Intrinsic and Extrinsic Regulation of Hematopoiesis in *Drosophila*

Ferdinand Koranteng¹,4, Bumsik Cho¹,4, and Jiwon Shim¹,²,³,*

¹Department of Life Science, Hanyang University, Seoul 04763, Korea, ²Research Institute for Natural Science, Hanyang University, Seoul 04763, Korea, ³Research Institute for Convergence of Basic Sciences, Hanyang University, Seoul 04763, Korea, *These authors contributed equally to this work.
*Correspondence: jshim@hanyang.ac.kr
https://doi.org/10.14348/molcells.2022.2039
www.molcells.org

*Drosophila melanogaster* lymph gland, the primary site of hematopoiesis, contains myeloid-like progenitor cells that differentiate into functional hemocytes in the circulation of pupae and adults. Fly hemocytes are dynamic and plastic, and they play diverse roles in the innate immune response and wound healing. Various hematopoietic regulators in the lymph gland ensure the developmental and functional balance between progenitors and mature blood cells. In addition, systemic factors, such as nutrient availability and sensory inputs, integrate environmental variabilities to synchronize the blood development in the lymph gland with larval growth, physiology, and immunity. This review examines the intrinsic and extrinsic factors determining the progenitor states during hemocyte development in the lymph gland and provides new insights for further studies that may extend the frontier of our collective knowledge on hematopoiesis and innate immunity.

**Keywords:** *Drosophila* hematopoiesis, hemocyte differentiation, inter-organ regulation, lymph gland, niche regulation, progenitor cell maintenance

**INTRODUCTION**

*Drosophila* hematopoiesis is similar to that of vertebrates (Crozatier and Vincent, 2011; Evans et al., 2003). Vertebrates exhibit two waves of hematopoiesis: the first wave, called primitive hematopoiesis, occurs in the yolk sac of the developing embryo at 7.5 days and 17 days post-coitum for mice and humans, respectively (Baron et al., 2012; Evans et al., 2003; Swain et al., 2014). Similarly, flies undergo the primitive wave as early as 2 h after gastrulation in the procephalic mesoderm of the embryo (Crozatier and Meister, 2007; Tepass et al., 1994). Definitive hematopoiesis, which is the second wave, leads to the generation of hematopoietic stem cells in vertebrates (Durand and Dzierzak, 2005; Dzierzak and Bigas, 2018). Hematopoietic stem cells generated in the second wave of hematopoiesis in the aorta-gonad-mesonephros are essential for the maintenance of the blood system in adult vertebrates (Orkin and Zon, 2002; Swain et al., 2014). Notably, in mice, the transition from primitive to definitive hematopoiesis occurs at about 12.5 days post-coitum in the fetal liver, and the definitive phase completely replaces the primitive wave (Crozatier and Vincent, 2011; Evans et al., 2003; Swain et al., 2014). In invertebrates such as the fruit fly, the lymph gland (LG), the organ where the second wave of blood cell development occurs, begins to form around stage 5 of the embryonic development; however, it becomes more prominent at stage 17, where it is observed as a kidney-like structure flanking either side of the dorsal vessel, an organ that behaves like the heart muscles in pumping hemocytes to maintain their circulation (Banerjee et al., 2019; Krzemien et al., 2010a; Lo et al., 2002; Rugendorff et al., 1994).
**Drosophila melanogaster** is a holometabolous organism and undergoes the following four stages of complete transformation: embryo, larva, pupa, and adult; the LG continues to develop well after the embryonic stage until pupariation under normal conditions (Banerjee et al., 2019; Snodgrass, 1954).

During the initial wave, a group of cells called plasmatocytes, which usually occupy ~95% of the total *Drosophila* blood population, are observed in circulation and perform functions that are analogous to those of vertebrate macrophages (Holz et al., 2003; Hontí et al., 2014; Rizki, 1957; Tepass et al., 1994). Crystal cells, named so due to crystalline inclusions within their cytosol, make up the remaining 5% of the fly blood population (Meister and Lagueux, 2003; Rizki, 1957; Shrestha and Gateff, 1982). They participate in immune responses and wound healing and are sensitive to changes in ambient O2 (Cho et al., 2018; Lebestky et al., 2014; Mukherjee et al., 2011; Rizki and Rizki, 1959). Notably, a third group of blood cells, called lamellocytes, exists in the fly blood system, but these are rarely observed under normal conditions (Lanot et al., 2001; Rizki, 1957). Nonetheless, when larvae are challenged by wasps, such as *Leptopilina boulardi*, which are their natural enemy, the LG produces a copious amount of lamellocytes and releases them into the circulation to mount an immune defense against the deposited wasp eggs (Carton et al., 2008; Keebaugh and Schlenke, 2014; Lemaitre and Hoffmann, 2007; Russo et al., 1996).

