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Drosophila immune cell migration and adhesion during embryonic development and larval immune responses

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The majority of immune cells in *Drosophila melanogaster* are plasmatocytes; they carry out similar functions to vertebrate macrophages, influencing development as well as protecting against infection and cancer. Plasmatocytes, sometimes referred to with the broader term of hemocytes, migrate widely during embryonic development and cycle in the larvae between sessile and circulating positions. Here we discuss the similarities of plasmatocyte developmental migration and its functions to that of vertebrate macrophages, considering the recent controversy regarding the functions of *Drosophila* PDGF/VEGF related ligands. We also examine recent findings on the significance of adhesion for plasmatocyte migration in the embryo, as well as proliferation, trans-differentiation, and tumor responses in the larva. We spotlight parallels throughout to vertebrate immune responses.

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Introduction

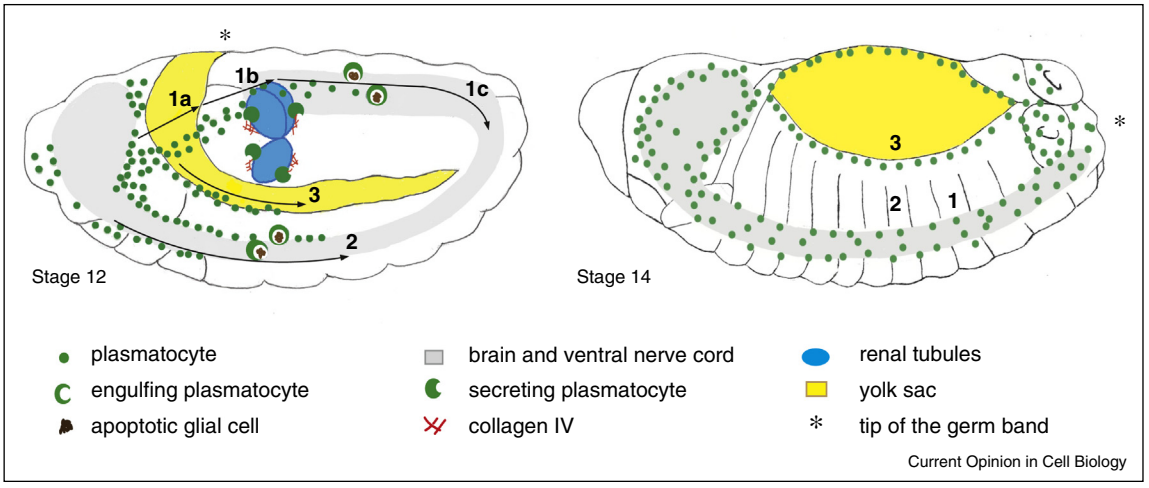
Immune cells are essential for survival, as they eliminate both foreign invaders and endogenous pathologies [1,2]. While vertebrates utilize a complex set of innate and adaptive immune cells, *Drosophila melanogaster* relies on an innate immune system consisting of only three cell types, jointly called hemocytes, to play a broad range of roles [3]. Plasmatocytes, the functional equivalent of vertebrate macrophages, are 95% of all *Drosophila* immune cells prior to infection and will be the focus of this review. They influence development [4•,5,6] and physiology [7] as well as defend against bacteria [8,9], fungi [8], viruses [10], and cancer [11,12••]. Plasmatocytes migrate actively during embryonic development [13] and pupation [14], as well as during responses to wounds [15,16]. In the larva, many of their positions are due to regulated adhesion [17••,18]. We have sought to avoid overlap with two recent excellent reviews [9,19]; here we focus on the conservation between *Drosophila* plasmatocyte and vertebrate macrophage migration in embryos, and examine the adhesion involved in larval plasmatocyte physiology and tumor responses. We highlight questions throughout that we consider intriguing for further exploration.

Conservation of embryonic macrophage migration paths and functions in *Drosophila* and vertebrates

Much of the embryonic migration of *Drosophila* plasmatocytes occurs along paths where their function is required for further development. Plasmatocytes are specified in the anterior mesoderm in the ventral side of the head [20,21]; they then ingress [22] and split into three main routes, two of which have at this time been shown to have clear developmental relevance (Figure 1). In route 1, plasmatocytes move over the yolk sac to the tip of the germband (route 1a). They then invade the epithelia of the extended germband [13,23,24•] on their way to kidney-like organs called the renal tubules (route 1b); plasmatocytes secrete collagen IV which facilitates BMP signaling required for the proper positioning of these organs [4•]. These plasmatocytes then migrate along the posterior ventral nerve cord (vnc) (1c), eventually joining the cells moving from their birthplace towards the posterior along the vnc in route 2; all along the vnc, plasmatocytes engulf apoptotic midline glia and facilitate vnc condensation [5,6,25]. Route 3 along the forming heart [16] has not yet been shown to have a developmental role but in any case serves to further disperse plasmatocytes in preparation for larval immune functions.

