

INSIGHTS
Death, eaters, and dark marks

 Matthew Collin 

The maintenance of macrophage populations, role of environmental cues in shaping their physiology, and the response of resident cells to perturbation are critical factors in tissue homeostasis with implications for many pathological scenarios. Pigment-containing cells of the dermis are the latest to come under the scrutiny of a mouse-induced depletion model.

The inlaying of tattoo pigment has been described in human remains dating back several millennia. Captain Cook, observing the Tahitians, reported that tattoos were painful, indelible, and “performed but once in a lifetime.” In humans, tattoo pigment is contained in long-lived low proliferative dermal macrophages (Zaba et al., 2007; Haniffa et al., 2009), suggesting that tattoos might endure simply because cell turnover is very slow. However, in this issue, Baranska et al. show in mice that depletion of macrophages induces a new generation of cells that engulf but does not translocate pigment granules. Under physiological conditions, the uptake of melanin is a natural activity of dermal macrophages, transforming them into high side scatter melanophages. The ability to ingest pigment is not merely of cosmetic interest, however; the scavenger function of macrophages is fundamental to pathological processes such as neurodegeneration and atheroma. The homeostasis of terminally differentiated macrophage populations has an important bearing on whether phagocytic cargo triggers potentially harmful local inflammation or can be removed quiescently. In locations such as the neuropil or intima, the balance between these processes is critical.

Baranska et al. (2018) developed a CD64-DTR mouse in which administration of diphtheria toxin (DT) results in a profound depletion of all dermal (and gut) monocyte-derived and macrophage populations as defined by previous descriptions (Tamoutounour et al., 2013). This tool promises to be extremely useful in the study of macrophages in other sites and was demonstrated to be selective and nontoxic.

In addition to depletion of the known monocyte-derived cells and macrophages, a population of side scatter high cells that map to the CCR2⁺ MHCII⁺ macrophage pool was also ablated. These cells were only observable in ear of a black mouse (B6), where melanocytes in the epidermis and dermis distribute melanin to the interstitium; in other sites, the hair follicles are selectively melanized to make the coat, rather than the skin, pigmented. High side scatter was shown to be directly attributable to the presence of melanin granules, and the population was restored to baseline between 3 wk and 3 mo after ablation by DT, as new macrophages gradually ingested melanin. Pigmented macrophages are not unique to the skin, and the study raises a technical question of whether high scatter macrophage populations have previously been inadvertently excluded in the analysis of other tissues.

Tattooing followed by ablation revealed a similar cycle of pigment release and recapture. Baranska et al. (2018) found that melanophages were labeled by a CX3CR1 reporter and showed that at least 50% were exchanged in a CCR2-dependent fashion in parabiosis models, suggesting a continual process of renewal from monocyte-origin cells. The data do not completely exclude the persistence of a population of prenatally derived melanophages in the steady state, but opening the niches with induced depletion clearly draws in bone marrow (BM)-derived precursors. Compared with nonmelanized macrophages, melanophage gene expression was enriched in annotations relating to aryl hydrocarbon receptor signaling, glutathione-mediated detoxification, and phagosome maturation, owing to the high



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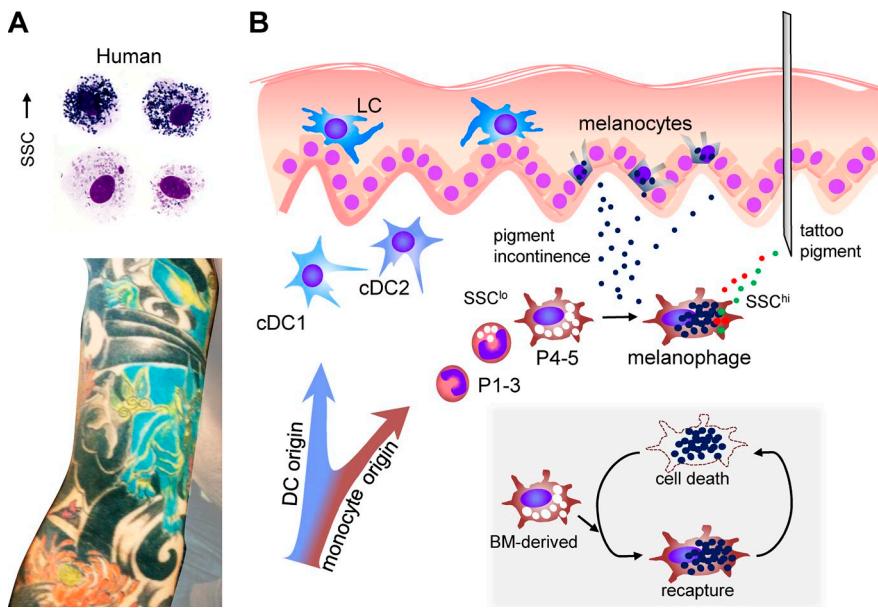
expression of a glutathione S-transferase gene, a lysosomal ATPase, and cysteine protease. Notably, they were also negative for Lyve-1, which is known to mark a subset of dermal macrophages associated with lymphatic endothelium (Wang et al., 2014).