The first wave of *Drosophila* hematopoiesis produces hemocytes that are functional at the embryonic and larval stages, and thereafter, the second-wave blood cells largely operate at the pupal and adult stages (Holz et al., 2003; Hontí et al., 2014; Lanot et al., 2001; Wood and Jacinto, 2007). A subset of hemocytes originating from the primitive wave is housed and maintained in segmental regions of the larval body wall called hematopoietic pockets. Blood cells in these pockets are released into the circulation on demand and can proliferate or differentiate into the other two cell types (Banerjee et al., 2019; Leitao and Sucena, 2015; Makhijani and Brückner, 2012; Márkus et al., 2009; Petraki et al., 2015). Recent studies have shown that the two groups of hemocytes survive to the adult stage with the ratio of primitive to definitive blood cells being approximately 3:2. Interestingly, no signs of hematopoiesis have been observed in the adult fly (Sanchez Bosch et al., 2019).

The complex system of hematopoiesis in flies is governed by several intrinsic and extrinsic factors that ensure that blood development is coherent with the homeostatic needs and developmental growth of the organism. This review aims to summarize the intrinsic and extrinsic regulators of *Drosophila* definitive hematopoiesis to provide novel insights for advancing the field.

**DROSOPHILA LYMPH GLAND**

At full maturity under normal conditions, the LG consists of four pairs of lobes on either side of the dorsal vessel: the first is called the primary lobe and lies anteriorly, while the second, third, and fourth are commonly called posterior lobes (Fig. 1) (El Shatoury, 1955; Jung et al., 2005; Krzemien et al., 2010b). The pairs of lobes are separated by pericardial cells, which are akin to nephrocytes in mammals, and the dorsal vessel (Crossley, 1972; Lo et al., 2002; Mills and King, 1965; Na and Cagan, 2013). The most inward zone and closest to the dorsal vessel in the primary lobe is the medullary zone, which consists of progenitors that give rise to plasmatocytes and crystal cells of the outermost cortical zone (Jung et al., 2005; Krzemien et al., 2010b; Sorrentino et al., 2002). An intermediate zone consisting of cells expressing markers for both the medullary and cortical zones was identified using genetic tracing experiments (Krzemien et al., 2010b; Spratford et al., 2021). With recent advances in single-cell RNA sequencing technology, significant heterogeneity of the progenitor population and endogenous gene expression have been established in the LG (Cho et al., 2020; Girard et al., 2021; Spratford et al., 2021). The complexity of hemocytes and mechanisms controlling multiple developmental paths in the LG hematopoiesis require further investigation. Nearest to the first pair of pericardial cells is a small cluster of cells called the posterior signaling center (PSC), which provides a microenvironment for progenitor maintenance (Krzemien et al., 2007; Minakhina and Steward, 2010). Recently, cardiac tubes have been shown to function as a second niche similar to that observed in vertebrates (Destalminil-Letourneau et al., 2021; Morin-Poulard et al., 2016).

Whereas previous findings on the LG were associated
with the primary lobe, recent studies have elucidated the composition and role of the other pairs of lobes. Posterior LG lobes are made up of progenitors that differentiate under specific stress conditions such as wasp infestation (Banerjee et al., 2019; Krzemień et al., 2007; Lan et al., 2020; Lanot et al., 2001; Letourneau et al., 2016). Rodrigues et al. (2021) revealed heterogeneity of posterior progenitors and characterized useful genetic tools to further study the population. In addition, Kanwal et al. (2021) highlighted the significance of Ultrabithorax (Ubx) and collier in posterior progenitor maintenance. These studies provide grounds for understanding the posterior lobe hematopoiesis; future studies will expand our knowledge regarding the posterior lobes and their function and proportion in the pupa and adult fly blood.

**INTRINSIC REGULATION OF HEMATOPOIESIS IN THE LYMPH GLAND**

Like vertebrate hematopoietic stem cells, LG progenitor cells require a niche microenvironment, called the PSC, that secretes various signaling factors for their maintenance (Mandal et al., 2007). At the first instar stage of larval development, Decapentaplegic (Dpp) signal from the PSC activates Notch in early progenitor cells. In the absence of Dpp, Notch-positive progenitor cells disappear, and the size of the LG decreases (Dey et al., 2016, 2019) (Fig. 2A). Serrate (Ser) is another Notch-activating regulator known to be expressed in the PSC. PSC-dependent Notch-Serrate signaling in progenitor cells is necessary to prevent their differentiation (Blanco-Obregon et al., 2020; Lebestky et al., 2003). In addition, the size of the PSC is reduced upon the expression of the dominant-negative form of Ser (Krzemień et al., 2007). These findings reveal the cell-autonomous and non-cell-autonomous functions of Notch in the PSC as well as the essential role of Notch in progenitor maintenance.

Progenitor cells express the Hh receptor patched (ptc), whose activation inhibits the cleavage of Cubitus interruptus (Ci) to attenuate their differentiation (Mandal et al., 2007; Fig. 2). In the late L3 (3rd instar) larvae, bnl and slit secreted from the cardiac tube (dorsal vessel) activate btl and robo, respectively. In the PSC, robo actuates dMyc while btl triggers JAK/STAT signaling via PLCγ, Ca²⁺ and CamKII in the MZ. Gap junctions contribute to the overall calcium levels in the MZ. In contrast to (A), N signaling in the MZ is propagated following interactions with Serrate (Ser) from the PSC while Relish (Rel) and collier (col) serve as inputs for Hedgehog signaling (HH). Pvr in the MZ is activated by traveling Pvf1 from the PSC. Active Pvr through STAT and Adgf-A regulates adenosine levels for MZ maintenance. Wnt/Wg signaling supports the MZ and inhibits Tiggrin (Tig) in the MZ while moderate levels of reactive oxygen species (ROS) as well as suppression of col by Jumu in the progenitors preserve the MZ. In posterior lobes, col maintains the posterior progenitors via Ultrabithorax (Ubx).