These embryonic migration paths and their purposes show similarities with those of vertebrate macrophages formed during primitive hematopoiesis (see Table 1) [26–28]. As in *Drosophila*, macrophages in zebrafish are specified in the anterior ventral mesoderm. They then move onto the yolk sac as in route 1 [29]; this step also precedes their penetration of epithelial tissues [30], and phagocytosis of apoptotic cells of the nervous system [31]. The precursors of mouse macrophages are also born in the anterior mesoderm and move onto the yolk sac; there they form blood islands in which they mature [32] before appearing in the head [33] and seeding the brain where they develop into microglia [34]. Movement analogous to route 2 along the vnc is observed in zebrafish and the chick, in which macrophages move into the spinal cord from anterior to posterior after their population of the head [29,35]. Mouse macrophages infiltrate the

Figure 1



Plasmatocyte migration routes and their functional roles during embryonic development. Schematic of two embryos (early Stage 12 on the left and Stage 14 on the right) showing that plasmatocytes specified in the head mesoderm migrate along three main routes during embryonic development. One sub population migrates in Stage 12 over the yolk sac to the edge of the extended germband indicated by an asterisk (route 1a). They then penetrate the germband epithelium and cluster around the renal tubules where they secrete collagen IV which ensheathes the tubules (route 1b). These and other plasmatocytes that have entered the germband continue along the posterior ventral nerve cord (vnc, route 1c in left embryo, route 1 in right embryo). Another subpopulation migrates out from the head (route 2 in both embryos) along the anterior ventral nerve cord. In both of these routes plasmatocytes engulf apoptotic midline glia. The third group of plasmatocytes migrates along the developing heart also towards the posterior of the embryo (route 3 in both embryos). Arrows indicate the migration routes.

developing kidney interstitium and may stimulate growth and ureteric bud branching [36]. Postnatally mouse macrophages also facilitate the branching of the mammary gland, a process requiring Bone morphogenetic protein (BMP) signalling [37,38]. Macrophage remodeling, although not secretion, of collagen appears to be involved [39]. Thus macrophages influence development in both *Drosophila* and vertebrates and migrate developmentally to many of the same tissues. This routing helps populate different vertebrate tissues with the resident macrophages that play later essential physiological and immunological roles [40].

PDGF/VEGF ligands in *Drosophila* and vertebrate macrophage migration

PDGF/VEGF-related ligands (Pvfs) have been thought to mediate migration along all three embryonic routes in *Drosophila* but this idea is now contested. The original hypothesis rested on the findings that each path expresses one of the 3 Pvfs [13,16] and that loss of function of the ligands or their plasmatocyte expressed receptor, the PDGF/VEGF-related Receptor, PVR, causes defects in movement along each route [13,16,23,41]. However, interpretation of these experiments is complicated; PVR signaling is also required for plasmatocyte survival [23]. PVR activation of Mbc and Rac has been implicated in its migratory function in another cell type [42,43], and signaling through Akt/Tor, and MEk/ERK in its role in hemocyte survival [13,23,42,44,45]. Thus to definitively demonstrate a migratory role for these ligands or their receptor requires the migration defects caused by their absence to remain when cell survival is restored. This has been shown for PVR and Pvf2/3 in penetration of the germband in route 1 [23,41]. In route 2 the importance of PVR [16] is established but that of Pvfs is not yet clear. One lab showed strong migratory defects after RNAi of Pvf2 and 3, but did not assess effects on plasmatocyte survival [16]. Another rescued survival and restored the migratory defects seen in a deletion affecting the two Pvfs, however this deletion causes only a reduction, not the elimination, of Pvf2 expression [41]. A role in route 3 is likely as migration there fails in the absence of only one Pvf [16]; eliminating two is required to see strong survival defects [13,23]. Whether these Pvfs are acting as chemoattractants is another open question. When Pvf2 is over-expressed in areas the plasmatocytes normally cross, it triggers plasmatocyte accumulation, which could be caused by attraction or adhesion [13,16,25]. Pvfs have not been used to redirect plasmatocytes to a new area, as was demonstrated with another migratory cell type, border cells [46]. Expression of Pvf2 or a dominant active (DA) form of PVR in the plasmatocytes themselves should block migration if a chemotactic response is required for guidance. Each appeared not to, but the expression was turned on only after much migration had already commenced [41] and in a background in which the endogenous protein was still present, albeit for Pvf2 at reduced levels. Thus the potential migratory functions for

