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Drosophila as a model for the two myeloid blood cell systems in vertebrates

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Abstract

Fish, mice and men rely on two coexisting myeloid blood cell systems. One is sustained by hematopoietic progenitor cells, which reside in specialized microenvironments in hematopoietic organs and give rise to cells of the monocyte lineage. The other system corresponds to the independent lineage of self-renewing tissue macrophages, which colonize organs during embryonic development and are maintained during later life by proliferation in local tissue microenvironments. However, little is known about the nature of these microenvironments and their regulation. Moreover, many vertebrate tissues contain a mix of both tissue-resident and monocyte-derived macrophages, posing a challenge to the study of lineage-specific regulatory mechanisms and function. This review highlights how research in the simple model organism *Drosophila melanogaster* can address many of these outstanding questions in the field. Drawing parallels between hematopoiesis in *Drosophila* and vertebrates, we illustrate the evolutionary conservation of the two myeloid systems across animal phyla. Much like vertebrates, *Drosophila* possesses a lineage of self-renewing tissue-resident macrophages, as well as a 'definitive' lineage of macrophages that derive from hematopoiesis in the progenitor-based lymph gland. We summarize key findings from *Drosophila* hematopoiesis that illustrate how local microenvironments, systemic signals, immune challenges and nervous inputs regulate adaptive responses of tissue-resident macrophages and progenitor-based hematopoiesis to achieve optimal fitness of the animal.

Introduction

For over a century, the fruit fly *Drosophila melanogaster* has been an invaluable genetic model for the identification of fundamental biological principles and signaling mechanisms in animal development. *Drosophila* research led to the discovery of innate immunity, and has enhanced our understanding of hematopoiesis and blood cell function [1-4]. Now, *Drosophila*

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is emerging as a promising model for the study of tissue macrophages. In vertebrates, as in invertebrates, tissue macrophages have roles in development and tissue homeostasis, and form the first line of defense against pathogens and environmental challenges [5]. Accordingly, tissue macrophages are involved in a wide range of diseases including neurodegeneration, atherosclerosis and fibrosis [5]. Nonetheless understanding the nature and ontogeny of resident macrophage lineages has remained a long-term unsolved problem in vertebrate hematopoiesis. Early reports emphasized the distinct phenotypes of two tissue-resident macrophage populations [6]. However, since the 1970s, the concept of the mononuclear macrophage system dominated the field, proposing that progenitors in the bone marrow or other hematopoietic organs give rise to monocytes, which then differentiate into macrophages that take residence in peripheral tissues [7]. Several studies challenged this view [8-10], but it was only recently that modern genetics and lineage tracing approaches provided definitive evidence that tissue-resident macrophages belong to an independent, self-renewing lineage that derives from primitive macrophages of the yolk sac and fetal liver [11-18]. Tissue macrophages are found in a multitude of organs, exemplified by the microglia of the brain, the Langerhans cells of the skin, the Kupffer cells of the liver, and resident macrophage populations of the pancreas and lung [17,18]. Yet little is known about the local microenvironments that maintain and expand tissue macrophages. Moreover, since many tissues harbor combinations of self-renewing tissue macrophages and monocyte-derived macrophages of the ‘definitive’ lineage [14,17,19], dissecting their regulatory mechanisms and specific functions is complicated [18]. Here we show how research in a simple invertebrate model can overcome many of these challenges. This review focuses on advances in the field of *Drosophila* hematopoiesis that provide evidence for an evolutionary conserved population of self-renewing tissue-resident macrophages, as distinct from *Drosophila* macrophages of the ‘definitive’ lineage that derive from the lymph gland, a progenitor-based hematopoietic organ. The *Drosophila* experimental toolkit for hematopoiesis research is powerful [20], offering versatile genetic approaches, lineage tracing methods and live imaging techniques, many of which remain challenging in vertebrate systems. In this review, we discuss hematopoiesis in *Drosophila* with respect to the two coexisting systems of myeloid cells and their regulation. We highlight the strengths, biological simplicity and evolutionary parallels of this invertebrate model, and illustrate how it can address specific questions relevant to self-renewing tissue macrophages and progenitor-dependent hematopoiesis in complex vertebrate systems.