**Fig. 2. Intrinsic regulation of progenitor maintenance in the lymph gland.** (A) Early progenitors of the L1 (1st instar)/L2 (2nd instar) lymph gland are sustained by Decapentaplegic (Dpp), Notch (N), or Pvf2 signaling prior to emergence of the CZ. Secreted Pvf2 from a group of cells near the dorsal vessel that are scalloped (sd)-positive activates Pvr in the MZ which, in turn, triggers STAT signaling to ensure formation of proper lymph gland size. Also, Dpp from the PSC progresses N signaling for progenitor maintenance. (B) In the late L3 (3rd instar) larvae, bnl and slit secreted from the cardiac tube (dorsal vessel) activate btl and robo, respectively. In the PSC, robo actuates dMyc while btl triggers JAK/STAT signaling via PLCγ, Ca²⁺ and CamKII in the MZ. Gap junctions contribute to the overall calcium levels in the MZ. In contrast to (A), N signaling in the MZ is propagated following interactions with Serrate (Ser) from the PSC while Relish (Rel) and collier (col) serve as inputs for Hedgehog signaling (HH). Pvr in the MZ is activated by traveling Pvf1 from the PSC. Active Pvr through STAT and Adgf-A regulates adenosine levels for MZ maintenance. Wnt/Wg signaling supports the MZ and inhibits Tiggrin (Tig) in the MZ while moderate levels of reactive oxygen species (ROS) as well as suppression of col by Jumu in the progenitors preserve the MZ. In posterior lobes, col maintains the posterior progenitors via Ultrabithorax (Ubx).
Sharma et al., 2019) (Fig. 2B). Interestingly, Hh signal is transferred by filopodia from the PSC to progenitor cells and directly binds to the ptc receptor. In addition, in the PSC, Relish (Rel) is required for proper cytoskeletal structure, and thus, loss of Rel traps the Hh signal, which impacts progenitor maintenance (Mandal et al., 2007; Ramesh et al., 2021). PDGF- and VEGF-related factor 1 (Pvf1) is secreted from the PSC and travels farther to indirectly promote Ci stabilization through Pvr, a Pvf receptor, in the cortical zone. Pvr in the cortical zone activates adenosine deaminase-related growth factor A (Adgf-A) via STAT92E, and consequently, Adgf-A lowers adenosine in progenitor cells (Mondal et al., 2011). A moderate concentration of adenosine in the LG regulates Hh-dependent Ci activation, and both Adgf-A and Hh signals ensure proper levels of Ci for progenitor cell maintenance. Like Pvf1, Pvf2 from scalloped (sd)-positive progenitor cells close to the dorsal vessel activate hemocyte proliferation and regulate the size of the LG (Ferguson and Martinez-Agosto, 2017).

In addition to the PSC, the cardiac tube also functions as a niche (Fig. 2B). The cardiac tube secretes slit (Jones et al., 2007), a ligand, to the PSC, which is recognized by roundabout (robo) and activates CDC42 and dMyc for proper PSC development (Morin-Poulard et al., 2016). The FGF ligand in D. melanogaster, namely branchless (bnl), is also secreted from the cardiac tube. The bnl activates its receptor breathless (btl) in progenitor cells and maintains its calcium levels by activating PLCγ. Therefore, blocking bnl-btl interaction causes progenitor cell differentiation (Destalminti-Letourneau et al., 2021).

Drosophila early B cell factor, collier (col), is expressed in progenitor cells and the PSC, although its targets are segregated (Fig. 2B). The level of col expression in the PSC is higher (col^{up}) than in the progenitors (col^{iso}), but col expression in both regions supports progenitor maintenance. While progenitor-specific overexpression of col represses blood cell differentiation, expression of col RNAi in the same cells leads to robust and precocious differentiation of plasmatocytes. Loss of col in the PSC reduces Hh expression as well as filopodia extension, whereas overexpression of col in the PSC does not alter the size of PSC or the number of crystal cells (Benmimoun et al., 2015; Oyallon et al., 2016; Pennetier et al., 2012). These studies imply that col exhibits bifurcated signaling pathways in the PSC and progenitor cells despite its identical function. As an upstream of col, Jumeau (Jumu), a member of the forkhead transcription factor family, cell-autonomously maintains a low level of col cells and indirectly suppresses the number of PSC, collectively maintaining progenitor homeostasis (Hao and Jin, 2017). In addition to col, the Wnt/Wg signal is expressed in the progenitors. Secreted Wg from progenitor cells is recognized by the Wnt receptor, frizzled 2 (fz2), and it maintains the level of Drosophila E-Cadherin, shotgun (shg) (Sinienko et al., 2009). Overexpression of Wg or constitutive activation of Wnt signaling pathway by the expression of armadillo (arm), a Drosophila homolog of beta-catenin, inhibits progenitor cell differentiation. Consistently, upregulation of Wnt suppresses Tiggrin (Tig), a cortical zone marker, and keeps the medullary zone intact (Zhang et al., 2014). These results indicate significant cell-autonomous roles of col and Wnt signaling in progenitor cell maintenance.