Table 1

Summary of *Drosophila* plasmatocyte embryonic migration routes, factors, functions and conservation with those of vertebrate macrophages

Drosophila plasmatocyte route	*Drosophila* route description	*Drosophila* ligands and receptors	*Drosophila* experiments and caveats	*Drosophila* functional relevance	Vertebrate route conservation	Vertebrate receptors involved	Vertebrate functional conservation
1a	Over the yolk sac to edge of posterior germband	PVR independent	PVR null mutant still moves up to edge of germband [13].	None yet identified.	Zebrafish and mouse macrophage precursors move over yolk sac [29,32,33].	VEGFR-2 needed for macrophage precursors to move onto yolk sac blood islands in mouse [47,48].	
1b	Penetration between posterior germ band epithelia on the way to the renal tubules.	PVR Pvf2 (Pvf3)	PVR null mutant rescued for cell survival shows no movement into germ band [23]. Pvf2/3Δ shows no movement into germ band. Phenotype rescued just by Pvf2 expression [41].	Collagen IV secretion to facilitate BMP signaling needed for renal tubule development [4].	Kidney infiltration by macrophages seen in mouse [36]. Epithelial penetration seen in zebrafish [30].	CSF1R needed for epithelial penetration in zebrafish (Fig. 9E,F in [30]).	Remodeling of collagen involved in mammary gland development seen in mouse [39].
1c	Along the posterior ventral nerve cord (vnc)	PVR Pvf2&3?	Pvf2 and 3 RNAi knockdown show migration defects along vnc, cell survival not assessed [16]. Pvf2/3Δ mutant defects restored upon rescue of cell survival [41]. Yet Δ mutant is not a complete null: reduces Pvf2, truncates Pvf3.	Engulfment of apoptotic midline glia [5,6], vnc condensation.	Zebrafish macrophages appear in posterior nerve cord (Fig. 8S in [29]).		Apoptotic neural cells engulfed in zebrafish [31].
2	Along the anterior vnc	PVR Pvf2&3?	PVR null mutant rescued for cell survival shows little movement along anterior vnc [16]. Pvf experiments and caveats same as above [41].	Engulfment of apoptotic midline glia [5,6], vnc condensation.	Zebrafish (Fig. 9E,F, in [30]), chick.	CSF1R (Fig. 9E,F in [30,35]).	Apoptotic neural cells engulfed in zebrafish [31].
3	Along the forming heart	PVR Pvf2	PVR null mutant rescued for cell survival shows little movement along forming heart [16]. Pvf2 transposon insert mutant and RNAi showed defects [16]. Cell survival not assessed but lacking one Pvf does not cause strong survival defects [13,23].	None yet identified.			

Each row corresponds to a route taken by *Drosophila* plasmatocytes during their embryonic migration. For each route, successive columns indicate the signals and receptors currently known to be required for the indicated migration and then the experiments underlying that conclusion and their caveats. A question mark indicates that the corresponding molecule has been contradictorily identified both as a plasmatocyte migratory cue and as solely a survival factor, as discussed in the caveat column. Further columns illustrate the potential conservation of the *Drosophila* plasmatocyte routes with those of vertebrate macrophages and the vertebrate receptor required for the vertebrate route indicated. The final column delineates the potential conservation of a functional role with vertebrates.

Pvfs are to facilitate invasion in route 1, mediate adhesion or guidance on several routes, or all of the above.

