Resident Macrophage Lookalikes of Unexpected Origin

Jean-Pierre Levraud, Philippe Herbomel

Title : **Resident macrophage lookalikes of unexpected origin**

Jean-Pierre Levraud[1,*] and Philippe Herbomel[1,*]
[1]Department of Developmental & Stem Cell Biology, Institut Pasteur, CNRS-UMR3738, Paris, 75015, France
*Correspondence: jlevraud@pasteur.fr (J.L.), herbomel@pasteur.fr (P.H.)

Abstract

In this issue of *Developmental Cell*, Lin et al. (2019) identify in zebrafish skin macrophage-like cells that sample the environment through trans-epithelial protrusions and import antigen from the water for traditional tissue-resident macrophages. Remarkably, these "metaphocytes" originate from the epidermis, challenging current assumptions about the lineage of tissue-resident macrophages.

Tissue-resident macrophages / dendritic cells (DCs) play key roles in local homeostasis, detection of foreign material, and repair. For historical reasons, these cells bear different names according to their tissue of residence: for example, microglia in the brain, Küpffer cells in the liver, and Langerhans cells in the epidermis. Since 1968 (van Furth and Cohn, 1968) and over the next 40 years, the prevailing view had been that all of these cells arise from mesoderm-derived hematopoietic progenitors, and more precisely from hematopoietic stem cell (HSC)-derived monocytes continually produced in the bone marrow. Then, over the last decade, following the work of a few pioneers around 1990, the second part of this model was deeply revised, with the demonstration that most tissue-resident macrophages in adult mice do not derive from HSC/monocytes, but from hematopoietic progenitors born in the yolk sac, that colonized tissues during embryogenesis and then self-renewed there throughout life (Ginhoux and Guilliams, 2016; Perdiguero and Geissmann, 2016). Yet even in this revised, current model, all tissue macrophages were still mesoderm-derived. This view may have to be reconsidered in the light of the findings reported in this issue of *Developmental Cell* from Lin et al. (2019).

Alongside mice, zebrafish has been an important vertebrate model to pinpoint the different hematopoietic waves and their progeny (Robertson et al., 2016). However, emphasis has largely been put on the transparent embryonic and larval stages, and the macrophages and DC populations of the adult zebrafish are still poorly characterized. DCs – functionally defined by their ability to present antigen to and prime naive T cells, requiring, among others, expression of MHC class II genes – have been shown to exist in zebrafish (Lugo-Villarino et al., 2010). Moreover, with the help of fluorescent reporter zebrafish lines driven by various promoters, cells with dendritic morphology and high MHC class II expression have been observed in several organs (Martins et al., 2019), notably in the skin, which remains easy to image in adult zebrafish, as pigment cells are located underneath the epidermis. The *mpeg1* promoter (Ellett et al., 2011) is currently the most commonly used promoter to fluorescently highlight macrophages in larval and adult zebrafish.

In an earlier work, the authors had already addressed the origin of epidermal resident myeloid cells (Langerhans cells) of the zebrafish (He et al., 2018). Their fate-mapping approach was based on temporally and locally controlled (via tamoxifen-dependence and targeted laser heating) activity of Cre recombinase, inducing red to green switch of a *mpeg1*-driven fluorescent reporter. This approach revealed that most skin *mpeg1*-expressing cells descended from definitive HSCs arising from the ventral floor of the dorsal aorta (VDA), but

not from embryonic macrophage progenitors in the yolk sac or posterior blood island. These HSC-derived *mpeg1*+ cells were distributed evenly in the entire epidermis, and present in other organs and in the blood.

Surprisingly however, in a substantial subset of zebrafish subjected to VDA-targeted laser-heating, Cre-switched cells were present in only some skin patches, and absent from the blood. This observation, which may have been disregarded as an experimental glitch, was instead investigated in more detail in a set of elegant experiments that led to the outstanding discovery reported in this issue of *Developmental Cell*.

