Drosophila cellular immunity: a story of migration and adhesion

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Journal of Cell Science 124, 1373-1382
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doi:10.1242/jcs.064592

Summary

Research during the past 15 years has led to significant breakthroughs, providing evidence of a high degree of similarity between insect and mammalian innate immune responses, both humoural and cellular, and highlighting *Drosophila melanogaster* as a model system for studying the evolution of innate immunity. In a manner similar to cells of the mammalian monocyte and macrophage lineage, *Drosophila* immunosurveillance cells (haemocytes) have a number of roles. For example, they respond to wound signals, are involved in wound healing and contribute to the coagulation response. Moreover, they participate in the phagocytosis and encapsulation of invading pathogens, are involved in the removal of apoptotic bodies and produce components of the extracellular matrix. There are several reasons for using the *Drosophila* cellular immune response as a model to understand cell signalling during adhesion and migration in vivo: many genes involved in the regulation of *Drosophila* haematopoiesis and cellular immunity have been maintained across taxonomic groups ranging from flies to humans, many aspects of *Drosophila* and mammalian innate immunity seem to be conserved, and *Drosophila* is a simplified and well-studied genetic model system. In the present Commentary, we will discuss what is known about cellular adhesion and migration in the *Drosophila* cellular immune response, during both embryonic and larval development, and where possible compare it with related mechanisms in vertebrates.

Key words: Cellular immunity, Haemocyte, Integrin, Phagocytosis, Parasitisation, Small GTPase

Introduction

Ever since Boman et al. (Boman et al., 1972) published their seminal paper showing that *Drosophila melanogaster* produced antibacterial agents in reaction to infection, research into the insect immune response has led to significant breakthroughs and underscored *Drosophila* as a suitable model system for studying the evolution of innate immunity. When *Drosophila* are invaded by pathogenic organisms, such as bacteria or fungi, induction of the immune response leads to the secretion of antimicrobial peptides into the haemolymph, and circulating immunosurveillance cells (haemocytes) attempt to phagocytise the invaders (Fig. 1). Parasites too large to undergo phagocytosis, such as eggs laid by endoparasitic wasps, provoke an encapsulation response, which involves the adhesion of numerous haemocytes around the invader, as well as inducing a melanisation response. Research has also established *Drosophila* as a valuable model for studying the innate immune response against viral pathogens (Kemp and Imler, 2009), although the contribution of circulating immune cells against viruses emerged only recently (Costa et al., 2009).

Although the production of antimicrobial peptides, and other host defence factors, mainly relies on fat body cell function, cellular immunity is provided by the haemocyte lineage, comprising three broad subtypes of cells – the plasmatocytes, crystal cells and lamellocytes – with each providing specific functions, namely phagocytosis, coagulation and encapsulation, respectively (Fig. 2). Haematopoiesis begins in the embryonic head mesoderm and gives rise to two haemocyte cell lineages: the plasmatocytes and crystal cells (Fossett et al., 2001; Fossett et al., 2003; Lebestky et al., 2000; Milchanowski et al., 2004; Waltzer et al., 2003). Embryonic plasmatocytes are involved in the phagocytosis of apoptotic bodies and bacteria, and in wound healing (Moreira et al., 2010; Stramer et al., 2005; Tepass et al., 1994; Vlisidou et al., 2009; Wood et al., 2006). They also produce antimicrobial peptides and secrete components of the extracellular matrix (ECM) (Lemaitre and Hoffmann, 2007; Martinek et al., 2008). In larvae, haemocytes are located in three main compartments: first in circulation; second in a haematopoietic organ, the lymph gland, which consists of multiple pairs of lobes and is located behind the brain; and third as a sessile haemocyte population found just underneath the larval cuticle (Crozatier and Meister, 2007; Lemaitre and Hoffmann, 2007; Williams, 2007). In healthy larvae, plasmatocytes are the most abundant haemocytes in circulation and are involved in phagocytosis, encapsulation and the production of antimicrobial peptides (Crozatier and Meister, 2007; Lemaitre and Hoffmann, 2007; Williams, 2007). Crystal cells make up the remaining circulating haemocytes and, owing to their ability to rupture and release components of the phenol oxidase cascade, are indispensable for the melanisation of invading organisms, for wound repair and for coagulation (Bidla et al., 2007; Meister, 2004). Melanisation involves a complex series of reactions that converts tyrosine into melanin, through phenol oxidase and other enzymes (Christensen et al., 2005). In addition to its role in coagulation and wound repair, insects also use melanisation as a means to confine parasites inside a hardened proteinaceous capsule. The third type of haemocyte, lamellocytes, are rarely seen in healthy larvae, but they circulate in large numbers after parasitisation. Lamellocytes are