Even if the Pvfs do guide migration, many questions remain. Movement along the first step (1a) of route 1 up to the germband can occur even in the absence of PVR [13], implying the existence of another migratory cue for this step. Each of the three main routes that the plasmatocytes split into contains Pvfs [13,16], thus how the cells decide which path to follow is unclear. Finally, along all three paths, consecutive waves of plasmatocytes move towards one source of Pvf, but then move beyond it to another. Thus, if Pvf guides movement during normal development, mechanisms must exist within the migrating hemocyte streams to create a gradient from the successive concentrations of Pvf expression, as in the zebrafish lateral line [47,48]. Alternatively, contact with the leading hemocyte could induce tissues to downregulate Pvfs or upregulate sequestering receptors [49] so that the leading hemocyte would receive greater signal from targets further ahead. This would require, however, that subsequent hemocytes follow cues not from their surroundings but from other hemocytes.

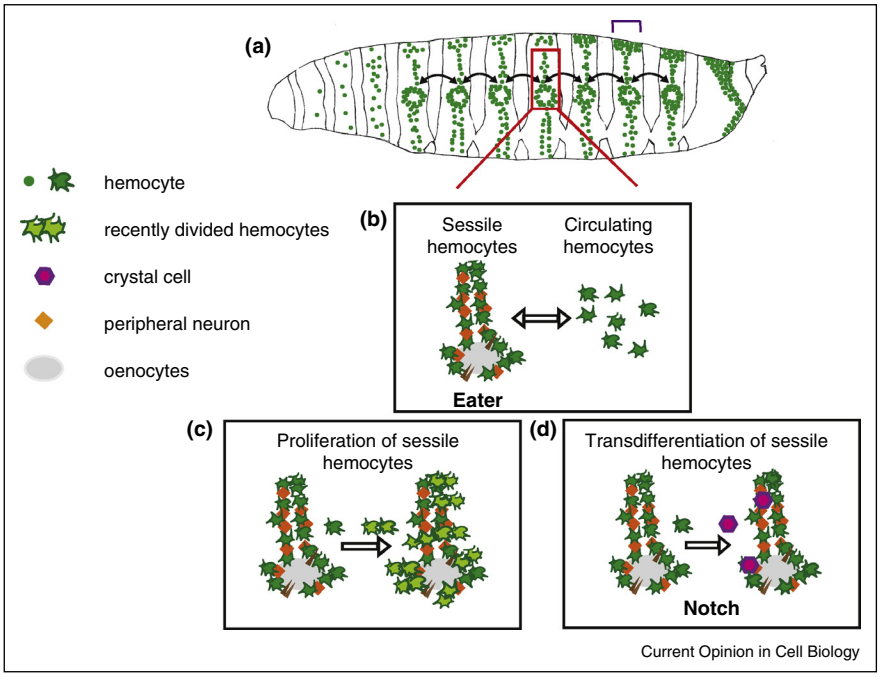
The closest vertebrate orthologs of *Drosophila* PVF are Vascular Endothelial Growth Factor (VEGF) and Platelet derived growth factor (PDGF). These can guide the migration of macrophages during development and of monocytes, the precursors of macrophages, during physiological responses. VEGF Receptor 2 (VEGFR-2) is needed for macrophage precursors to appear in blood islands in mice [50]; this is thought to be due to a defect in their migration as VEGFR-2-mutant cells can differentiate properly *in vitro* [51]. A role for PDGFRβ in migration of macrophage precursors to blood islands or from the yolk sac has not been assessed, but it is not required for the developmental migration of hematopoetic stem cells from the fetal liver [52]. Purified VEGF can guide human monocytes across endothelial monolayers [53]; both VEGF and PDGF can direct monocyte chemotaxis *in vitro* [54–56]. The next closest ortholog of *Drosophila* PVR, after PDGFR and VEGFR, is the Colony Stimulating Factor 1 Receptor (CSF1R), which is involved in monocyte/macrophage precursor chemotaxis [57]. Interestingly, in zebrafish the invasion of macrophages from the yolk sac into the brain, retina and epidermis depends on CSF1R, which starts to be expressed in pre-macrophages maturing in the yolk sac [30]. Thus as evolution proceeded, the migratory functions of *Drosophila* PVR may have been split between VEGFR, PDGFR, and CSF1R [58] during development and immunological responses.