Lin et al. (2019) hypothesized that these spatially restricted *mpeg1*+ cells could arise from collaterally laser-irradiated cells while targeting HSC precursors in the VDA. They indeed found that these cells also appeared after targeting dorsal regions devoid of any hematopoietic cell at this stage. Furthermore, the distribution of these cells in the adult skin matched the region irradiated in the embryo. Mid-blastula cell transplantation assays then showed that presence of donor-derived spatially restricted *mpeg1*+ skin cells (but not of universally distributed ones) required the presence of donor epidermal cells in the same region. Finally, by using epidermis- or mesoderm-driven CreERT2 transgenes, they confirmed that the population of skin *mpeg1*+ cells could be subdivided in two subsets: 70% were mesoderm-derived conventional Langerhans Cells (cLCs), with circulating counterparts, while the remaining 30%, designated myeloid-like cells (MLCs), were ectoderm-derived and found exclusively in the skin.

Lin et al. (2019) then compared the properties of the two skin cell subsets. Morphologically they looked identical. Transcriptionally, they were very similar – much closer to each other than to T cells or keratinocytes – but distinguishable with proper markers. The authors observed a strong difference in cell mobility by time-lapse imaging in scale explant assays: whereas MLCs were spontaneously more mobile than cLCs, only the latter were attracted to a wound or a bacterial infection. Remarkably, upon adding labelled ovalbumin to the fish water and monitoring its internalization in the skin, MLCs were the first to acquire OVA, in just a few hours. However, over a few days of chase, the signal disappeared from MLCs and appeared in cLCs, with evidence suggesting that cLCs engulfed apoptotic antigen-laden MLCs. High-resolution 3D imaging of skin indicated that MLCs sent protruding dendrites between the superficial keratinocytes, reaching the external mucus layer – much like CX3CR1+ DCs in the mammalian gut (Niess et al., 2005). This strongly suggests that MLCs sampled antigens from the outside environment, which led the authors to rename them “metaphocytes” referring to their role as antigen carriers.

The findings of Lin et al. (2019) immediately raise a question: in mammals, are there also myeloid-like cell subsets of ectodermal origin? Despite a considerable body of work in rodents, this remains possible, considering the caveats of irradiation and bone-marrow transplantation, and that the more recent retrospective fate mapping studies have focused on known hematopoietic lineages (Ginhoux and Guilliams, 2016; Perdiguero and Geissmann, 2016). If anything, transcriptome-based cellular “phylogenies” should not be taken as proof of ontogeny, as shown in this study by the similar gene expression of ectoderm-derived metaphocytes and mesoderm-derived Langerhans cells. There are precedents for such situations: among cells of ectodermal origin, neural crest cells are well known to be able to

differentiate into cell types primarily derived from mesoderm, such as chondrocytes or smooth muscle cells. Could metaphocytes also derive from neural crest? This seems unlikely, considering the shared localization of donor-derived keratinocytes and metaphocytes following mid-blastula transplantation, but it remains to be more directly examined.

There are also many exciting questions regarding the functions and behavior of metaphocytes for future exploration. Why would these cells express high levels of MHC class II if they only serve to transfer antigen to cLCs? How do they maintain their population; is there a metaphocyte-generating stem cell, and does it produce other epidermal cells? Why are they so spatially restricted if they are so mobile? Considering this mobility, how do they manage to maintain transepithelial dendrites with tight junctions? Their analysis will probably illuminate the workings of cells much harder to visualize in vivo, such as the CX3CR1+ DCs of the mammalian gut.

REFERENCES

Ellett, F., Pase, L., Hayman, J.W., Andrianopoulos, A., and Lieschke, G.J. (2011). Mpeg1 Promoter Transgenes Direct Macrophage-Lineage Expression in Zebrafish. Blood *117*, e49-56.

van Furth, R., and Cohn, Z.A. (1968). The origin and kinetics of mononuclear phagocytes. J. Exp. Med. *128*, 415–435.

Ginhoux, F., and Guilliams, M. (2016). Tissue-Resident Macrophage Ontogeny and Homeostasis. Immunity *44*, 439-449.