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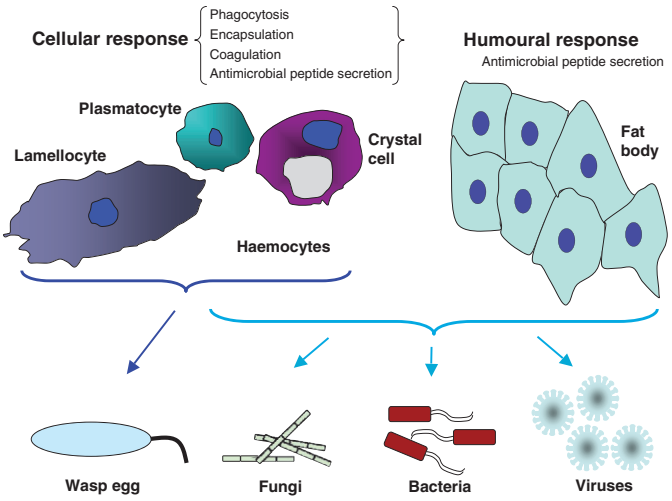

Fig. 1. Schematic representation of the *Drosophila* larval immune response. Microbial infections initiate responses by both the cellular and humoural immune tissues. Haemocytes and the fat body can produce and secrete antimicrobial peptides in response to bacterial and fungal infections. Both haemocytes and the fat body might be involved in the anti-viral response, whereas haemocytes are essential for the anti-parasitic encapsulation response.

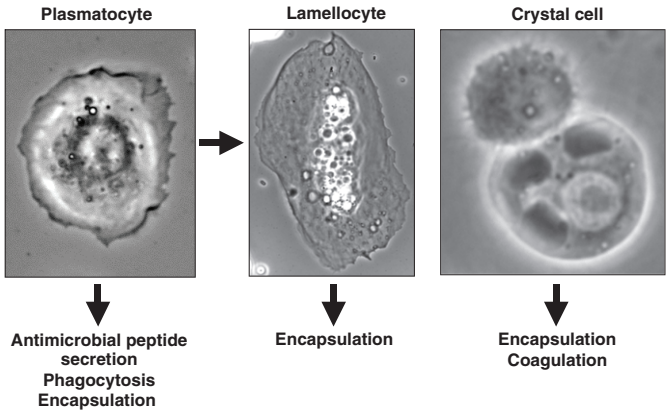

Fig. 2. *Drosophila* haemocyte subtypes. Plasmatocytes resemble the mammalian monocyte macrophage lineage and are involved in phagocytosis, encapsulation and the production of antimicrobial peptides. Lamellocytes, which are rarely seen in healthy larvae, are larger than other haemocytes and are involved in the encapsulation of invading pathogens. Many lamellocytes derive directly from plasmatocytes, as indicated by the arrow. Crystal cells rupture to release components of the phenol oxidase cascade, involved in the encapsulation process of invading organisms, coagulation and wound repair. The image of the crystal cell has been kindly provided by Ulrich Theopold.

larger than other haemocytes and seem to be a specialised cell type that is involved in the encapsulation of foreign pathogens that are too large to undergo phagocytosis (Meister, 2004; Rizki and Rizki, 1992; Williams, 2007). Recently, it was demonstrated that, in addition to their genesis in the larval lymph gland, many lamellocytes derive directly from plasmatocytes (Fig. 2) (Honti et al., 2010; Stofanko et al., 2010).

To date, most of our knowledge on phagocytosis and cell migration in response to infection or tissue damage comes from studies in human cell culture (Groves et al., 2008; Dupuy and Caron, 2008). Chemotaxis and phagocytosis have also been extensively studied in the unicellular free-living amoeba *Dictyostelium discoideum*, which actively feeds on bacteria by phagocytosis, thus enabling the deciphering of crucial mechanisms and molecules involved in cell chemotaxis and bacterial phagocytosis and killing (Cosson and Soldati, 2008; Jin et al., 2009; Bozzaro et al., 2008; Lee et al., 2010). Complementary studies show that *Drosophila* is also a particularly relevant model organism for genetic in vivo studies of phagocytic cell function during development and for studies on the elimination of pathogens or transformed cells (Stuart and Ezekowitz, 2008). One of the main advantages of using *Drosophila* for studying cell immune functions, compared with using other invertebrate models, is the complexity of its immune response. Indeed, *Drosophila* immunity relies on interconnected humoural and cellular processes, which both show striking similarities with those in mammalian innate immunity (Lemaitre and Hoffmann, 2007; Ferrandon et al., 2007). Studies using the embryonic *Drosophila* cellular immune system confirmed that it was a relevant model system for understanding the activity of circulating immunosurveillance cells during developmental processes, wound healing and the host response to infection. Moreover, specific advantages of using *Drosophila* as a model system for the cellular immune response are the abilities to follow cell migration in vivo (Stramer et al., 2005) and to assess the contribution of immune cells in the defence against infection (Stramer et al., 2005; Tingval et al., 2001). In this Commentary, we discuss the current knowledge regarding the *Drosophila* embryonic and larval cellular immune response in the context of cellular adhesion and migration, and where possible compare it with related mechanisms in vertebrates.