Overview of *Drosophila* hematopoietic waves and the ontogeny of blood cell lineages

Many elements of vertebrate hematopoiesis are evident in *Drosophila*. *Drosophila* blood cells, which are collectively called hemocytes, comprise undifferentiated prohemocyte progenitors and at least three differentiated blood cell lineages [2,3,21-23]. With the exception of the early embryo, more than 90% of the *Drosophila* blood cell pool corresponds to differentiated macrophages, also known as plasmatocytes [2,23,24]. *Drosophila* macrophages have active roles in immunity, development and wound healing through engulfing invaders and cellular debris, secreting antimicrobial peptides and producing extracellular matrix, much like their vertebrate counterparts [2,4,25]. *Drosophila* blood cell formation also gives rise

to smaller fractions of invertebrate-specific cell types. Crystal cells, named for their crystalline inclusions of Prophenoloxidase, mediate melanization reactions in innate immunity and wound healing [21,26]. Lamellocytes have roles in the encapsulation of large immune targets and melanization, but emerge only in the larva and mainly upon immune challenge [2,21,23,27-29].

Three hematopoietic waves have been described during *Drosophila* development, namely embryonic, larval and lymph gland hematopoiesis [2,3,23,24,30-34]. The embryonic and larval phases of hematopoiesis correspond to the formation and expansion of self-renewing 'primitive', or tissue-resident, macrophages (Figure 1A). Like their vertebrate counterparts [11-16,35,36], *Drosophila* tissue macrophages fulfill three criteria: (1.) they derive from the earliest macrophages that emerge during development; (2.) they colonize local microenvironments in peripheral tissues; and (3.) they are functional macrophages that self-renew in the differentiated state, bypassing the need for an undifferentiated progenitor [33,34]. Tissue macrophages execute all routine immune and phagocytic functions in the *Drosophila* larva [27,33,37]. Only in cases of extreme immune or environmental challenge are larval tissue macrophages supported by the second lineage of *Drosophila* blood cells, the lymph gland hemocytes, which then respond by undergoing precocious maturation and early entry into circulation [23,27,38].

In contrast to *Drosophila* tissue macrophage formation, lymph gland hematopoiesis (Figure 1B) resembles vertebrate definitive hematopoiesis. Lymph gland progenitors give rise to all three differentiated *Drosophila* lineages. The ontogeny of the lymph gland in the embryo is somewhat analogous to the aorta-gonad-mesonephros (AGM) region of vertebrates [2,3]. Lymph gland progenitors share a common origin with cells of the vascular (dorsal vessel) and excretory (pericardial nephrocyte) lineages, all of which develop from the cardiogenic mesoderm [2,39]. Specifically, lymph gland cells derive from a hemangioblast-like progenitor that also gives rise to the dorsal vessel, the *Drosophila* heart-like vascular organ [39]. This bears similarity to the differentiation of vertebrate hematopoietic and endothelial lineages from a common hemangioblast progenitor, as has most clearly been demonstrated during the development of the mammalian primitive streak [40,41]. However, in contrast to the vertebrate AGM, where blood cells are produced by specialized hemogenic endothelial cells [41,42], there is no evidence for a similar hemogenic mechanism in *Drosophila*. This suggests that hemogenic endothelium may have emerged later during evolution. Additionally, *Drosophila* lymph gland hematopoiesis appears to be finite: blood progenitors of the lymph gland differentiate synchronously and the organ disintegrates during metamorphosis [43]. Severe immune challenges accelerate the differentiation of lymph gland progenitors, but no condition is known to facilitate the preservation of a larger pool of progenitor cells beyond larval development [23,38,43].