He, S., Chen, J., Jiang, Y., Wu, Y., Zhu, L., Jin, W., Zhao, C., Yu, T., Wang, T., Wu, S., et al. (2018). Adult zebrafish Langerhans cells arise from hematopoietic stem/progenitor cells. Elife *7*, e36131.

Lin, X., Zhou, Q., Zhao, C., Lin, G., Xu, J., and Wen, Z. (2019). An Ectoderm-Derived Myeloid-Like Cell Population Functions as Antigen Transporters for Langerhans Cells in Zebrafish Epidermis Dev Cell *49*, this issue.

Lugo-Villarino, G., Balla, K.M., Stachura, D.L., Bañuelos, K., Werneck, M.B.F., and Traver, D. (2010). Identification of dendritic antigen-presenting cells in the zebrafish. Proc. Natl. Acad. Sci. U. S. A. *107*, 15850–15855.

Martins, R.R., Ellis, P.S., MacDonald, R.B., Richardson, R.J., and Henriques, C.M. (2019). Resident Immunity in Tissue Repair and Maintenance: The Zebrafish Model Coming of Age. Front. Cell Dev. Biol. *7*, 12

Niess, J.H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B.A., Vyas, J.M., Boes, M., Ploegh, H.L., Fox, J.G., et al. (2005). CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science *307*, 254–258.

Perdiguero, E.G., and Geissmann, F. (2016). The development and maintenance of resident macrophages. Nat. Immunol. *17*, 2–8.

Robertson, A.L., Avagyan, S., Gansner, J.M., and Zon, L.I. (2016). Understanding the regulation of vertebrate hematopoiesis and blood disorders – big lessons from a small fish. FEBS Lett. *590*, 4016-4033

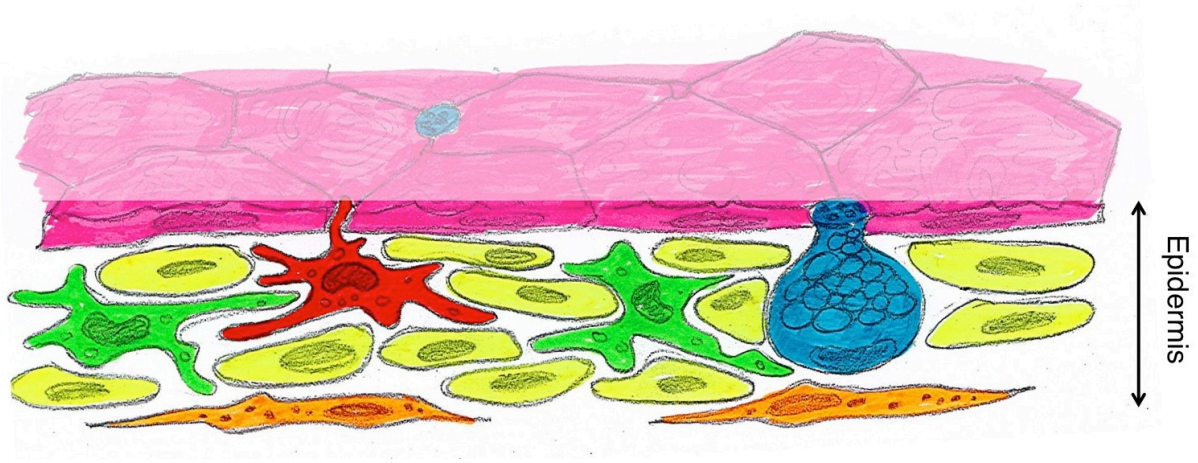

Figure 1. Macrophages in the zebrafish epidermis

In the zebrafish epidermis, Langerhans cells (green) and the very similar, but epidermis-derived, metaphocytes (red) wander amongst basal keratinocytes (yellow), above the pigment cells (orange) that overlay the dermis (not shown). Metaphocytes send protrusions between surface keratinocytes (pink), sample antigens trapped in the mucus layer secreted by goblet cells (blue & light blue) on the skin surface (light pink), and transfer these to Langerhans cells.