Migration of *Drosophila* embryonic macrophages

Real-time studies in living *Drosophila* embryos have demonstrated that the migration of differentiated plasmatocytes depends on similar growth factors to that of mammalian blood phagocytic cells (Stramer et al., 2005). Notably, developmentally controlled plasmatocyte migration requires the *Drosophila* platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) receptor Pvr, which, in mammals, directs neutrophils and/or macrophage migration during development and in response to infection (Brückner et al., 2004; Cho et al., 2002; Duchek et al., 2001; Heino et al., 2001). Pvr has three potential ligands, the PDGF- and VEGF-related factors 1 to 3 (Pvf1, Pvf2 and Pvf3); of these, Pvf2 and Pvf3 contribute to embryonic plasmatocyte migration (Brückner et al., 2004; Munier et al., 2002; Olofsson and Page, 2005; Wood et al., 2006). Developmental migration of embryonic plasmatocytes occurs in three distinct stages. First, plasmatocytes migrate out of the cephalic (head) mesoderm to populate the head region of the embryo. In the next phase, plasmatocytes leave the head region and follow Pvf-regulated routes around the embryo, including along the ventral nerve chord (VNC) and the embryonic dorsal vessel (heart) (Fig. 3). During this process, plasmatocytes, in a manner similar to mammalian macrophages, start to ingest apoptotic bodies that arise from naturally occurring developmental processes (Tepass et al., 1994). In the latter stages of embryogenesis, plasmatocytes are found scattered throughout the embryo, but maintain their ability to migrate to wound sites (Fig. 3) (Moreira et al., 2010; Paladi and Tepass, 2004; Tepass et al., 1994; Wood et al., 2006).

The molecular basis of plasmatocyte migration during development has been elucidated by in vivo genetic studies. It has

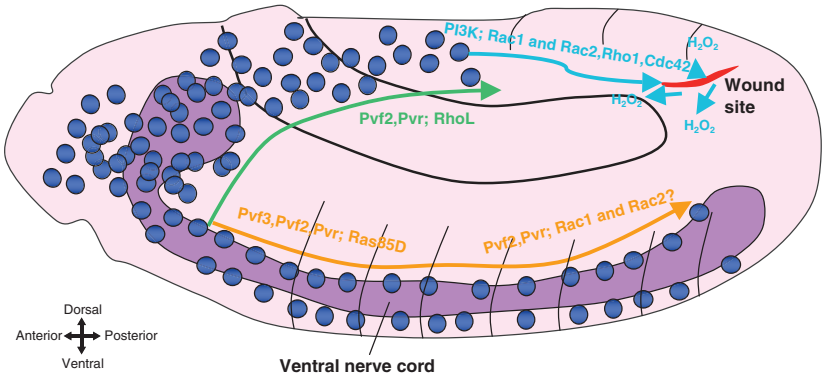

Fig. 3. Schematic illustration of the pathways controlling embryonic plasmatocyte migration during normal development and after wounding. The illustration shows a *Drosophila* embryo at late embryogenesis. Pvf3 and Pvr might signal through the small GTPase Ras85D to initiate plasmatocyte (represented by the blue circles) migration along the anterior portion of the VNC. Later in development, Pvf2 signals through Pvr and possibly also through Rac1 and Rac2 (indicated by a question mark) and results in plasmatocyte migration along the posterior VNC (along the orange arrow). The wound response is illustrated in the upper right-hand corner of the embryo (light blue). After epithelial wounding, the damaged cells or cells near the wound (red area) release H_2O_2, which is sensed by plasmatocytes and initiates their migration to the wound site (along the blue arrow). Migration to wound sites does not require Pvr signalling, but instead relies on PI3K activity and the small GTPases.

been proposed that Pvf3 is the first Pvr ligand that is involved, and that it regulates the migration of plasmatocytes out of the head region. Indeed, *Pvf3* is expressed in the VNC earlier in development than *Pvf2*, and in *Pvf3* mutant flies plasmatocytes do not begin their migration along the VNC (Table 1) (Wood et al., 2006). Although the signalling pathways that drive plasmatocyte migration downstream of Pvr are still not fully established, the small GTPases Rac1 and Rac2 are good candidates for mediating Pvr-dependent signalling; in *Drosophila* embryos lacking the activity of these two GTPases, plasmatocytes fail to migrate along the posterior end of the VNC (Fig. 3). Furthermore, Rac1 is required downstream of Pvr for border cell migration during *Drosophila* oogenesis and for thorax closure during metamorphosis; thus, it is likely to play a similar role during plasmatocyte migration (Duchek et al., 2001; Ishimaru et al., 2004; Mathieu et al., 2007). Interestingly, a similar pathway (i.e. PDGF receptor signalling through the small GTPase Rac1) controls directional cell migration in mammalian cells (Kawada et al., 2009). In mammalian NIH3T3 cells, the PDGF receptor sequentially activates Ras-related protein 1 (Rap1) and Rac1 to induce lamellipodia formation, and this activation is necessary for PGDF-regulated cell migration in the cell line (Takahashi et al., 2008). Similarly, *Drosophila* Rap1 and its activator PDZ-GEF, also known as Dizzy, are necessary for the migration of embryonic haemocytes along the posterior half of the VNC (Huelsmann et al., 2006; Paladi and Tepass, 2004; Wood et al., 2006). These results suggest that Pvr signals through Rap1, Rac1 and Rac2 to coordinate the second stage of plasmatocyte migration in response to activation by the Pvf2 ligand (Fig. 3).