In the adult fly, the production of new blood cells has not been reported. Both *Drosophila* tissue macrophages and lymph gland macrophages persist into adulthood [33,44,45] and likely coexist as a mixed population. However, neither blood cell population seems to expand further under unchallenged conditions (Brückner lab, in preparation), consistent with the progressive cellular immunosenescence observed as flies age [46]. Biologically, this places greater emphasis on the larval stage of development in *Drosophila*, when the expansion and

differentiation of the two blood cell lineages takes place (Figure 1). During this sensitive phase, multiple regulatory mechanisms allow the blood cell pool to undergo adaptive responses to environmental, nutritional and immune conditions, as outlined in detail below. The two myeloid systems in *Drosophila* offer great experimental versatility, owing to their anatomical and temporal separation during development.

Tissue macrophages in the *Drosophila* embryo: specification and migration

Hematopoiesis in the *Drosophila* embryo begins in the head mesoderm (procephalic mesoderm), which generates the earliest pool of blood cells (Figure 1A) [24,47]. Embryonic blood formation has been studied extensively with respect to the transcriptional regulation of blood progenitors and their descending lineages. The GATA factor Serpent (Srp) [47], in combination with the friend of GATA (FOG) transcription factor U-shaped (Ush) [48,49] is a master regulator of *Drosophila* blood cell fate, which is reminiscent of the role of GATA factors and GATA-FOG complexes in vertebrate hematopoiesis [50,51]. *Drosophila* macrophage fate is determined by the Zinc-finger transcription factors Glial Cells Missing (Gcm) and Gcm2, [52-54]. In vertebrates, putative blood-specific roles for *gcm* orthologs have yet to be determined, owing to early embryonic lethality of *gcm* mutants [55]. *Drosophila* macrophages are characterized by expression of the extracellular matrix enzyme Peroxidasin (Pxn) [56], the scavenger receptors Croquemort (Crq) [57], Eater [58] and antigen P1, identified as the phagocytosis receptor Nimrod C (NimC) [59]. These *Drosophila* macrophage-specific molecules belong to highly conserved protein families that also function in vertebrate immune regulation, such as the Peroxidasin orthologs Myeloperoxidase (MPO) and Eosinophil Peroxidase (EPO) [60,61], the Croquemort ortholog CD36 [62], and a diverse class of EMI domain phagocytic receptors with similarities to NimC1 and Eater [63]. Crystal cell specification requires the AML-1/ RUNX homolog Lozenge (lz) [54], and mature crystal cells are marked by continued expression of lz and Prophenoloxidase (PPO) [64]. The vertebrate *lz* ortholog *RUNX1* (*AML1*) has important functions in vertebrate hematopoiesis [65,66] and *AML1* fusions are well-known drivers of human leukemias [67]. Other markers of *Drosophila* blood cells include the clotting factor Hemolectin (Hml), which is expressed by the majority of *Drosophila* macrophages and crystal cells [68] and is similar in its domain structure and biochemistry to von Willebrand Factor (VWF), essential for hemostasis and blood clotting [69]. In addition, the *Drosophila* membrane protein Hemese (He) is expressed by all differentiated lineages, as well as many maturing blood cells [70], and shares features with the glycophorins expressed by vertebrate erythrocytes [71]

Embryonic macrophages are a convenient model for studying blood cell survival. Prohemocyte progenitors originate in the head mesoderm, where they complete four divisions by embryonic stage 11[24] and then cease proliferation, differentiating into a defined set of 600-700 macrophages and 36 crystal cells [2,24]. Subsequently, these embryonic macrophages remain quiescent, allowing researchers to quantify absolute blood cell numbers [72]. The model has been used to identify regulators of embryonic macrophage survival, first and foremost the *Drosophila* PDGF/VEGF Receptor (Pvr) [72,73]. These findings highlight parallels with Colony stimulating factor 1 receptor (CSF1R), an evolutionarily related receptor expressed in virtually all vertebrate macrophages [5,74,75]. Likewise, other members of the large family of vertebrate PDGF/VEGF receptors have

important roles in regulating cell survival, proliferation and differentiation in macrophages and other blood cell lineages [73-77], but confounding factors such as the early embryonic lethality of mutants and pleiotropic functions have hindered analyses [78-81]. *Drosophila* embryonic macrophages therefore offer unique advantages as a model to specifically dissect pro- and anti-apoptotic signaling pathways [72] (Sopko et al. in revision).