In the medullary zone, free radical oxygen species (ROS) function as signaling molecules to support progenitor cells (Fig. 2B) (Owusu-Ansah and Banerjee, 2009). High ROS up-regulates FOXO and induces the JNK pathway, which triggers the precocious differentiation of progenitor cells. Likewise, scavenging ROS induce the same phenotype, indicating that moderate ROS levels are required for progenitor maintenance. In contrast to progenitor cells, PSC cells do not exhibit the presence of ROS under normal conditions. However, under wasp infestation, an increase in the level of ROS in the PSC stimulates the secretion of spitz (spi) and activates the EGFR signaling pathway, causing lamellocyte differentiation (Sinienko et al., 2011).

JAK/STAT signaling is involved in the intrinsic regulation of progenitor cell maintenance (Fig. 2B) (Jung et al., 2005; Makki et al., 2010). Through single-cell RNA sequencing, the expression of domeless, a JAK/STAT receptor, in the medullary zone has been confirmed, and Rodrigues et al. (2021) have shown that progenitor cells in the posterior lobes also express domeless-Gal4 (Girard et al., 2021; Rodrigues et al., 2021). Additionally, the expression of JAK/STAT is highlighted in the earliest progenitor cells in the first to second instars (Cho et al., 2020), indicating that JAK/STAT is a universal pathway for progenitor cell fate. The level of calcium, shared through gap junctions, induces phosphorylation of calcium/calmodulin-dependent protein kinase II (CamKII), and inhibits the trafficking of domeless receptors to prevent progenitor cell differentiation (Ho et al., 2021). In response to wasp infestation, JAK/STAT pathway is attenuated in the primary lobe through an antagonistic effect of Latran (lat), a short type I cytokine-related receptor (Makki et al., 2010). However, JAK/STAT pathway in posterior lobes is enhanced against wasp parasitism, implying functional compartmentalization of JAK/STAT in the progenitors (Rodrigues et al., 2021).

**EXTRINSIC REGULATION OF HEMATOPOIESIS IN THE LYMPH GLAND**

Developmental homeostasis of progenitor and differentiated hemocyte populations in the LG is regulated by systemic factors from other organs (Fig. 3). Drosophila insulin-like peptide 2 (dIlp2) is secreted from insulin producing cells (IPC) in the brain and promotes growth and metabolism similar to mammalian insulin (Boulain et al., 2015; Brogiolo et al., 2001; Graham and Rick, 2017). Malnutrition reduces the release of dIlp2 from the IPCs into the hemolymph and subsequently decreases Wnt signaling in progenitor cells. Consequently, starved larvae show a smaller population of progenitor cells and an increase in differentiated hemocytes (Shim et al., 2012). Insulin signaling in the PSC supports the number of PSC cells and promotes progenitor maintenance (Benmimoun et al., 2012; Tokusumi et al., 2012).

Sensory input from olfactory neurons triggers γ-amino butyric acid (GABA) secretion from the brain into the hemolymph. Circulating GABA, in turn, regulates calcium signaling in the progenitors to systemically control hematopoiesis in the LG (Fig. 3) (Shim et al., 2013). From an immune perspec-
Inter-Organ Communication in Fly Hematopoiesis

Ferdinand Koranteng et al.

GABA, secreted by the brain as a metabolite in progenitor cells through succinate conversion to enhance lamellocyte differentiation (Madhwal et al., 2020). The level of food odors through olfactory receptor 42a (Or42a) sets up the basal GABA concentration enough to activate the GABA-B receptor (GABABR) in the lymph gland support progenitor maintenance through IP3 receptor (IP3R), cytosolic calcium, and CamKII/CaM. Under wasp infestation, combination of Or49a and Or42a produces high levels of GABA secretion from the brain. High GABA is transported and internalized by GABA transporter (Gat) after which it is converted through the GABA shunt pathway to generate succinate. Succinate stabilizes sima and enhances lamellocyte (LM) differentiation. A portion of succinate is utilized by the TCA cycle to create ROS for progenitor maintenance. Nutrient including amino acids is sensed by the fat body which in turn increases Drosophila insulin-like peptide 2 (dIlp2) release from the brain insulin producing cells (IPC). dIlp2 progresses insulin receptor (InR) signaling in the MZ which eventually sustains progenitors through dTOR and Wnt/wg signaling. Alternatively, Slimfast (Sifl) directly uptakes amino acids and merges into dTOR pathway. Also, in the PSC, dIlp2 activates InR and dTOR to control PSC number. Ambient CO2 is recognized by Gr63a/Gr21a inhibiting sima (Hif-α) in the ventral nerve cord (VNC). sima stabilization induces release of unpaired 3 (upd3) which is detected by domeless in the fat body. Fat body secretes dIlp6 which increases Serrate in the IZ and causes crystal cell differentiation. Red arrows, olfaction and GABA axis; Blue arrows, nutrient and insulin axis; Green arrows, CO2 and upd3/dIlp6 axis.