However, the initial Pvf3-induced migration of plasmatocytes out of the head region does not require the Rac1 and Rac2 GTPases. This indicates that other factors downstream of Pvr might control plasmatocyte migration in response to Pvf3 during the initial stages of migration out of the head region. In mammalian cells, receptor tyrosine kinases, including the PDGF receptor, signal through Ras to regulate cell proliferation, cell polarity and cell migration (Lahsnig et al., 2009; Ogita et al., 2009). By analogy, in *Drosophila* embryos, Pvr signalling in response to Pvf3 expression during the initial phases of plasmatocyte migration might occur through the *Drosophila* Ras protein Ras85D. Indeed, a deletion removing two closely located GTPase-encoding genes, *Ras85D* and the insect-specific Rho-family GTPase *RhoL*, prevents plasmatocytes from migrating out of the head region (Paladi and Tepass, 2004). As a mutation in *Ras85D* is known to affect larval haemocyte behaviour and cell morphology, *Ras85D* might be required for

Table 1. *Drosophila* developmental stages and plasmatocyte migration

Stage of development	Hours after fertilisation	Developmental process	Plasmatocyte migration
Stage 10	4.0–5.0	Gnathal and clypeolabral lobe formation (head features)	Plasmatocytes can be first identified
Stage 11	5.0–7.0	Epidermal parasegmentation evident; mesectodermal cell ingress; end of third postblastoderm mitosis; end of neuroblast formation	Plasmatocytes migrate throughout the head region; Pvf3 expressed in VNC
Stage 12	7.0–9.5	Germ band retraction; ventral closure; segment formation; fusion of anterior and posterior midgut	Plasmatocytes start spreading throughout the embryo; Pvf2 expressed in anterior portion of the VNC; Pvf-induced migration along the anterior portion of the VNC
Stage 13	9.5–10.0	End of germ band retraction; central nervous system and peripheral nervous system differentiation	Pvf2 expressed along entire VNC; plasmatocytes migrate along VNC
Stage 14	10.0–11.0	Dorsal closure of epidermis; head involution begins	Beginning anteriorly and moving in a posterior direction; *Pvf2* RNA levels decrease in the VNC; plasmatocytes migrate along the posterior portion of VNC
Stage 15	11.0–13.0	End of dorsal closure; head involution; cuticle deposition begins	Plasmatocytes are evenly distributed throughout the embryo
Hatching	21–22	Hatching to first-instar larva	

Journal of Cell Science

Journal of Cell Science

embryonic haemocyte migration (Bakal et al., 2007; Rogers et al., 2003; Zettervall et al., 2004). It has been demonstrated that *RhoL* is not necessary for Pvr-induced plasmatocyte migration along the VNC (Paladi and Tepass, 2004; Siekhaus et al., 2010).

Taken together, however, the above results suggest that *Drosophila* Pvr activates Ras and Rac GTPases (*Rap1*, *Ras85D*, *Rac1* and *Rac2*) to ensure appropriate cellular migration in response to developmental signals, in a manner similar to that with the mammalian PDGF receptor.

Among the underlying processes that are absolutely required for cell migration and transmigration is integrin-mediated cell adhesion at the leading edge of the cell (Caswell et al., 2009). A recent study demonstrated that, in *Drosophila* embryonic plasmatocytes, RhoL interferes with Rap1 GTPase-induced integrin adhesion, by inhibiting the localisation of Rap1 to the leading edge. Inhibition of integrin-based adhesion is necessary to regulate the cadherin interactions that allow plasmatocytes to transmigrate from the head region, through the epithelium, to the posterior of the embryo (Siekhaus et al., 2010). The molecular events underlying this transmigration are very similar to those in the migration of vertebrate immune cells during inflammation (Basoni et al., 2005; Ebisuno et al., 2009; M'Rabet et al., 1998). In fact, although developmental and pro-inflammatory cytokines use different types of receptors in *Drosophila* and mammalian phagocytic cells, they employ common downstream effectors for driving cell adhesion changes during migration.