Drosophila embryonic hemocytes also provide a versatile system for studying blood cell invasion, migration and guidance. After specification from the head mesoderm, differentiating embryonic macrophages migrate into central parts of the embryo from the anterior and posterior ends. In the process, macrophages need to invade an epithelial barrier at the posterior end of the embryo, where the GTPases RhoL and Rap1 are required to activate integrin affinity in the migrating macrophages [82]. The PVR ligands Pvf2 and −3 also play roles in this invasion, most likely acting in the epithelial barrier cells [73]. Several studies have focused on blood cell migration in other regions of the *Drosophila* embryo [4]. Macrophage migration along the ventral nerve cord revealed additional roles for Pvf2 and −3 as local guidance cues [83], and identified many membrane and cytoskeletal factors necessary for blood cell migration, including beta PS integrin, Rho family GTPases, the microtubule-binding protein Clasp, the Vasp family member Enabled (Ena), and the Arp2/3 activator SCAR/WAVE [84-88]. Researchers have made further use of the *Drosophila* embryo to uncover similarities and differences between developmental and wounding-induced macrophage migration [89-92]. The system continues to provide a versatile platform to study the inflammatory response and its coordination with epithelial repair [93]. The mechanisms underlying *Drosophila* embryonic macrophage migration resemble those involved in the chemoattraction, migration and invasion of vertebrate leukocytes and macrophages [76,94-97], making the highly tractable *Drosophila* model an attractive experimental alternative.

Tissue macrophages in the *Drosophila* larva: self-renewal and adaptive responses

Drosophila larval hematopoiesis sheds light on the expansion and dynamics of tissue-resident macrophages, in particular their regulation by local inductive microenvironments and systemic signals. The self-renewal potential of tissue macrophages and other differentiated cells of ‘self-duplicating’ or ‘static’ tissues has raised considerable interest in the field of regenerative medicine [17,98,99], but studying the underlying mechanisms has remained challenging in vertebrate systems. Lineage tracing has demonstrated that *Drosophila* Pxn+ embryonic macrophages persist into the larva, where they colonize local microenvironments and expand through self-renewal in the differentiated state (Figure 1A) [33,34]. Interestingly, these macrophages undergo a switch from quiescence in the embryo [23,24,33] to high proliferation in the larva, expanding from ~ 300 cells in the 1st instar to more than 6000 cells in the 3rd instar [23,33,34] (Brückner lab in preparation). Lineage tracing experiments did not provide any indication that Pxn+ tissue macrophages of the larva derive from undifferentiated progenitors. However other investigations have detected a small fraction of potentially undifferentiated, (Wingless (Wg)+ Hml−) cells among the resident/circulating blood cell population, which remain to be studied in more detail [100].

Tissue macrophage expansion relies on local microenvironments, in particular the segmentally repeated Hematopoietic Pockets (HPs) of the larval body wall [33,34]. HPs harbor sensory neuron clusters of the peripheral nervous system (PNS) [101,102], which are essential in promoting the localization, trophic survival and proliferation of *Drosophila* tissue macrophages; this was recently demonstrated by genetic ablation and silencing of sensory neurons [33,34] (Brückner lab, submitted). These crucial interactions are likely mediated through neuronal membrane surfaces or dendritic synapses [103-107]. HPs also contain oenocytes, which are metabolically active cells with a liver-like function [108]. However, under steady-state conditions, oenocytes do not appear to play an instructive role for blood cells in the HPs [33].

In addition to the Hematopoietic Pockets, *Drosophila* larval tissue macrophages colonize the proventriculus, a cardia-like area of the gastrointestinal system [109] that is flanked by peripheral innervation [110,111], possibly mirroring functional elements of the Hematopoietic Pockets. Resident tissue macrophages also accumulate in dorsal vessel-associated clusters [22,27,112]. These have been proposed to act as larval hematopoietic organs [27], although their dynamics suggest that they may result from the accumulation of circulating hemocytes [33,113].

