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Letter to the Editor

Technical caveats in identifying the source of endothelial cells in cultures derived from brain microvessels

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To the editor: We would like to take this opportunity to respond to an article, by Coisne *et al*,[1] which recently appeared in *Laboratory Investigation*. The article, 'Mouse syngenic *in vitro* blood–brain barrier model: a new tool to examine inflammatory events in cerebral endothelium, 'purports to describe a blood–brain barrier (BBB) model in which ECs (endothelial cells) are specifically isolated from cerebral capillaries,' thus distinguishing it from other murine brain EC culture paradigms, which, these authors claim, are constituted of 'mainly macrovascular or microvascular EC.'

While the authors of this article are correct and astute in emphasizing the importance of recognizing endothelial cell heterogeneity in developing brain endothelial cell cultures, we cannot see that they provide any evidence that differentiates their model from the one we described 2 years earlier[2]—especially as regards a derivative source of the EC. Coisne *et al* report, 'The resulting suspension containing vascular component was filtered through a 59 μm nylon mesh, in order to retain large vessels on the mesh surface while the capillaries passed through.' In our earlier paper,[2] we described first passing the brain microvascular suspension 'through a 75 μm nylon mesh,' digesting that which was retained on the filter surface with collagenase/dispase, then further filtering the digested microvascular fragments through a 40 μm mesh and collecting only the filtrate for plating EC. Importantly, as we described in our paper, our digestion protocol merely serves to disrupt the basement membrane, and leaves the vascular fragments otherwise intact. It is thus logical to conclude that the population of microvascular fragments plated in both studies are likely to be similar—and, most certainly, we did not plate macrovascular-derived fragments as was implied by Coisne *et al*. While we too recognize the provenance of capillary endothelial cells in formulating the BBB, we consider it unlikely that the 59 μm filter employed by Coisne *et al* completely resolved capillaries from artertioles and venules. This conclusion stems, in part, from our own observations that even sequential filtration of microvascular segments through 75, 40, 20 and 10 μm meshes does not produce a pure capillary fraction, although it certainly yields one enriched in capillaries. Also, standard histology texts[3] classify capillaries as having diameters of 4–10 μm, arterioles being 10–100 μm and postcapillary venules being 10–50 μm. Accordingly, the 59 μm mesh employed by Coisne *et al* can reasonably be assumed to have allowed significant passage of smaller arterioles and postcapillary venules.

This issue of contamination of capillaries by venular and arteriolar fragments is of critical importance, and bears heavily on the argument by Coisne *et al* that their EC cultures are derived from capillaries. In this regard, both our own observations and those of Spatz *et al*[4] impart that larger microvessel fragments (possibly venules), when placed in culture, sprout EC at a far greater rate than the smallest diameter vessel fragments (capillaries). Such a discrepancy is cause for considering that EC grown from capillary-enriched fractions might actually derive largely, if not exclusively, from a much lesser population of larger microvessels.

That this is a distinct possibility is underscored by the findings of Coisne *et al* that the EC in their cultures exhibited both basal and inducible expression of adhesion molecules ICAM-1, ICAM-2 and VCAM-1, which are critically involved in extravasation of leukocytes. Inasmuch as leukocyte extravasation across brain endothelium, as well as across most peripheral endothelial beds, is thought to occur at the level of postcapillary venules[5,6]—not capillaries—it is difficult to reason that adhesion molecule expression exemplifies derivation of the EC from capillaries. Also, expression of von Willebrand factor should not be taken, *de facto*, as evidence of capillary derivation, as this antigen has been shown, in some microvascular beds, to be absent from capillaries while present in the larger microvascular branches.[7–9]

These concerns notwithstanding, we very much welcome modifications to our protocol, and those of others, which serve to further advance the fidelity of murine brain EC cultures in simulating the BBB.

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References

1 Coisne C, Dehouck L, Faveeuw C, *et al.* Mouse syngenic *in vitro* blood–-brain barrier model: a new tool to examine inflammatory events in cerebral endothelium. Lab Invest 2005;85:734–746.
2 Song L, Pachter JS. Culture of murine brain microvascular endothelial cells that maintain expression and

cytoskeletal association of tight junction-associated proteins. *In Vitro* Cell Dev Biol Anim 2003;39:313–320.

3 Ross MH, Kaye GI, Pawlina W. Histology: A Text and Atlas, 4th edn. Lippincott Williams & Wilkins: Philadelphia, 2002.

4 Spatz M, Kawai N, Merkel N, *et al.* Functional properties of cultured endothelial cells derived from large microvessels of human brain. Am J Physiol 1997; 41:C231–C239.

5 Villringer A, Dirnagl U, Them A, *et al.* Imaging of leukocytes within the rat cortex *in vivo*. Microvasc Res 1991;42:305–315.

6 Battastini L, Piccio L, Rossi B, *et al.* CD8+ T cells from patients with acute multiple sclerosis display selective increase of adhesiveness in brain venules: a critical role for P-selectin glycoprotein ligand-1. Blood 2003;101: 4775–4782.

7 Hollestelle MJ, Thinnes T, Crain K, *et al.* Tissue distribution of factor VIII gene expression *in vivo*—a closer look. Thromb Haemost 2001;86:855–861.

8 Zhang EG, Smith SK, Charnock-Jones DS. Expression of CD105 (endoglin) in arteriolar endothelial cells of human endometrium throughout the menstrual cycle. Reproduction 2002;124:703–711.

9 Ebina M, Shimizuka M, Shibata N, *et al.* Heterogeneous increase in CD34-positive alveolar capillaries in idiopathic fibrosis. Am J Respir Crit Care Med 2004;169: 1203–1208.