Migration of embryonic macrophages in response to epithelial wounding

In mammals, studies have shown that leukocyte polarisation and migration in response to wounding can be induced by a variety of factors, including cytokines, ATP, bacterial factors (e.g. lipopolysaccharides and peptidoglycans) and ECM breakdown products (Hammer, 2005; Jones, 2000). Many of these factors stimulate G-protein-coupled receptor (GPCR) pathways and lead to the activation of phosphoinositide 3-kinase (PI3K). One major role of PI3K is to phosphorylate phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] to give rise to phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] (Cantley, 2002). PtdIns(3,4,5)P_3 is known to serve as a docking site for the Dbl-homology domain and Dock-type Rho guanine-nucleotide-exchange factors (RhoGEFs), as well as for the serine/threonine protein kinase Akt (also known as PKB). The recruitment of RhoGEFs leads to localised activation of Rho GTPase pathways that are involved in inducing protrusions, such as filopodia and lamellopodia, and thus in directional movement (Alahari, 2003; Kolsch et al., 2008; Lavenburg et al., 2003; Sachdev et al., 2002).

A number of elegant studies, in which the epithelium of developing *Drosophila* embryos was wounded with a laser, have assessed the ability of plasmatocytes to migrate to the wound site in a living organism (Stramer et al., 2005; Wood et al., 2006). Upon tissue damage, plasmatocytes migrate to the wound site in a manner that is independent of the Pvf–Pvr pathway but is dependent on PI3K, demonstrating that plasmatocytes are able to distinguish between developmental cytokines, such as Pvf2 and Pvf3, and PI3K-mediated wound-induced signals (Wood et al., 2006). The receptor that activates PI3K in embryonic plasmatocytes is yet to be identified, but it is possible that, similar to the pathway in mammalian leukocytes, *Drosophila* plasmatocytes also use a GPCR-coupled pathway to recognise as yet unknown wound-induced signals, which redirect them from their developmentally regulated Pvf–Pvr-mediated pathway of migration towards the site of wounding. Wood and collaborators have also attempted to determine whether additional chemotactic signals, known to attract macrophages to wound sites, elicit a haemocyte response to wounding [e.g. ATP, epidermal growth factor (EGF) and fibroblast growth factor (FGF)], but found that only H_2O_2 was able to redirect plasmatocytes to the wound site (Moreira et al., 2010). It should be noted that, similar to mammalian embryos (Morris et al., 1991; Wood et al., 2000), there is a refractile period early in haemocyte development, in which haemocytes do not respond to H_2O_2, and that they only become responsive to wound signalling after stage 15 (mid-to-late embryogenesis) (Moreira et al., 2010).

In the future, the knowledge gained from the above-mentioned initial studies will allow researchers to, for instance, use the *Drosophila* embryonic cellular immune system to understand how circulating immunosurveillance cells differentiate between, and prioritise, two competing signals in physiologically relevant situations in vivo.

Cytoskeleton regulatory proteins control larval haemocyte cell shape changes

During the course of an infection, immune cells migrate towards the site of microbial entry with the aim of eliminating pathogens and, furthermore, contributing to the repair of the wound caused by the microbe (Martin and Leibovich, 2005; Nishio et al., 2008). This process requires not only cell migration but also changes in cell adhesion, as well as phagocytosis, which universally depend on the dynamics of the actin network (i.e. the polymerisation or depolymerisation of actin filaments) (Fig. 4). Briefly, actin dynamics mainly relies on the Arp2/3 complex, a few nucleation-promoting factors and formins, which control filament polymerisation and depolymerisation through interactions with regulatory proteins (Campellone and Welch, 2010). Actin nucleation depends upon the activation of Arp2/3, which directly binds to members of the Wiskott–Aldrich syndrome protein (Wasp) family, including Scar, whereas debranching and depolymerisation of actin filaments are controlled by cofilin and cofilin-like proteins (Chan et al., 2009). The concerted activity of Rho-family GTPases (e.g. Rho, Rac and Cdc42) then directs the formation of different cellular protrusions, such as filopodia, which contain narrow actin projections (so-called actin spikes), membranes ruffles or large lamellipodial extensions (Etienne-Manneville and Hall, 2001; Nobes and Hall, 1995). As might be expected, studies in cultured *Drosophila* S2 cells (a cell line derived from embryonic haemocytes) or primary larval plasmatocytes revealed a similar requirement for cytoskeleton regulatory proteins to that previously found in mammalian cells during the processes of migration (see above), phagocytosis and the control of cell shape changes upon their spreading on glass (Table 2). A reverse genetic approach that investigated the contribution of 90 cytoskeleton regulatory proteins in lamellopodia formation in S2 cells, through systematic RNA interference (RNAi), has demonstrated the requirement for actin nucleation proteins (Arp2/3 and Scar), capping proteins, filament depolymerisation factors [cofilin and actin-interacting protein 1 (Aip1)] and actin-monomer-binding proteins (profilin and cyclase-associated protein) (Rogers et al., 2003). Moreover, initiation of cell spreading requires Rac GTPases and the adaptor protein Dreadlocks (Dock), which is a known stimulator of Scar and Arp2/3 (Kunda et al., 2003; Rogers et al., 2003). Direct genetic approaches have also revealed the necessity of cytoskeleton regulatory proteins in mediating phagocytosis. For example, the *Drosophila* Scar mutant was isolated in a screen that focused on

