Developmental Cell
Commentary

Creating a Buzz about Macrophages: The Fly as an In Vivo Model for Studying Immune Cell Behavior

Helen Weavers[1,2] and Will Wood[1,*]
[1]School of Cellular and Molecular Medicine, Faculty of Biomedical Sciences, University of Bristol, Bristol BS8 ITD, UK
[2]School of Biochemistry, Faculty of Biomedical Sciences, University of Bristol, Bristol BS8 1TD, UK
*Correspondence: w.wood@bristol.ac.uk
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Drosophila macrophages exhibit functional parallels with their vertebrate counterparts in both their early developmental roles and later diverse roles in health and disease. This, together with the fly's genetic tractability and opportunities for live imaging, has recently established *Drosophila* as a powerful model to study macrophage behavior in vivo.

Macrophage Migrations and Developmental Functions

Fly macrophages share striking parallels with their vertebrate counterparts, in terms of both their migrations within the embryo and their functions during development. This, together with the optical translucency of *Drosophila* embryos, enabling high-resolution in vivo imaging of macrophage behavior, makes the fly an ideal system in which to study macrophage developmental functions (Figure 1). The fly's embryonic macrophages (often referred to as plasmatocytes or, more generally, hemocytes) are derived from the anterior head mesoderm of the developing embryo (Evans and Wood, 2014). After birth, embryonic macrophages disperse from this point of origin to distribute themselves throughout the embryo, such that at the end of embryogenesis they are evenly distributed throughout the body (Figure 2). This is achieved through a developmentally hard-wired pattern of migrations that are orchestrated at least in part by chemotactic signals provided by members of the PDGF/VEGF or Pvf family of growth factors. These migrations direct macrophages along a number of specific routes, where they are critical for the correct development of many of the tissues and organs with which they come into contact (Evans and Wood, 2014).

One migratory route chosen by a subset of macrophages takes them from the head mesoderm into the extended germband. This invasive migration across the germband resembles transepithelial migration of vertebrate immune cells. In the fly, this migration is dependent on integrin function regulated by the GTPase Rap1 (Ratheesh et al., 2015), and both of these are required for the transepithelial migration of vertebrate neutrophils and monocytes out of the vasculature. Macrophages that have moved across the yolk sac and into the extended germband come into contact with the fly equivalent of the developing kidney, the renal or Malpighian tubules, where they play a key role in influencing the development of this critical organ. Macrophages secrete collagen IV around the renal tubules, which is essential for the effective activation of BMP signaling within the tubule buds that in turn directs the outgrowth and positioning of these organs within the body cavity (Bunt et al., 2010). The role of *Drosophila* macrophages in the correct BMP-regulated development of the renal tubules is reminiscent of the requirement of mouse macrophages in regulating BMP-driven mammary gland branching morphogenesis or instances in which mouse macrophages infiltrate the developing kidney interstitium, stimulating growth and uteric bud branching (reviewed in Pollard, 2009).

Another well-conserved key developmental function of macrophages in the fly is the clearance of apoptotic cells within the developing embryo. As is the case in vertebrates, *Drosophila* macrophages are the professional phagocytic cell within the animal and are responsible for efficiently engulfing and degrading the large number of apoptotic corpses generated during normal developmental tissue sculpting. The mechanisms by which macrophages detect, engulf, and degrade apoptotic corpses have been intensively studied, and this has been covered in many excellent recent reviews (Shklover et al., 2015) so will not be covered in detail here; but again, the mechanisms underlying each of these stages of apoptotic processing show strong conservation from the fly to vertebrates, with *Drosophila* macrophages using evolutionarily conserved scavenger receptors such as the homolog of vertebrate CD36, croquemort, and the CED-1 homolog Draper—among others—to recognize their apoptotic prey (reviewed in Shklover et al., 2015).

Later in development, once corpse engulfment is complete, *Drosophila* macrophages disperse into their final positions using contact inhibition of locomotion (CIL); by exploiting the live-imaging capabilities of the fly, recent work in *Drosophila* has begun to shed light on the mechanisms regulating CIL in vivo. In *Drosophila* macrophages, contact between neighboring macrophages triggers a repolarizing event such that macrophages actively migrate away from one another, ensuring that macrophages are evenly spread across the embryo by the end of embryogenesis (reviewed in Evans and Wood, 2014). One recent study has revealed mechanistic insight into this process and demonstrated that the rapid migration away from a neighboring cell is driven by the sudden release of tension that builds up at the interface between two colliding cells (Davis et al., 2015).