Atmospheric gaseous molecules control blood cell development in the LG (Fig. 3). Gr63a/Gr21a in the terminal organ of larvae senses ambient CO2 levels and normally triggers behavioral responses (Jones et al., 2007; Kwon et al., 2007). However, during the development, low levels of CO2 cause sima accumulation in the ventral nerve cord (VNC) and up-regulates unpaired 3 (upd3) secretion from the brain. Subsequently, upd3 targets the fat body, an organ akin to the liver, and induces the secretion of Drosophila insulin-like peptide 6 (dIlp6) (Arese and Soulages, 2010). dIlp6 augments the expression of Serrate in the intermediate zone and leads to excessive crystal cell differentiation (Cho et al., 2018). Likewise, hypoxia stabilizes sima to non-canonically activate Notch receptor and promote crystal cell survival (Mukherjee et al., 2022).
Together, these studies suggest a close relationship between the level of ambient gases and hemocyte differentiation.

**PERSPECTIVE**

In a nutshell, LG progenitor maintenance is regulated by various intrinsic signaling factors, such as Dpp, Notch, Hh, col, Wnt, ROS, and JAK/STAT, largely mirroring the mechanisms underlying myeloid differentiation in vertebrates (Chavakis et al., 2019; Dzierzak and Bigas, 2018; Kim et al., 2021; Yamashita et al., 2020; Zhu and Emerson, 2002). Notably, the heterogeneity of the LG progenitor population and existence of intermediate progenitor cells have been frequently proposed (Cho et al., 2020; Ferguson and Martinez-Agosto, 2014; Krzemien et al., 2010b; Spratford et al., 2021). These studies suggest that signaling factors may not work in the same way for all progenitor subtypes, and hence, more complex signaling mechanisms may be active. Further studies may elucidate such unknown cascades in diverse progenitor populations.

Extrinsic signaling factors reflect environmental condition—examples include nutritional content, odor diversity, and atmospheric gaseous composition. However, previous studies involving external factors have heavily focused on behavioral and physiological contexts (van Breugel et al., 2018; Vermehren-Schmaedick et al., 2010; Wang et al., 2013). This may be due to the difficulty in observing direct interactions between these factors and in vivo hematopoietic systems. Since *Drosophila* LG is a sentinel for stress responses and provides intuitive phenotypes for observation, it serves as a potent model to further characterize the effects of extrinsic factors on hematopoiesis and immunity.

**ACKNOWLEDGMENTS**

This work was supported by the National Research Foundation (NRF) of Korea (2019R1A2C2006848) to J.S. and the National Research Foundation (NRF) of Korea (2020R1A6A3A13076568) to B.C.

**AUTHOR CONTRIBUTIONS**

F.K., B.C., and J.S. wrote the manuscript. F.K. and B.C. drew figures. J.S. supervised the manuscript.

**CONFLICT OF INTEREST**

The authors have no potential conflicts of interest to disclose.

**ORCID**

Ferdinand Koranteng https://orcid.org/0000-0002-0545-2423

Burnsik Cho https://orcid.org/0000-0003-1989-0624

Jiwon Shim https://orcid.org/0000-0003-2409-1130

**REFERENCES**

Arrese, E.L. and Soulages, J.L. (2010). Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomol. 55, 207-225.

Banerjee, U., Girard, J.R., Goins, L.M., and Spratford, C.M. (2019). Drosophila as a genetic model for hematopoiesis. Genetics 211, 367-417.

Baron, M.H., Isern, J., and Fraser, S.T. (2012). The embryonic origins of hematopoiesis in mammals. Blood 119, 4828-4837.

Benmimoun, B., Polesello, C., Haenlin, M., and Waltzer, L. (2015). The EBF transcription factor Collier directly promotes Drosophila blood cell progenitor maintenance independently of the niche. Proc. Natl. Acad. Sci. U. S. A. 112, 9052-9057.

Benmimoun, B., Polesello, C., Waltzer, L., and Haenlin, M. (2012). Dual role for Insulin/TOR signaling in the control of hematopoietic progenitor maintenance in Drosophila. Development 139, 1713-1717.

Blanco-Obregon, D., Katz, M.J., Durrieu, L., Gándara, L., and Wapnner, P. (2020). Context-specific functions of Notch in Drosophila blood cell progenitors. Dev. Biol. 462, 101-115.

Boulan, L., Milán, M., and Léopold, P. (2015). The systemic control of growth. Cold Spring Harb. Perspect. Biol. 7, a019117.

Brogiole, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., and Hafen, E. (2001). An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. Curr. Biol. 11, 213-221.