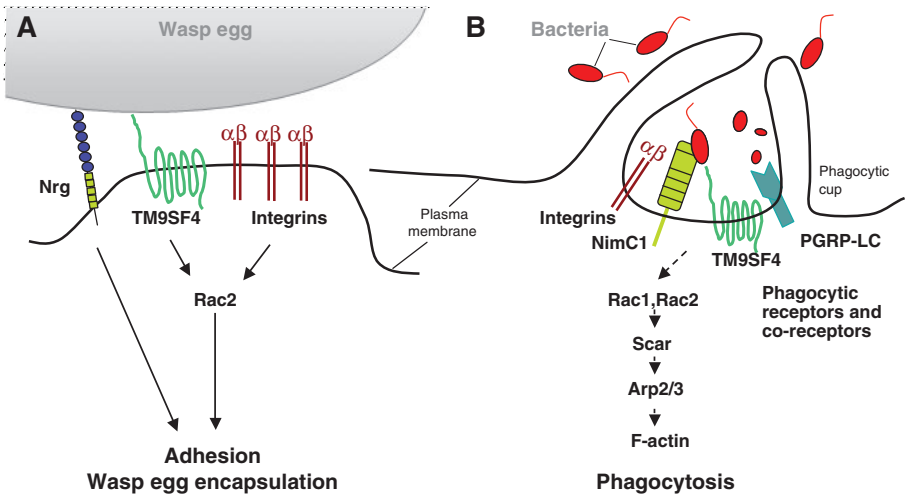

Fig. 4. Pathways inducing changes in haemocyte shape upon infection. (**A**) Integrin βPS defective (*mys* mutant) (Irving et al., 2005), *TM9SF4* (Bergeret et al., 2008) and *Rac2* (Williams et al., 2005) mutant larvae fail to encapsulate wasp eggs properly following larval parasitisation, and their larval plasmatocytes display either defective adhesion and/or phagocytosis defects associated with an abnormal F-actin cytoskeleton network. This suggests that Rac2 plays an essential function in signal transduction from adhesion transmembrane proteins to the cytoskeleton during the encapsulation processes. Similar to platelets during thrombosis, plasmatocytes require the L1CAM homologue Neuroglian (Nrg) to properly adhere to and spread over parasitoid wasp eggs (Williams, 2009). (**B**) Phagocytosis of smaller pathogens (such as bacteria or yeast, shown in red) depends upon their opsonisation by circulating receptors or complement-like proteins (not shown) (Garver et al., 2006; Strochein-Stevensen et al., 2006), and on their recognition by transmembrane phagocytic receptors, such as PGRP-LC, Eater or NimC1 (Ramet et al., 2002; Kocks et al., 2005; Kurucz et al., 2007). How exactly phagocytic receptors induce the formation of a phagocytic cup and actin reorganisation required for pathogen engulfment has been poorly investigated to date in *Drosophila*. Putatively, phagocytic receptors might interact with co-receptors, such as integrins, which are also required for phagocytosis, thereby possibly transmitting an internalisation signal to Rho GTPases that regulate the cytoskeleton. Deciphering the signalling molecules between these receptors and the cytoskeleton regulatory proteins known to be required for phagocytosis, such as the Rac1 and Rac2 (dashed arrow), is one of the future challenges to better understand *Drosophila* cellular immunity. Rho GTPases, notably Rac1 and Rac2, affect haemocyte cell shape changes, upon infection, by activating cytoskeleton-associated proteins, such as Scar and Arp2/3.

the phagocytic properties of circulating primary macrophages isolated from mutant larvae. *Drosophila* Scar, and the closely related protein *Drosophila* Wasp, is required for the internalisation of *Escherichia coli* and *Staphylococcus aureus* particles (Pearson et al., 2003; Rogers et al., 2003). A second mutation, in *chickadee* (*chic*), was also isolated in the screen; the *chic* mutant macrophages exhibit an opposite phenotype, leading to enhanced engulfment properties (Rogers et al., 2003). The *chic* gene encodes the *Drosophila* homologue of profilin, and mammalian profilin was shown to bind and regulate Scar in vitro (Pollard and Borisy, 2003) and to promote actin dynamics in vivo (Bottcher et al., 2009; Witke, 2004). In addition to causing alterations in phagocytosis, the circulating plasmatocytes of both *scar* and *chic* mutant larvae display aberrant actin cytoskeleton structures and abnormal shape. Specifically, the plasmatocytes of *scar* mutants are enlarged and exhibit numerous actin spikes, whereas *chic* mutant cells spread more widely on glass, when compared with spreading of wild-type cells, and can be distinguished by a large lamellipodium around the cell. Therefore, it has been speculated that abnormal cell shape and defects in actin dynamics could be the underlying reason for the altered cell adhesion and phagocytosis observed in mutant plasmatocytes.