Drosophila larval tissue macrophages are an interesting model system for studying blood cell dynamics and adhesion. In the 1st instar larva, tissue macrophages are very adherent and almost exclusively form resident clusters. Yet from the late 2nd instar onward, increasing numbers lose their tight association and are found in a dynamic steady state between residence and circulation [33,113,114]. This progression culminates in the mobilization of the majority of tissue macrophages at the transition to metamorphosis [23,33,34,112]. The dynamics of *Drosophila* tissue macrophages raise conceptual parallels with the cycling of vertebrate hematopoietic stem and progenitor cells between defined microenvironments and peripheral blood [115,116]. Experimentally, resident *Drosophila* macrophages can be dispersed by mechanical manipulation, resulting in rapid spontaneous return or ‘homing’ to Hematopoietic Pockets [33,34], thus permitting the study of the attractive and/or adhesive properties of tissue macrophages and their microenvironment.

Under certain circumstances, tissue macrophages leave their constitutive resident locations. For example, starvation triggers macrophage infiltration into the larval fat body, a fat-storing tissue with roles in metabolism and immunity [117]. Malignant tumors or injury to imaginal discs induce local aggregations of macrophages, which may be accompanied by an increase in the number of circulating tissue macrophages [118,119]. Future studies will reveal whether these macrophage-accumulating tissues act as inductive microenvironments, or correspond to sites of macrophage activity. Vertebrate equivalents for both scenarios exist, such as the inducible niches that attract vertebrate hematopoietic stem cells to peripheral sites [120], or metabolically-induced inflammation responses [121].

Sterile wounding of the *Drosophila* epidermis, or immune challenges such as parasitic wasp infestations, lead to a number of responses which parallel injury-induced inflammation in vertebrates. These include promoting the entry of resident blood cells into circulation, inducing macrophage differentiation toward the lamellocyte fate [27,81,122,123], and

stimulating macrophage accumulation at sites of injury [113,124-128]. Although immune challenges in the *Drosophila* larva are known to induce antimicrobial peptide expression [129], few studies have elucidated the systemic and/or local signals that feed back to tissue macrophages and regulate cellular immunity. However, screens for genes involved in the mobilization, proliferation and differentiation of larval macrophages have identified several signaling pathways [37,112], and directed studies have established roles for both Rac1 and JNK signaling [130,131]. The systemic steroid ecdysone, an inducer of metamorphosis and other developmental transitions [132], is required for lamellocyte formation following wasp infestations of *Drosophila* larvae [28]. It may also be involved in the mobilization and enhanced phagocytic activity of tissue-resident macrophages at the transition to pupariation [133].

Signals from a variety of sources must be integrated to modulate the tissue-resident macrophage pool in *Drosophila*. The discovery of *Drosophila* Hematopoietic Pockets heralds a new system for studying communication between the sensory nervous system and the blood. Other tissues present in the Hematopoietic Pockets, such as oenocytes, muscle and epidermis, may also play roles in the local relay of signals to resident tissue macrophages under specific circumstances of injury, metabolic or immune challenges. The regulation of stem cell niches and tissue microenvironments through direct innervation by the peripheral nervous system is a new paradigm in development and homeostasis [134,135]. In the mouse, all hematopoietic sites are innervated by the sympathetic nervous system, and bone marrow and lymph nodes are further innervated by sensory neurons from the dorsal root ganglia [136,137]. The peripheral nervous system plays roles in the developmental emergence of blood cells from the AGM [138], and the homeostasis and induction of blood cells in the bone marrow and other hematopoietic and immune organs [139-145]. Comparably little is known about the regulation of vertebrate tissue macrophages. Studies on their proliferation and survival have focused on Colony Stimulating Factor-1 (CSF1) Receptor signaling, which is triggered by Macrophage colony-stimulating factor (M-CSF) during development, or Interleukin-34 (IL-34) during inflammation. Alternative pathways are induced by Interleukin-4 (IL-4) or Granulocyte macrophage colony-stimulating factor (GM-CSF) [17,75], and M-CSF and IL-4 stimulate local tissue macrophage proliferation in response to infection [146,147]. However, the nature and regulation of local microenvironments of vertebrate tissue macrophages remain elusive. Research in *Drosophila* may point toward fundamental new regulatory principles in vertebrate systems, particularly regarding the neuronal control of tissue macrophages. Interestingly, neural reflex circuits that regulate inflammatory responses have already been described, further reinforcing the possibility that vertebrate tissue macrophage self-renewal, and other cellular adaptive responses, are regulated by the nervous system and its inputs [148,149].