In the last year, *Drosophila* has also emerged as a valuable system in which

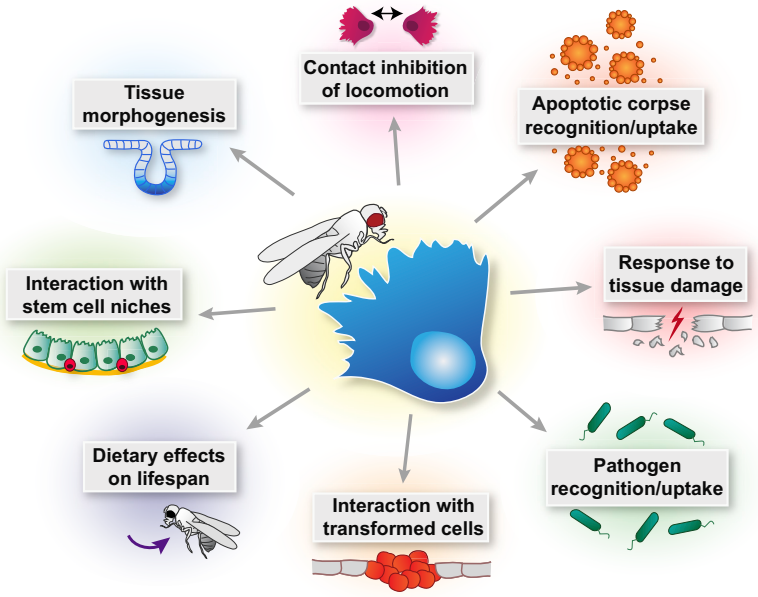

Figure 1. The Fly Is a Powerful In Vivo Model to Study Diverse Macrophage Functions
Drosophila macrophages have been used to study multiple functions in vivo, ranging from their response to various cues (including those produced by apoptotic corpses, damaged tissue, pathogens, and transformed cells) to communicating with other tissues to help direct tissue morphogenesis or maintain stem cell niches. They also sense and respond to each other, exhibiting contact inhibition of locomotion during development. These impressive multi-tasking cells have also recently been shown to play a role in mediating dietary effects on lifespan.

to study the role that macrophages play on the maintenance of stem cell niches. In the adult fly, macrophages regulate stem cell activity both in the intestine (Ayyaz et al., 2015) and in the ovaries (Van De Bor et al., 2015). In the intestine, fly macrophages respond to tissue damage by inducing Dpp-dependent proliferation of intestinal stem cells to regenerate and restore the integrity of the intestinal barrier, which protects the fly against further infection (Ayyaz et al., 2015). In the fly female gonad, macrophages that are tightly associated with the developing ovaries deposit collagen IV in a basement membrane around the stem cell niche, which is required for the BMP-dependent regulation of stem cell number and organization (Van De Bor et al., 2015). These and other developmental parallels are covered in more detail in recent reviews (Ratheesh et al., 2015), but it is becoming clear that the fly can provide a powerful model to inform vertebrate studies as to how macrophages can influence the development of many tissues both within the embryo and later during adult life.

Detecting Damage, Disease, and Infection

Drosophila macrophages, in addition to their important roles during development, play an important "sentinel" function within the immune system, where they efficiently protect the individual against invading pathogens. Again, by exploiting the live-imaging capabilities and genetic tractability of *Drosophila*, the fly embryo has become established as a valuable model to study these host-pathogen interactions in vivo. Using time-lapse imaging, researchers have been able to closely follow the processes by which fly macrophages recognize and phagocytose bacterial pathogens that have been introduced into the extracellular space and begun to dissect the molecular mechanisms and hormonal signaling controlling the immune cell response to host infection (Vlisidou and Wood, 2015).

However, not only do fly macrophages detect "non-self," but studies over the past decade have also discovered that these immune sentinels—just like their vertebrate counterparts—can also detect and be recruited to "altered or damaged self," such as sites of tissue damage or tumors. Perhaps the best characterized of these damage responses is the embryonic macrophage's rapid inflammatory-like chemotactic response toward wounds (reviewed in Evans and Wood, 2014). We now know a considerable amount about the immediate signaling that triggers the recruitment of macrophages to a wound in the fly, and once again the mechanism shows strong conservation through to vertebrates. A recent study has gleaned additional insight from the time-lapse imaging of macrophage behavior by employing novel computational analysis on the spatiotemporal inflammatory response to wounding. This analysis has revealed new details of the properties and behavior of the wound chemoattractant signal that is released upon tissue damage to draw macrophages into the wound site (Weavers et al., 2016a).

Another recent study where the fly has provided a significant advance in our understanding of the wound inflammatory response involves the process of innate immune priming, or "trained immunity." Emerging evidence from vertebrate studies has demonstrated that innate immune cells can develop a form of "immunological memory," a trait previously associated with the adaptive system alone. New work in the fly has revealed the existence of this innate immune memory in *Drosophila*, where the phagocytosis of apoptotic cells by macrophages is an essential primer for their subsequent inflammatory response to tissue damage and infection (Weavers et al., 2016b). Both fly and vertebrate studies reveal a mechanism whereby, through changing levels of PAMP and DAMP receptors on their surface, macrophages are able to build a memory of their previous encounters and thus reshape their response to subsequent insults.

The wound response in *Drosophila* has also been studied during larval stages, which has provided additional insight into the mechanisms by which macrophages adhere to damaged tissue in vivo. At the larval stage of fly development, the primitive fly heart has begun to beat and macrophages are therefore pumped around the extracellular space within the larva. These circulating larval macrophages can be captured at sites of wounding by a process that resembles the rolling and tethering of vertebrate