Although studies in *Drosophila* have permitted in vivo investigation of the function of cytoskeleton regulatory proteins, purposeful studies to decipher their activity in the physiological context, such as in the immune response, had largely not been undertaken. Along these lines, our groups and others have performed detailed analyses of the in vivo function of the Rho-family GTPases in haemocytes in order to show that they make essential and non-redundant contributions to embryonic migration (see above), to the cell shape changes of haemocytes and to bacterial phagocytosis. Notably, expression of wild-type and mutant forms of Rac1 in *Drosophila* specifically in plasmatocytes revealed that Rac1 induces F-actin accumulation and lamellipodia formation through two distinct pathways involving either the Jun kinase Basket (Bsk) or Twinstar (the *Drosophila* cofilin homologue) (Williams et al., 2006). A complementary study has shown that Rac1 induces Rho1 activation and F-actin stress fibre formation, which allows filopodia to differentiate, and, moreover, that Rac2 is also required for this process (Williams et al., 2007). Finally, experiments with injected or in-vivo-expressed bacterial toxins that target GTPases also confirm that Rho GTPases contribute to embryonic haemocyte motility (Vlisidou et al., 2009) and to larval and adult haemocyte-dependent phagocytosis of bacteria (Avet-Rochex et al., 2005; Avet-Rochex et al., 2007). Such in vivo models, in which a bacterial toxin is either injected into the living *Drosophila* embryos or expressed in host cells by transgenic means, open new avenues to study the functions of bacterial toxins in vivo. These experiments might also lead to the discovery of new host genes and proteins whose functions are modified by pathogens and which are therefore likely to be involved in innate immunity.

Although most reverse genetic approaches were initiated from data obtained from human cell culture models, a further protein involved in cell adhesion and phagocytosis in *Drosophila* was deduced using data obtained in the phagocytic unicellular organism *D. discoideum*. The nonaspanin TM9SF4, also known as Phg1A, is an evolutionarily conserved protein of nine transmembrane segments, whose function in adhesion and phagocytosis was first described in *Dictyostelium* (Benghezal et al., 2003; Cornillon et al., 2000) and subsequently also observed in *Drosophila* haemocytes

Journal of Cell Science

Table 2. Comparison of a non-exhaustive set of proteins regulating cell shape in *Drosophila* haemocytes and their mammalian counterparts

Proteins [mammalian name (*Drosophila* homologue)]	Function in *Drosophila* haemocytes	References	Known functions in mammalian phagocytes	References
Membrane proteins				
β-integrin (Myospheroid)	Adhesion; encapsulation	(Irving et al., 2005)	Adhesion; migration; signalling	(reviewed by Dupuy and Caron, 2008)
L1CAM (Neuroglian)	Adhesion	(Nardi et al., 2006)	Cell adhesion; homophilic and heterophilic interactions	(reviewed by Hortsch, 2000)
	Encapsulation	(Williams, 2009)	Cell migration	(Maddaluno et al., 2009)
			Platelet aggregation	(Prevost et al., 2002)
TM9SF4 (TM9SF4 or Phg1A)	Adhesion; phagocytosis; encapsulation	(Bergeret et al., 2008)	Phagocytosis and cannibalism (in tumour cells)	(Lozupone et al., 2009)
Rho GTPases				
Common functions	Haemocyte migration	(Paladi and Tepass, 2004)	Adhesion; migration; phagocytosis; cytoskeleton dynamics; integrin complex assembly; FAK turnover	(reviewed by Ridley, 2001; Bokoch, 2005)
	Wound-induced migration	(Kawada et al., 2009; Moreira et al., 2010; Wood et al., 2006)	Macrophage adhesion and migration	(Ridley, 2007)
	Adhesion	(Stramer et al., 2005)	Neutrophil chemotaxis	(Zhang et al., 2009)
	Phagocytosis	(Avet-Rochex et al., 2007)		
Rac1	Recruitment of sessile haemocytes upon immune challenge	(Williams et al., 2005; Williams et al., 2006)	Cell spreading; membrane ruffling; phagocytosis	(Wells et al., 2004; Cox et al., 1997)
	Filopodia and lamellipodia differentiation		Platelet aggregation	(McCarty et al., 2005; Ridley, 2007)
Rac2[a]	Phagocytosis and host defence	(Avet-Rochex et al., 2007)	NADPH oxidase activation	(Knaus et al., 1991)
	Encapsulation	(Williams et al., 2005; Williams et al., 2006)	Podosome formation	(Linder and Aepfelbacher, 2003; Ridley, 2007)
Cdc42	Cell polarity; velocity	(Stramer et al., 2005)	Cell chemotaxis	(Allen et al., 1998)
	Cell shape	(Rogers et al., 2003)	Cell polarity	(Etienne-Manneville and Hall, 2001)
			Wasp activation	(Park and Cox, 2009)
Rho1	Tail retraction	(Stramer et al., 2005)	Cell migration; tail retraction	(Worthylake et al., 2001)
	Dynamics of cell–cell contact			
Other cytoskeleton regulatory molecules				
SCAR (SCAR)	Phagocytosis; lamellipodia	(Pearson et al., 2003; Rogers et al., 2003)	Actin nucleation; activation of Arp2/3 complex	(Machesky et al., 1999)
Arp2/3	Phagocytosis; lamellipodia	(Pearson et al., 2003; Rogers et al., 2003)	Actin nucleation	(Etienne-Manneville and Hall, 2002)
			Polarisation and migration	(Linder et al., 2000)
			Activation of Rho GTPases	(reviewed by Campellone and Welch, 2010)
Cofilin (Twinstar)	Lamellipodia; spreading	(Rogers et al., 2003)	Arp2/3 dissociation; actin branching	(Chan et al., 2009)
Profilin (Chickadee)	Lamellipodia; spreading; phagocytosis	(Pearson et al., 2003; Rogers et al., 2003)	Actin monomer binding; actin assembly	(Coppolino et al., 2001; Machesky et al., 1999)
Diaphanous	Filopodia; Rho-dependent signalling	(Williams et al., 2007)	Actin polymerisation; phagocytic cup; Rho-dependent signalling	(Colucci-Guyon et al., 2005; Brandt et al., 2007)
Signalling molecules				
Rho kinase	Rac1 activation; actin stress fibres	(Williams et al., 2007)	Rho-induced actin reorganisation	(Watanabe et al., 1999)
Slingshot	Lamellipodia formation; F-actin distribution	(Rogers et al., 2003)	Dephosphorylation of cofilin	(Niwa et al., 2002)