Modulation of adhesion during the *Drosophila* plasmatocyte life cycle

Integrin adhesion plays an essential and dynamic role in facilitating and influencing the migration of plasmatocytes in the embryo. Integrin affinity is regulated by the GTPase Rap1 [59], as in vertebrates in which both of these proteins are required for the movement of neutrophils and monocytes between endothelial cells out of the vasculature [60]. *Drosophila* plasmatocytes also penetrate a tissue barrier as they move into the germband along route 1b and analogously require α-Integrin, Inflated, as well as Dizzy, a GEF for Rap1 [24•] for this step. Modulation of this adhesion appears to be crucial as the GTPase RhoL, which regulates Rap1 localization and thus Integrin affinity, is essential for this process. Plasmatocytes could use Integrins to bind the germband's epithelial cells and change their junctional properties to permit penetration, as vertebrate monocytes do while exiting blood vessels [61]. Alternatively, Integrins could facilitate homotypic adhesion since plasmatocytes migrate in chains during germband entry, contacting the rear of the cell ahead [24•]; indeed strong plasmatocyte β-Integrin dependent clustering can be induced at later stages by over expressing Dizzy or Rap1DA [59]. In contrast, at these later stages, overlap that arises normally between lamellipods leads to repulsion, facilitating the dispersal and movement of hemocytes [62•,63••]. The contacting lamellipods form an adhesion that leads to the coordinated reorganization of the colliding cytoskeletal networks and a build up of accumulated tension [63••]; its release seems to propel repulsion. Integrins could be involved in this event, as in its absence the cells maintain contact longer and move more slowly away from one another [64]. Thus plasmatocytes seek contact at early stages and are repelled by it at later ones; this change could be due to a temporal shift in plasmatocyte signaling pathways downstream of Integrins.

Embryonic plasmatocytes persist into the larval stage, but in this period active migration plays a more limited role than adhesion. During all larval stages, plasmatocytes circulate passively in the lymph that bathes the internal organs and are then recruited to tissue surfaces and wound sites by adhesion [65,66]. In the early larvae, plasmatocytes also home based on cues provided by neurons to segmentally repeated pockets between muscles and the epidermis where they attach to the internal surface of the body wall [17••,67] (Figure 2). Localization in these pockets permits these sessile plasmatocytes to undergo a faster rate of division, receive survival signals, and transdifferentiate. Their presence at these locations requires Eater, a hemocyte specific EGF-like repeat receptor [68•]. These sites maintain their attractive capacities over time because plasmatocytes return after mechanical disruption displaces them [17••]. Yet this localization is also dynamic; at later larval stages these plasmatocytes undergo exchanges between the body wall pockets [17••]. Trans-differentiation of a few plasmatocytes into crystal cells occurs in a Notch-dependent manner even in the

Figure 2



Larval hemocytes exist in sessile patches and in circulation. **(a)** Schematic showing hemocyte distribution in a 3rd instar larva. Hemocytes colonize segmentally repeated epidermal-muscular pockets found along the side of the embryo (indicated in one segment by the red box) and attach to the internal body wall from early larval stages. At later stages hemocytes are also found in association with the dorsal vessel (indicated in one segment by a purple bracket). Sessile hemocytes undergo exchanges between the pockets on the body wall (shown with bi-directional arrows) and during immune challenges return to circulation. Cartoons depicted below correspond to the boxed region in the larva and demonstrate different sessile hemocyte behaviors. **(b)** Sessile hemocytes in the epidermal-muscular pockets cluster around the oenocytes and associate with cells of the peripheral nervous system (PNS), which are essential for their trophic survival. Hemocyte association with the sessile compartment requires the plasmatocyte specific EGF-like repeat receptor, Eater. Hemocytes also exchange between sessile patches and the circulation. **(c)** Plasmatocytes attached to the sessile patches undergo proliferation. **(d)** Plasmatocytes attached to the sessile compartment can trans-differentiate into crystal cells in a Notch dependent manner.