Progenitor-based lymph gland hematopoiesis

Drosophila lymph gland (LG) hematopoiesis allows researchers to address many questions regarding cell lineage, and the local and systemic signals that mediate blood cell progenitor maintenance, proliferation and differentiation [2,31,150,151]. Progenitors of the lymph gland originate in the embryo and slowly proliferate until the 2nd larval instar, forming the primary, secondary and sometimes additional lobes of the lymph gland that line the anterior part of

the dorsal vessel (Figure 1B). In the 3rd instar larva, lymph gland blood cell differentiation becomes apparent. The lymph gland consists of a central Medullary Zone (MZ), where prohemocyte progenitors are maintained, a peripheral Cortical Zone (CZ), comprising macrophages and small numbers of crystal cells and lamellocytes [2,30,152], and a group of cells at the posterior tip of the primary lobes termed the Posterior Signaling Center (PSC), which has been proposed to function as local microenvironment [38,152,153] (Figure 1B). Once metamorphosis is initiated, most if not all lymph gland progenitors differentiate by 8 hours after puparium formation, and all cells of the organ are released into circulation [43] (Figure 1B).

Multiple signaling pathways have roles in the specification of lymph gland cells, and in the regulation of progenitors and differentiated blood cells. Notch signaling is required for the early specification of the lymph gland [39] and for the subsequent differentiation of crystal cells [152]. A local Hedgehog signal from the Posterior Signaling Center maintains progenitors in the Medullary Zone [38,153], and progenitor maintenance also depends on the autonomous activation of the Wingless pathway [154]. Furthermore, differentiated blood cells from the Cortical Zone contribute to progenitor maintenance through the secretion of adenosine deaminase-related growth factor (ADGF), which is expressed downstream of the receptor tyrosine kinase PVR [151]. Many other genes and pathways have reported functions in lymph gland cell proliferation and differentiation, including the Rel/I(kappa)B-family related Toll/cactus pathway, Jak/Stat signaling, Dpp (BMP) signaling, the Polycomb group (PcG) gene *multi sex combs* (*mxc*) and the transcription factor Zfrp8 [155-161].

The lymph gland also responds to a variety of systemic factors, enabling the integration of signals relating to nutritional status and sensory inputs [162]. A recent study demonstrated an interesting molecular link between odorant sensing, GABA production in the brain and calcium signaling in the lymph gland, which drives blood cell differentiation [163]. Starvation of *Drosophila* larvae leads to the premature differentiation of lymph gland progenitors, demonstrating a role for the insulin/TOR pathway [117,164-166]. Further, systemic insulin signaling, or amino acid sensing through the transporter Slimfast, triggers the premature differentiation of lymph gland progenitors, in a process that also involves Wingless signaling [117]. Tor (Target of rapamycin) pathway activity may impinge on the levels of reactive oxygen species (ROS) in the lymph gland [165], consistent with findings showing that high ROS levels also drive lymph gland expansion [167].

Immune challenges, such as parasitic wasp infestations, trigger precocious lymph gland differentiation and the mobilization of macrophages and lamellocytes [23,29,122]. Several studies have examined the engagement of local and systemic signaling mechanisms in this context [23,29,38,168]. The contribution of the lymph gland blood population to larval immune responses is somewhat delayed compared to the immediate response of larval tissue macrophages [27]. However the dual origin of macrophages, and the concerted action of the two lineages in mounting an immune defense against external challenges, is a common motif that can also be seen in the mixed populations of macrophages guarding vertebrate tissues [17,146].