Carton, Y., Poinié, M., and Nappi, A.J. (2008). Insect immune resistance to parasitoids. Insect Sci. 15, 67-87.

Chavakis, T., Mitroulis, I., and Hajishengallis, G. (2019). Hematopoietic progenitor cells as integrative hubs for adaptation to and fine-tuning of inflammation. Nat. Immunol. 20, 802-811.

Cho, B., Spratford, C.M., Yoon, S., Cha, N., Banerjee, U., and Shim, J. (2018). Systemic control of immune cell development by integrated carbon dioxide and hypoxia chemosensation in Drosophila. Nat. Commun. 9, 2679.

Cho, B., Yoon, S.H., Lee, D., Koranteng, F., Tattikota, S.G., Cha, N., Shin, M., Do, H., Hu, Y., Oh, S.Y., et al. (2020). Single-cell transcriptome maps of myeloid blood cell lineages in Drosophila. Nat. Commun. 11, 4483.

Crossley, A.C. (1972). The ultrastructure and function of pericardial cells and other nephrocytes in an insect: Calliphora erythrocephala. Tissue Cell 4, 529-560.

Crozatier, M. and Meister, M. (2007). Drosophila haematopoiesis. Cell. Microbiol. 9, 1117-1126.

Crozatier, M. and Vincent, A. (2011). Drosophila: a model for studying genetic and molecular aspects of haematopoiesis and associated leukaemias. Dis. Model. Mech. 4, 439-445.

Destalminil-Letourneau, M., Morin-Poulard, I., Tian, Y., Vanzo, N., and Crozatier, M. (2021). The vascular niche controls Drosophila hematopoiesis via fibroblast growth factor signaling. Elife 10, e46672.

Dey, N.S., Ramesh, P., Chugh, M., Mandal, S., and Mandal, L. (2016). Dpp dependent Hematopoietic stem cells give rise to Hh dependent blood progenitors in larval lymph gland of Drosophila. Elife 5, e18295.

Dey, N.S., Ramesh, P., Chugh, M., Mandal, S., and Mandal, L. (2019). Correction: Dpp dependent Hematopoietic stem cells give rise to Hh dependent blood progenitors in larval lymph gland of Drosophila. Elife 8, e51742.

Durand, C. and Dzierzak, E. (2005). Embryonic beginnings of adult hematopoietic stem cells. Haematologica 90, 100-108.

Dzierzak, E. and Bigas, A. (2018). Blood development: hematopoietic stem cell dependence and independence. Cell Stem Cell 22, 639-651.

El Shatoury, H.H. (1955). The structure of the lymph glands of Drosophila larvae. Wilhelm Roux Arch. Entwickl. Mech. Org. 147, 489-495.

Evans, C.J., Hartenstein, V., and Banerjee, U. (2003). Thicker than blood: cell dependence and independence. Cell Stem Cell 22, 639-651.

Ferguson, G.B. and Martinez-Agosto, J.A. (2014). Kicking it up a Notch for the best in show. Scalloped leads Yorkie into the hematopoietic arena. Fly (Austin) 8, 206-217.

Ferguson, G.B. and Martinez-Agosto, J.A. (2017). The TEAD family
transcription factor Scalloped regulates blood progenitor maintenance and proliferation in Drosophila through PDGF/VEGFR receptor (Pvr) signaling. Dev. Biol. 425, 21-32.

Girard, J.R., Goins, L.M., Vuu, D.M., Sharpley, M.S., Spratford, C.M., Mantri, S.R., and Banerjee, U. (2021). Paths and pathways that generate cell-type heterogeneity and developmental progression in hematopoiesis. Elife 10, e67516.

Goyal, M., Tomar, A., Madhwal, S., and Mukherjee, T. (2022). Blood progenitor redox homeostasis through olfaction-derived systemic GABA in hematopoietic growth control in Drosophila. Development 149, dev199550.

Graham, P. and Pick, L. (2017). Drosophila as a model for diabetes and diseases of insulin resistance. Curr. Top. Dev. Biol. 121, 397-419.

Hao, Y. and Jin, L.H. (2017). Dual role for Jumu in the control of hematopoietic progenitors in the Drosophila lymph gland. Elife 6, e25094.

Ho, K.Y.L., Khadilkar, R.J., Carr, R.L., and Tanentzapf, G. (2021). A gap-junction-mediated, calcium-signaling network controls blood progenitor fate decisions in hematopoiesis. Curr. Biol. 31, 4697-4712.e6.

Holt, A., Bossinger, B., Strasser, T., Janning, W., and Klapper, R. (2003). The two origins of hemocytes in Drosophila. Development 130, 4955-4962.

Honti, V., Csdorás, G., Kurucz, E., Markus, R., and Ando, I. (2014). The cell-mediated immunity of Drosophila melanogaster: hemocyte lineages, immune compartments, microanatomy and regulation. Dev. Comp. Immunol. 42, 47-56.

Jones, W.D., Cayirlioglu, P., Grunwald Kadow, I., and Vosshall, L.B. (2007). Two chemosensory receptors together mediate carbon dioxide detection in Drosophila. Nature 445, 86-90.