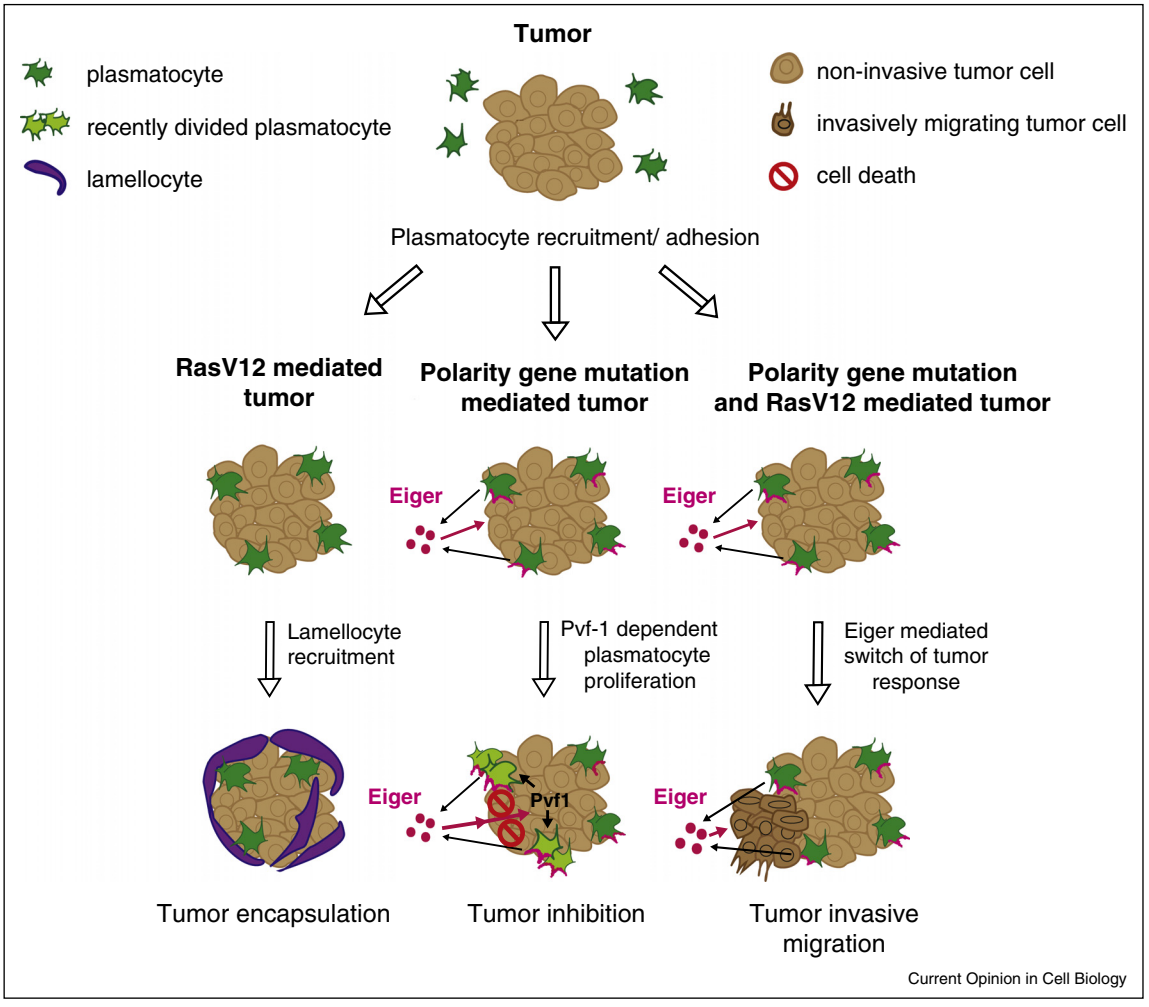
absence of the wounds and parasites that the crystal cells serve to melanize [69–71]. These crystal cells remain in the pockets as long as plasmatocytes express Eater and are also located there [68•]. Immune challenge leads to the return to circulation of plasmatocytes and crystal cells [72,73]; if the infecting agent is a parasite, these released sessile plasmatocytes also transdifferentiate into lamellocytes which wrap around the invaders [72].

What molecular mechanisms trigger the alterations in adhesion underlying their dynamic cycling between pockets in the normal larva or their mobilization in the infected one is an open question. Expression in plasmatocytes of two genes, either of which should disrupt Wg signaling, releases sessile plasmatocytes [74]. Constitutive Toll signaling in the fat body can also lead to disruption of the plasmatocyte pattern [73]. These results argue that unknown external signals heralding the presence of infection can decrease adhesive strength directly in sessile hemocytes or in the muscles or epidermis they bind to. As Eater also binds to bacteria to permit their phagocytosis [75,76], plasmatocytes that are triggered to leave and then encounter bacteria might be temporarily precluded from rebinding to the pockets. Whether plasmatocytes returning after exposure to pathogens can shift the proliferation or differentiation rate of the sessile ones and thus act analogously to macrophages and dendritic cells presenting antigen to T cells in lymph nodes is an intriguing area to explore [77]. In any case, larval plasmatocyte adhesion in these pockets is required for their expansion and responses to infection, behaviors also observed in vertebrate tissue resident macrophages which they have been proposed to be analogous to [27,33,78,79].