Many of the molecular mechanisms involved in regulating *Drosophila* lymph gland hematopoiesis have subsequently or in parallel been found to play key roles in progenitor-based hematopoeisis in vertebrates. Notch (N) signaling in vertebrates is required for the specification of the hematopoietic and vascular systems and the generation of hematopoietic stem cells, and also functions in T cell differentiation and as a tumor suppressor in leukemias [169,170]. Wingless/Wnt signalling has highly conserved roles as fundamental regulator of hematopoietic development, and acts in hematopoietic stem cell (HSC) self-renewal and leukemogenesis [171,172], while Hedgehog signaling has diverse functions in normal and malignant hematopoiesis [173]. Jak/Stat signalling is crucial for multiple aspects of hematopoiesis and immune function, and misregulation of this pathway drives hematologic malignancies [174-176]. Reactive oxygen species also have reported roles in hematopoiesis and leukemias [177,178]. As in *Drosophila*, PI3K/Akt/TOR signaling links cell signaling with metabolic regulation and has multi-faceted functions in vertebrate hematopoiesis, immunity and leukemia development [179-182]. Future studies will determine whether odorant sensing, or other sensory systems, provide systemic signals in the regulation of progenitor-based hematopoiesis also in vertebrates.

Outlook

Comparing hematopoietic mechanisms across animal phyla suggests that the tissue-resident macrophage lineage is the more ancient and widely-conserved of the two myeloid blood cell systems in vertebrates. *Drosophila* promises to become an excellent model to investigate basic principles of tissue macrophage regulation, as it has proven in the study of innate immunity. In *Drosophila,* just as in vertebrates, tissue-resident macrophages are complemented by a distinct lineage of progenitor-based ‘definitive’ macrophages. Combining two myeloid lineages bolsters the cellular immune response of the animal and affords a broader range of upstream regulatory mechanisms that shape the number, differentiation and availability of blood cells. This allows the animal to respond adaptively to nutritional, immune and sensory inputs, in order to achieve the best possible overall fitness. The importance of integrating developmental, physiological and environmental responses is a key concept that likely applies to multiple tissues and throughout the life of an animal.

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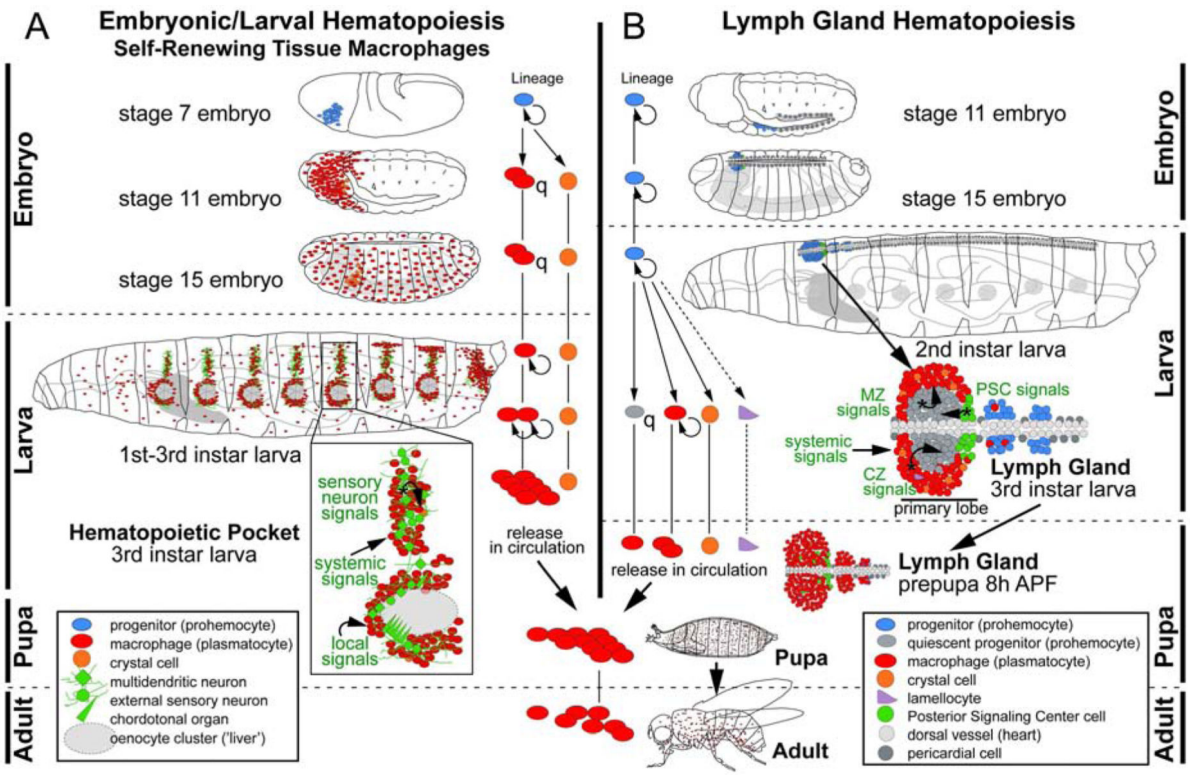