Keebaugh, E. and Schlenke, T. (2014). Insights from natural host-parasite interactions: the Drosophila model. Dev. Comp. Immunol. 42, 111-123.

Kim, H.I., Park, J.W., Kang, J.Y., and Seo, S.B. (2021). Negative regulation of erythroid differentiation via the CBX8-TRIM28 axis. Mol. Cells 44, 444-457.

Krzemien, J., Crozatier, M., and Vincent, A. (2010a). Ontogeny of the Drosophila larval hematopoietic organ, hemocyte homeostasis and the dedicated cellular immune response to parasitism. Int. J. Dev. Biol. 54, 1117-1125.

Krzemien, J., Dubois, L., Makki, R., Meister, M., Vincent, A., and Crozatier, M. (2007). Control of blood cell homeostasis in Drosophila larvae by the posterior signalling centre. Nature 446, 320-324.

Krzemień, J., Bourbon, H.M., Zhou, R., Vincent, A., et al. (2010). A short receptor downregulates JAK/STAT signalling to control the Drosophila cellular immune response. PLoS Biol. 8, e1000441.

Mandal, L., Martinez-Agosto, J.A., Evans, C.J., Hartenstein, V., and Banerjee, U. (2007). A Hedgehog- and Antennapedia-dependent niche maintains Drosophila haematopoietic precursors. Nature 446, 320-324.

Márkus, R., Laurinyecz, B., Kurucz, É., Honti, V., Bajusz, I., Sipos, B., Somogyi, K., Kronhamn, J., Hultmark, D., and Andó, I. (2009). Sesile hemocytes as hematopoietic cell progenitor. Proc. Natl. Acad. Sci. U. S. A. 106, 4805-4809.

Meister, M. and Lagueux, M. (2003). Drosophila blood cells. Cell. Microbiol. 5, 573-580.

Mills, R. and King, R. (1965). The pericardial cells of Drosophila melanogaster. Q. J. Microsc. Sci. 106, 261-268.

Minakshina, S. and Steward, R. (2010). Hematopoietic stem cells in Drosophila. Development 137, 27-31.

Mondal, B.C., Mukherjee, T., Mandal, L., Evans, C.J., Sinenko, S.A., Martinez-Agosto, J.A., and Banerjee, U. (2011). Interaction between differentiating cell- and niche-derived signals in hematopoietic progenitor maintenance. Cell 147, 1589-1600.

Morin-Poulard, I., Sharma, A., Louradour, I., Vanzo, N., Vincent, A., and Crozatier, M. (2016). Vascular control of the Drosophila haematopoietic microenvironment by Slit/Robo signalling. Nat. Commun. 7, 11634.

Mukherjee, T., Kim, W.S., Mandal, L., and Banerjee, U. (2011). Interaction between Notch and Hif-alpha in development and survival of Drosophila blood cells. Science 332, 1210-1213.

Na, J. and Cagan, R. (2013). The Drosophila nephrocyte: back on stage. J. Am. Soc. Nephrol. 24, 161-163.

Orkin, S.H. and Zon, L.I. (2002). Hematopoiesis and stem cells: plasticity versus developmental heterogeneity. Nat. Immunol. 3, 323-328.

Owusu-Ansah, E. and Banerjee, U. (2009). Reactive oxygen species prime Drosophila haematopoietic progenitors for differentiation. Nature 461, 537-541.

Oyallon, J., Vanzo, N., Krzemien, J., Morin-Poulard, I., Vincent, A., and Crozatier, M. (2016). Two independent functions of Collier/Early B Cell Factor in the control of Drosophila blood cell homeostasis. PLoS One 11, e0148978.

Pennetier, D., Oyallon, J., Morin-Poulard, I., Dejean, S., Vincent, A., and Crozatier, M. (2012). Size control of the Drosophila hematopoietic niche by bone morphogenetic protein signaling reveals parallels with mammals. Proc. Natl. Acad. Sci. U. S. A. 109, 3389-3394.

