer across both cell types, al-
though the magnitude of this effect was greater in microvascular than in macrovascular cells. Bradykinin did not increase the transfer of 77-kDa dextrans, suggesting a size restriction in gaps that formed. Microvascular endothelial cells also responded to thrombin with an increase in permeability, whereas macrovascular endothelial cells did not. Water permeability was not measured in these studies. In the future, it may be important to determine whether microvascular endothelial cells isolated from the bronchial mucosa possess enhanced water permeability, which contributes to airway humidification (12).

It is interesting that BAEC exhibit increased growth and permeability responses compared with bronchial microvascular endothelial cells. These parameters have been assessed in other vascular beds, but perhaps the most intriguing comparison is with endothelial cells from the pulmonary circulation. In this case, lung microvascular endothelial cells grow faster than their macrovascular counterparts (unpublished observations), and they also exhibit a more restrictive barrier function (5, 10, 13). Macro- and microvascular endothelial cells isolated from the bronchial circulation, therefore, exhibit exactly opposite behavior than do respective cell types isolated from the pulmonary circulation. These findings illustrate the need to determine influences, environmental and epigenetic, that underlie site-specific endothelial phenotype. Ultimate success of therapeutic strategies to target endothelia will require an ability to discern between organ- and site-restricted phenotypes.

In summary, Moldobaeva and Wagner (11a) report an important advance in airway and endothelial cell biology. Evidence for distinct behavior of bronchial macro- and microvascular endothelial cells, even under similar environmental conditions, suggests the cell types possess fundamentally different phenotype and function. Successful isolation and culture of these cells may now reveal mechanistic insight into the phenotype of these cell populations for rigorous comparison with their behavior in vivo.

REFERENCES