Plasmatocyte tumor responses initiated by adhesion

Circulating plasmatocytes are captured by adhesion to larval tumors where they can block or promote aberrant cell growth, depending on the tumor type (Figure 3). Tumors induced in salivary glands solely by oncogenic

Figure 3



Tumor associated hemocytes can lead to tumor promotion and invasion or tumor regression. Schematic depiction of *Drosophila* hemocyte and tumor interactions. Plasmatocytes are recruited to adhere to tumors of all genetic types. The further responses of both cell types depend on the genetic makeup of the tumor, as indicated below. In tumors induced in salivary glands by Ras^{V12}, lamellocytes and crystal cells are recruited to the tumor, leading to its encapsulation. In tumors induced in imaginal discs by mutations in the polarity genes, *scribble* and/or *discs large*, plasmatocyte derived Eiger causes tumor cells to upregulate Pvf1, leading to further plasmatocyte proliferation. Plasmatocyte Eiger also triggers tumor inhibition in combination with factors from the fat body. Eiger is a transmembrane protein; it may act through direct contact with tumor cells or be secreted after cleavage. In imaginal disc tumors deficient for *scribble* but overexpressing Ras^{V12}, plasmatocyte derived Eiger mediates a switch in tumor response from *in situ* residence to invasive migration.

Ras^{V12} are bound by plasmatocytes, lamellocytes, and crystal cells. These immune cells encapsulate and melanize the transformed tissues, isolating it as they do with wasp eggs [80]. Tumors elicited in imaginal discs by mutations in the polarity genes, *scribble*, *discs large* or *lethal giant larvae* [81], lead to the adhesion of plasmatocytes at areas where the basement membrane is disrupted [11]. These plasmatocytes inhibit tumor growth by producing Eiger, the only identified member in *Drosophila* of the Tumor Necrosis Factor (TNF) α superfamily [11,12••]. Plasmatocyte Eiger leads to a positive feedback loop of tumor control; it induces tumor cells to die and to express Pvf1 which results in plasmatocyte proliferation through PVR signalling [12••].

Finally, if the tumors induced by polarity gene mutations in imaginal discs also express Ras^{V12}, plasmatocytes are again captured from the circulation by adhesion, but lead to a different response. Eiger produced by these plasmatocytes causes not tumor death, but rather overgrowth and invasive migration [82,83•]. This final case shows similarities to vertebrates, in which tumor associated macrophages promote tumor functions through TNFα as well as pro inflammatory cytokines [84]. There are likely to be common signals, perhaps a disrupted basement membrane, through which all *Drosophila* tumors induce plasmatocyte adhesion. Yet there must also be distinct tumor signaling pathways that lead to the specific plasmatocyte responses to different

tumor types and divergent tumor responses to plasmatocyte produced Eiger.

Conclusions

Due to the relative ease of genetic manipulation and imaging in *Drosophila*, its immune system serves as an excellent system to study how cellular migration occurs within diverse *in vivo* environments. While migration plays the major role in bringing plasmatocytes to locations where they play essential developmental roles in the embryo, during larval life adhesion predominates and must be dynamically regulated to permit both normal proliferation and infectious responses. Plasmatocyte binding to tumors can lead to their inhibition or promote their invasion, depending on the genetic state of the tumor. In many of these steps similarities are evident to vertebrate macrophages and monocytes. The molecular mechanisms governing the movements, adhesion, and functions of the *Drosophila* immune system likely represent ancient programs upon which evolution has elaborated to permit the complex repertoire of immune cell behavior seen in vertebrates. Identifying new aspects of these mechanisms and their relevance for vertebrate immunology will occupy many exciting years ahead.

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This interesting work shows the potential of *Drosophila* tumor models to study long and short-range interactions between epithelial tumors and the immune system. In an elegant and thorough series of genetic and imaging experiments, Parisi and colleagues show that tumor associated plasmatocytes (TAP) induce a feedback loop leading to tumor inhibition. Plasmatocytes produce the *Drosophila* TNFα ortholog, Eiger, which stimulates tumor production of a factor, Pvf1, that promotes plasmatocyte proliferation. Plasmatocytes also kill tumor cells by producing Eiger and the Toll ligand Spaetzle which induces Toll activation in the distant fat body; this analog of the mammalian liver produces some factor that acts with Eiger to facilitate tumor cell death.

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