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Identity of pigmented subretinal cells in age-related macular degeneration

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Dear Editor

We were very interested to read the letter by Curcio and Ach in *Graefes Archives* in response to our manuscript. We are pleased that our work has generated interest by expert investigators in the AMD research community.

The authors question our identification of the pigmented CD163+ cells in the subretinal space of cases with Sarks stage IV (intermediate AMD) to VI (severe) AMD as monocyte-derived. In high-resolution light microscopy studies [1, 2], Curcio and colleagues identified spherical subretinal cells as “sloughed” retinal pigment epithelium (RPE) cells since they contain RPE granules similar in morphology and autofluorescence patterns to granules in the RPE cells. Pathologists often encounter phagocytic cells engulfing melanin pigment (“melanophages”) in the skin [3] and eye [4] and have found the morphology of melanosomes and melanin granules to be that of the tissue of provenance. Therefore, extreme caution is required before using pigment granule characteristics to classify the pigmented CD163+ subretinal cells.

Curcio and Ach cite the manuscript of Sennlaub and collaborators [5] as evidence that the inflammatory marker CCR2 can be present within the RPE layer. However, the paper by Sennlaub et al. agrees with our histopathology findings, detailing the presence of monocytic cells (CCR2+) in the subretinal and sub-RPE space in human eyes with AMD.

We acknowledge the significant technical challenges in differentiating between migrated, “sloughed” RPE cells and pigment-laden macrophages. A conclusive answer cannot be obtained solely from morphological classification but requires immunohistochemical analysis of antigens specific for RPE and monocytes/macrophages. Thus, we have now performed immunohistochemical staining of two postmortem human eyes with GA and two eyes with early choroidal neovascularization (CNV) displaying pigmented subretinal cells. We stained serial sections of the eyes using monoclonal antibodies to CD163 (clone 10D6)

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and cytokeratin 8 (clone CAM5.2). Cytokeratins (CK) 8 and 18 are present in human RPE cells [6, 7] but not in any immune cells including monocytes [8]. Immunostaining employed a Ventana Discovery Ultra automated staining system (Tucson, AZ) and the Discovery Red Kit for detection, as immunofluorescent co-localization of CAM5.2 and CD163 would be confounded by the strong autofluorescence displayed by RPE cells. Normal human RPE was CK8+ and CD163-, as expected (Figs. 1a and b). Towards the edge of areas of GA or CNV, there was usually abrupt loss of CK8 within RPE cells, with occasional plump CD163+ cells interspersed among RPE cells (Figs. 1c and d). Pigmented cells insinuated between the RPE and Bruch's membrane were CK8- but CD163+ both at the periphery (Figs. 1e and f) and center (Figs. 1g and h) of GA and CNV. While occasional cells beneath the neurosensory retina were CK8+ (Fig. 1g), most were CD163+ (Fig. 1h). Our new results support the proposition that subretinal pigmented cells are macrophages that have engulfed melanin granules. However, since we have now shown for the first time that many RPE cells in GA and CNV are CK8-, additional studies are needed to identify RPE markers that are not lost in AMD and that can be employed for two-color co-localization.

We fully agree with Curcio and Ach that additional work to define the pigmented cells is essential to answer this question of central importance for AMD pathobiology and treatment development. This is critical since both monocytes [9] and RPE [10] may derive from human stem cells recruited to the eye during injury.

References

1. Rudolf M, Vogt SD, Curcio CA, Huisinck C, McGwin G Jr, Wagner A, Grisanti S, Read RW. Histologic basis of variations in retinal pigment epithelium autofluorescence in eyes with geographic atrophy. *Ophthalmology*. 2013; 120:821–828. [PubMed: 23357621]
2. Zanzottera EC, Messinger JD, Ach T, Smith RT, Curcio CA. Subducted and melanotic cells in advanced age-related macular degeneration are derived from retinal pigment epithelium. *Invest Ophthalmol Vis Sci*. 2015; 56:3269–3278. [PubMed: 26024109]
3. Schamburg-Lever G, Lever WF. Electron microscopy of incontinentia pigmenti. *J Invest Dermatol*. 1973; 61:151–158. [PubMed: 4200407]
4. Fineman MS, Eagle RC, Shields JA, Shields CL, De Potter P. Melanocytomalytic glaucoma in eyes with necrotic iris melanocytoma. *Ophthalmology*. 1998; 105:492–496. [PubMed: 9499781]
5. Sennlaub F, Auvynet C, Calippe B, Lavalette S, Poupel L, Hu SJ, Dominguez E, Camelo S, Levy O, Guyon E. CCR2+ monocytes infiltrate atrophic lesions in age-related macular disease and mediate photoreceptor degeneration in experimental subretinal inflammation in Cx3cr1 deficient mice. *EMBO Mol Med*. 2013; 5:1775–1793. [PubMed: 24142887]
6. Owaribe K, Kartenbeck J, Rungger-Brändle E, Franke WW. Cytoskeletons of retinal pigment epithelial cells: interspecies differences of expression patterns indicate independence of cell function from the specific complement of cytoskeletal proteins. *Cell Tissue Res*. 1988; 254:301–315. [PubMed: 3058315]
7. Kasper M, Moll R, Stosiek P, Karsten U. Patterns of cytokeratin and vimentin expression in the human eye. *Histochemistry*. 1988; 89:369–377. [PubMed: 2457569]
8. Grossniklaus HE, Ling JX, Wallace TM, Dithmar S, Lawson DH, Cohen C, Elner VM, Elner SG, Sternberg P Jr. Macrophage and retinal pigment epithelium expression of angiogenic cytokines in choroidal neovascularization. *Mol Vis*. 2002; 8:119–126. [PubMed: 11979237]
9. Shepard JL, Zon LI. Developmental derivation of embryonic and adult macrophages. *Curr Opin Hematol*. 2000; 7:3–8. [PubMed: 10608497]

10. Klimanskaya I, Hipp J, Rezai KA, West M, Atala A, Lanza R. Derivation and comparative assessment of retinal pigment epithelium from human embryonic stem cells using transcriptomics. *Cloning Stem Cells*. 2004; 6:217–245. [PubMed: 15671670]

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Fig. 1.

Normal RPE in the eyes with GA or CNV were CK8+ (a) and CD163- (b). At the periphery of the GA and CNV, there was usually abrupt loss of CK8 expression (c) with a few rounded CD163+ cells on the inner surface of Bruch's membrane (d). Sloughed RPE cells beneath artifactually detached neurosensory retina were CK8+ (c), but the majority of the cells beneath the neurosensory retina were CD163+ (d). At the peripheral edge of GA in an area where the overlying RPE had retained CK8 expression, pigmented cells insinuated between the RPE and Bruch's membrane (arrowhead) were CK8- (e) and CD163+ (f). The pigmented cells at the arrowheads are shown at higher magnification in the insets. In the center of GA and CNV, cells morphologically resembling RPE cells were CK8- (g), while plumper cells resembling macrophages (arrowhead) and microglial cells were CD163+ (h). A plump cell containing pigment and resembling a macrophage is shown at higher magnification in the insets. All photomicrographs are at the same magnification (bar = 100 μ m) as are the insets (bar = 25 μ m)