Sufficient Evidence for Lymphatics in the Developing and Adult Human Choroid?

We read with interest the recent article by Koina et al.[1] suggesting evidence for the presence of lymphatic vessels in the developing and adult human choroid. However, this study does not meet the recently published consensus criteria on the immunohistochemical detection of ocular lymphatic vessels,[2] and therefore, in our opinion, requires critical revision.

First, appropriate positive and unequivocal negative controls are not presented in the study of Koina et al. In particular, when describing novel anatomical structures for the first time, and in order to change an existing dogma, a detailed documentation of blood and lymphatic vessel detection in the control tissue is mandatory. The provided supplementary data do not fulfill these criteria.

Second, the immunohistochemical marker panel used is critical. Endomucin does not represent an established lymphatic marker,[3,4] but is rather expressed by "endothelial cells along the whole vascular tree including lymphatic vessels."[5] Thus, an unequivocal discrimination between blood and lymphatic vessels is impossible with this marker. A further discrepancy is the use of the transcription factor prospero-related homebox gene-1 (Prox-1) as an extranuclear lymphatic endothelial precursor marker. Although reports of the extranuclear presence of PROX-1 in cell types other than lymphatic endothelium exist,[6–8] PROX-1 clearly shows a nuclear expression in lymphatic endothelia in human,[9] as well as mouse[10] and avian,[11] embryos, retaining its nuclear localization into adulthood.[12–14] On the other hand, it is not clear why lymphatic endothelial surface markers, such as podoplanin, lymphatic vascular endothelial-specific hyaluronic acid receptor-1 (LYVE-1), and the vascular endothelial marker CD34 display nuclear expression in this study. Additionally, the only lymphatic endothelial cell marker used in whole mounts is VEGFR-3, which is also expressed in fenestrated blood vessels, and, as such, also in the choriocapillaris.[15,16] Morphologically, the supposed lymphatic VEGFR-3–positive vessels are indistinguishable from the honeycomb-like lobular pattern of the choriocapillaris.[17]

Furthermore, the study of Koina et al. includes a blatant inconsistency in the use and documentation of immunohistochemical markers between fetal and adult eyes. Although one has to acknowledge that certain lymphatic markers might be expressed during embryogenesis, this pattern easily changes during maturation.[18] Therefore, such an approach would require extensive comparison of the same markers in different ages, thus representing an extensive survey in its own right. However, this is not the case in the study of Koina et al.

Third, the ultrastructural study would be greatly strengthened by immunoelectron microscopy. Indeed, anchoring filaments with a diameter of 40 to 100 Å—becoming readily identifiable only at magnifications of 40,000× to 50,000×—are present in lymphatics,[19] but could be easily present in the choroid as well without any association to lymphatic vessels,[20–22] particularly in aged eyes with typical alterations of the extracellular matrix. For this purpose, as well as for ruling out Weibel-Palade bodies, serial ultrathin sectioning with appropriate labeling would be necessary. Despite possible postmortem tissue alterations, numerous previous studies successfully applied different detection systems for ultrastructural investigations using ocular human donor tissue.[23–29] A limited use of immunomarkers for these investigations, as claimed, seems therefore not justified.

In regard to the above-mentioned criticisms, the evidence presented in the study of Koina et al. does not justify the hypothesized paradigm shift that functional lymphatic vessels are present in the human choroid. Rather, the findings of Koina et al. confirm previous reports of net-like structures with a "pseudo-vessel" appearance in the human choroid endowed with lymphatic vascular precursor cells (represented as LYVE-1^+ macrophages).[25] Those "atypical" lymphatic-like cells (i.e., endothelial cells with divergent or uncommon immunohistochemical phenotypes) may also exist in other parts of the eye. For example, the endothelial cells of Schlemm's canal display many, but not all, features of terminally differentiated lymphatic endothelial cells, including responsiveness to VEGF-C–induced lymphangiogenesis.[30]

In closing, we acknowledge that the work of Koina et al. is a further contribution to our understanding of the choroid, but although the existence of lymphatics in the human choroid cannot be ruled out per se, because of the aforementioned points and the sheer volume of evidence to date, we maintain that the inner human eye and in particular the choroid should still be considered an immune-privileged site devoid of lymphatic vessels. Further unequivocal evidence of "typical lymphatic vessels" in the human choroid is still missing.

Ludwig M. Heindl[1]
Alexandra Kaser-Eichberger[2]
Simona L. Schlereth[1]
Felix Bock[1]
Birgit Regenfuss[1]
Herbert A. Reitsamer[2]
Paul McMenamin[3]
Gerard A. Lutty[4]
Kazuichi Maruyama[5]
Lu Chen[6]
Reza Dana[7]
Dontscho Kerjaschki[8]
Kari Alitalo[9]
Maria Egle De Stefano[10]
Barbara M. Junghans[11]
Falk Schroedl[2,12]
Claus Cursiefen[1]

[1]Department of Ophthalmology, University of Cologne, Cologne, Germany; [2]Department of Ophthalmology, Paracelsus Medical University, Salzburg, Austria; [3]Department of Anatomy and Developmental Biology, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia; [4]Wilmer Ophthalmological Institute, Johns Hopkins University, Baltimore, Maryland, United States; [5]Department of Ophthalmology, Tohoku University Graduate School of Medicine, Sendai, Japan; [6]Center for Eye Disease & Development, Program in Vision Science and School of Optometry, University of California at Berkeley, Berkeley, California, United States; [7]Massachusetts Eye and Ear Infirmary and Schepens Eye Research Institute, Harvard Medical School, Boston, Massachusetts, United States; [8]Department of Pathology, Medical University Vienna, Vienna, Austria; [9]Institute of Biomedicine, University of Helsinki, Helsinki, Finland; [10]Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Biology and Biotechnology "Charles Darwin," Sapienza University of Rome, Rome, Italy; [11]School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales, Australia; [12]Department of Anatomy, Paracelsus Medical University, Salzburg, Austria.

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FS and CC contributed equally to the work presented here and should therefore be regarded as equivalent last authors.
E-mail: Ludwig M. Heindl; ludwig.heindl@uk-koeln.de.

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