Figure 1. Ontogeny of blood cell lineages and regulation of hematopoiesis in *Drosophila*

(A) Self-renewing tissue macrophages, corresponding to *Drosophila* embryonic and larval hematopoiesis. *Drosophila* tissue-resident macrophages originate as prohemocyte progenitors (blue) in the head mesoderm at around embryonic stage 7. After four rounds of division, progenitors cease proliferation and differentiate into 600-700 macrophages (red) and a small number of crystal cells (orange). Crystal cells remain clustered around the proventriculus (‘cardia of the stomach’). Differentiating macrophages start migrating on routes from the anterior, and into the folded-over posterior end of the embryo (stage 11). By stage 15, macrophages have evenly populated the embryo. All macrophages remain quiescent (q) until the end of embryogenesis. At the transition to the larval stage, macrophages and crystal cells persist from the embryo. Macrophages colonize local microenvironments, in particular the segmentally repeated Hematopoietic Pockets (HPs), which also contain sensory neuron clusters (green). Localization to the HPs re-initiates macrophage proliferation, or ‘self-renewal’, which continues throughout larval life. Sensory neurons regulate the localization and expansion of tissue macrophages, raising the possibility that sensory stimuli from the environment and neuronal activity provide another layer of regulation. Macrophages are further regulated by systemic and/or local signals (green) stemming from immune challenges and signaling pathway activity. Many of these conditions cause premature mobilization of resident macrophages and induce differentiation into lamellocyte fate (not shown). Conversely, during normal larval development, resident tissue macrophages only gradually contribute to the pool of circulating macrophages in the hemolymph, and are released from their microenvironments at the onset of metamorphosis. Throughout larval development, crystal cells are found at locations similar to tissue macrophages but show only marginal increases in cell number. (B) Lymph Gland

hematopoiesis. Prohemocytes are specified from hemangioblast precursors, which derive from the cardiogenic mesoderm of the embryo. Blood progenitors undergo four divisions in the embryo and continue to proliferate at a low rate until second larval instar. By the 3rd larval instar, blood cells in the Cortical Zone of the primary lobes (CZ) have differentiated into macrophages that expand further by proliferation, small numbers of crystal cells, and occasional lamellocytes. Progenitors in the Medullary Zone (MZ) have become quiescent (q). The proliferation and differentiation of LG blood cells is under the tight control of a wide range of signals, which arise from within the LG (PSC signals, CZ signals, MZ signals), and from systemic sources, such as neurotransmitters and growth factors from the brain, and nutritional compound levels. As development proceeds, virtually all blood cells of the lymph gland differentiate, and by 8h after puparium formation (APF), all lymph gland cells have been released into circulation. Adult flies appear devoid of significant hematopoietic activity, but carry over macrophages that persist from previous developmental stages. This places greater emphasis on the production and maintenance of blood cells in the embryo and larva, and explains the need for a multitude of regulatory mechanisms (signals and inductive tissues in green), which ensure adaptive responses of the blood cell pool during the sensitive period of larval development.