Petraki, S., Alexander, B., and Bruckner, K. (2015). Assaying blood cell
populations of the Drosophila melanogaster larva. J. Vis. Exp. (105), 52733.
Ramesh, P., Dey, N.S., Kanwal, A., Mandal, S., and Mandal, L. (2021). Relish plays a dynamic role in the niche to modulate Drosophila blood progenitor homeostasis in development and infection. Elife 10, e67158.
Rizki, M.T. and Rizki, R.M. (1959). Functional significance of the crystal cells in the larva of Drosophila melanogaster. J. Biophys. Biochem. Cytol. 5, 235-240.
Rizki, M.T.M. (1957). Alterations in the haemocyte population of Drosophila melanogaster. J. Morphol. 100, 437-458.
Rodrigues, D., Renaud, Y., VijayRaghavan, K., Waltzer, L., and Inamdar, M.S. (2021). Differential activation of JAK-STAT signaling reveals functional compartmentalization in Drosophila blood progenitors. Elife 10, e61409.
Rugendorff, A., Younossi-Hartenstein, A., and Hartenstein, V. (1994). Embryonic origin and differentiation of the Drosophila heart. Roux Arch. Dev. Biol. 203, 266-280.
Russo, J., Dupas, S., Frey, F., Carton, Y., and Brehelin, M. (1996). Insect immunity: early events in the encapsulation process of parasitoid (Leptopilina boulardi) eggs in resistant and susceptible strains of Drosophila. Parasitology 112 (Pt 1), 135-142.
Sanchez Bosch, P., Makhijani, K., Herbosco, L., Gold, K.S., Baginsky, R., Woodcock, K.J., Alexander, B., Kukar, K., Corcoran, S., Jacobs, T., et al. (2019). Adult Drosophila lack hematopoiesis but rely on a blood cell reservoir at the respiratory epithelia to relay infection signals to surrounding tissues. Dev. Cell 51, 787-803.e5.
Sharma, S.K., Ghosh, S., Geetha, A.R., Mandal, S., and Mandal, L. (2019). Cell adhesion-mediated actomyosin assembly regulates the activity of Cubitus interruptus for hematopoietic progenitor maintenance in Drosophila. Genetics 212, 1279-1300.
Shim, J., Mukherjee, T., and Banerjee, U. (2012). Direct sensing of systemic and nutritional signals by hematopoietic progenitors in Drosophila. Nat. Cell Biol. 14, 394-400.
Shim, J., Mukherjee, T., Mondal, B.C., Liu, T., Young, G.C., Wijewarnasuriya, D.P., and Banerjee, U. (2013). Offactory control of blood progenitor maintenance. Cell 155, 1141-1153.
Shrestha, R. and Gateff, E. (1982). Ultrastructure and cytochemistry of the cell-types in the tumorous hematopoietic organs and the hemolymph of the mutant lethal (1) malignant blood neoplasm (l(1)mbn) of Drosophila melanogaster. (drosophila/mutant blood cells/ultrastructure/cytochemistry). Dev. Growth Differ. 24, 83-98.
Sinenko, S.A., Mandal, L., Martinez-Agosto, J.A., and Banerjee, U. (2009). Dual role of wingless signaling in stem-like hematopoietic precursor maintenance in Drosophila. Dev. Cell 16, 756-763.
Sinenko, S.A., Shim, J., and Banerjee, U. (2011). Oxidative stress in the haematopoietic niche regulates the cellular immune response in Drosophila. EMBO Rep. 13, 83-89.
Snodgrass, R.E. (1954). Insect Metamorphosis (Washington: Smithsonian Institution).
Sorrentino, R.P., Carton, Y., and Govind, S. (2002). Cellular immune response to parasite infection in the Drosophila lymph gland is developmentally regulated. Dev. Biol. 243, 65-80.
Spratford, C.M., Goins, L.M., Chi, F., Girard, J.R., Macias, S.N., Ho, V.W., and Banerjee, U. (2021). Intermediate progenitor cells provide a transition between hematopoietic progenitors and their differentiated descendants. Development 148, dev200216.
Swain, A., Inoue, T., Tan, K.S., Nakanishi, Y., and Sugiyama, D. (2014). Intrinsic and extrinsic regulation of mammalian hematopoiesis in the fetal liver. Histol. Histopathol. 29, 1077-1082.
Tepass, U., Fessler, L.I., Aziz, A., and Hartenstein, V. (1994). Embryonic origin of hemocytes and their relationship to cell death in Drosophila. Development 120, 1829-1837.
Tokusumi, Y., Tokusumi, T., Shoue, D.A., and Schulz, R.A. (2012). Gene regulatory networks controlling hematopoietic progenitor niche cell production and differentiation in the Drosophila lymph gland. PLoS One 7, e41604.
van Breugel, F., Huda, A., and Dickinson, M.H. (2018). Distinct activity-gated pathways mediate attraction and aversion to CO2 in Drosophila. Nature 564, 420-424.
Vermehren-Schmaedick, A., Ainsley, J.A., Johnson, W.A., Davies, S.A., and Morton, D.B. (2010). Behavioral responses to hypoxia in Drosophila larvae are mediated by atypical soluble guanylyl cyclases. Genetics 186, 183-196.
Wang, Y., Pu, Y., and Shen, P. (2013). Neuropeptide-gated perception of appetitive olfactory inputs in Drosophila larvae. Cell Rep. 3, 820-830.
Wood, W. and Jacinto, A. (2007). Drosophila melanogaster embryonic haemocytes: masters of multitasking. Nat. Rev. Mol. Cell Biol. 8, 542-551.
Yamashita, M., Dellorusso, P.V., Olson, O.C., and Passegue, E. (2020). Dysregulated haematopoietic stem cell behaviour in myeloid leukaemogenesis. Nat. Rev. Cancer 20, 365-382.
Zhang, C.U., Blauwkamp, T.A., Burby, P.E., and Cadigan, K.M. (2014). Wnt-mediated repression via bipartite DNA recognition by TCF in the Drosophila hematopoietic system. PLoS Genet. 10, e1004509.
Zhu, J. and Emerson, S.G. (2002). Hematopoietic cytokines, transcription factors and lineage commitment. Oncogene 21, 3295-3313.