The description of melanophage homeostasis is another example of BM-derived monocyte-lineage cells replacing tissue macrophages under conditions of minimal inflammation. A foundation macrophage population is laid down in prenatal life by early myeloid progenitors from the yolk sac, fetal liver monocytes, or definitive hematopoietic stem cells (Ginhoux and Guilliams, 2016). Foundation macrophages established at birth are renewed from BM-derived precursors to different degrees depending on the anatomical site and the level of perturbation (Bain et al., 2016; Scott et al., 2016; van de Laar et al., 2016). The skin, gut, and serous cavity are notable for relatively high rates of CCR2-dependent macrophage turnover in

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(A) In parallel with the mouse, human dermal macrophages show side scatter (SSC) low and high properties, depending on the level of melanin ingestion, as indicated by sorting, cytopsin, and Giemsa stain (top). Tattoo ink remains intact after bone marrow (BM) transplantation, as illustrated by the highly decorated arm of a patient 2 yr after transplantation (bottom). **(B)** Schematic showing dendritic cell (DC)- and monocyte-related populations of the skin of mice and the maturation of melanophages from P4-5 macrophages after uptake of melanin or pigment. Inset illustrates the cycle of cell death and pigment recapture proposed by Baranska et al. Homologous populations of monocyte-derived cells and melanophages are also found in humans. LC, Langerhans cell.

the steady state (Tamoutounour et al., 2013; Bain et al., 2014, 2016), although dermal MHCII⁺ macrophages and melanophages have among the slowest kinetics (Bain et al., 2016; Baranska et al., 2018). At the other end of the spectrum, alveolar macrophages and Kupffer cells are robustly self-maintaining but can be restored from the BM after depletion, most likely by monocytes (Scott et al., 2016; van de Laar et al., 2016). In recent years, it has often been overlooked that the original authors of the mononuclear phagocyte system did not categorically state that all tissue macrophages were monocyte derived. Two populations of macrophages were described: “free” and “fixed.” The latter were known to proliferate *in situ* and were described as “probably also of monocytic origin, but definitive proof on this point has not yet been obtained” (van Furth et al., 1972).

In humans, a little information on macrophage homeostasis in the dermis may be gleaned from BM transplantation (Haniffa et al., 2009) and genetic conditions in which monocytes are absent, such as reticular dysgenesis and GATA2 and IRF8 mutation (Bigley et al., 2017). Melanophage turnover occurs but is slower than in the mouse with a half-life of ~6 mo after transplantation. At least half of dermal macrophages depend on monocytes because their numbers are de-

creased to ~30% in severe moncytopenia, roughly consistent with the fraction that remains in CCR2^{-/-} mice. Tattoos are not disrupted by BM transplantation, even many years later, and although the kinetics may be species dependent, both observations are consistent with a model in which a major population of human dermal macrophages is dependent on BM-derived precursors.

The results of Baranska et al. (2018) might be perceived as bad news for tattoo removal, but elucidation of the cell turnover process in this *in vivo* model now opens the way for rational testing of agents that attenuate or block the cycle of reuptake by a new generation of cells. Tattoo removal is dependent on the selective absorption of pulsed laser light and subsequent fragmentation of the pigment granules. This is increasingly challenging because the monochrome art form of Polynesia has become a fully-fledged polychromasia, requiring multiple laser bandwidths. Laser treatment may still be required to initiate a renewal cycle, but topical or systemic therapy that inhibits the recruitment of macrophages could prove strongly synergistic. Liposomal clodronate, which is toxic to phagocytes, is already under development as a potential tattoo removal cream. Postinflammatory hyperpigmentation caused by increased melanin loading in

the dermis resulting from pigment incontinence is a distressing consequence of acne that could benefit similarly.

The model also opens opportunities to ask more fundamental questions, such as the signals that keep macrophages fixed and the impact of phagocytosis on macrophage gene regulation and physiology. It is possible that fixed macrophages focus tissue immune responses by retaining antigen at a discrete location. However, in pathology, the recalcitrance of macrophages to move along and dispose of their phagocytic cargo in less critical environs is of major interest in the development of neurodegeneration, atheroma, granulomatous disease, and fibrosis. The mechanisms that lead to macrophage accumulation in atheroma and the potential of cellular egress to promote plaque resolution have been extensively discussed (Tabas and Lichtman, 2017). Several recent studies have also highlighted the connection between genome-wide association study hits for neurodegenerative disease, gene expression, and function of microglia (Hansen et al., 2018). Phagocytosis itself induces context-dependent differential gene regulation in specific populations of macrophages (A-Gonzalez et al., 2017). With these studies in mind, it is clear that understanding the molecular cues that fix macrophages to a niche, the regulation of flux from their more numerous and transient precursors, and the environmental signals that influence their responses will continue to have far-reaching consequences for medicine.

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