[a]*Drosophila* Rac2, also named Rac1b, is approximately equally related to human Rac1 [171 of 191 identical amino acids (89% identity)] and human Rac2 protein [169 of 192 identical amino acids (88% identity)].

(Bergeret et al., 2008) and in human tumour cells (Lozupone et al., 2009). Similar to the phenotype of *scar* and *Rac1* mutants, *Drosophila TM9SF4* mutant haemocytes are larger than control haemocytes and can be distinguished by their numerous actin spikes. *Drosophila TM9SF4* mutant larvae also fail to correctly encapsulate parasitoid wasp eggs (Bergeret et al., 2008), a process that requires Rac2 and integrin-mediated adhesion of plasmatocytes to the foreign parasite (Irving et al., 2005; Williams et al., 2005). Owing to the phenotypic similarities to those induced by mutant components of the cytoskeleton regulatory networks, such as Myospheroid (a *Drosophila* integrin β), Rac1, Rac2, Scar and Twinstar (*Drosophila* cofilin), TM9SF4 is considered a candidate for coupling changes in the actin cytoskeleton to adhesion and, putatively, for controlling integrin-dependent activation of Rho

Journal of Cell Science

Journal of Cell Science

GTPases during the adhesion of plasmatocytes to pathogens (Irving et al., 2005; Zhuang et al., 2008).

In addition, we note that there are several phagocytic receptors, which have been discovered through genetic and reverse genetic approaches, that mediate the recognition and internalisation of various pathogens by larval plasmatocytes or *Drosophila* cultured S2 cells. These include Eater, Nimrod C1 (NimC1), and the peptidoglycan recognition proteins (PGRPs) PGRP-LC and PGRP-SC1 (Kocks et al., 2005; Kurucz et al., 2007; Pearson et al., 2003; Ramet et al., 2002; Garver et al., 2006). However, the signalling pathways for the subsequent activation of Rho GTPases and reorganisation of the actin network, which are required for the initiation of the phagocytic cup, have not yet been elucidated in *Drosophila*. Deciphering these pathways constitutes a challenge for building an integrated view of pathogen recognition, phagocytosis and killing in multicellular organisms.

The *Drosophila* encapsulation response has aspects in common with thrombosis

The first step in mammalian thrombosis is the adhesion of platelets to sites of endothelial damage in vessels where the ECM has been exposed, and the activation of platelets by inflammatory triggers might be a crucial component leading to atherothrombosis. Insertion of a parasitoid wasp egg into the *Drosophila* larval open circulatory system (haemocoel) mimics vascular injury as it results in deposition of ECM onto the egg (Russo et al., 1996). Parasitisation also induces changes in haemocyte morphology, and in their inherent adhesive properties, thus allowing haemocytes to form a cellular capsule around the ECM. In a manner similar to processes occurring during thrombosis, cell–cell contacts between haemocytes on the surface of the wasp egg promote the stabilisation of a growing cellular capsule (Russo et al., 1996; Williams et al., 2005). Analogous to activated platelets inducing the inflammation response, which can enhance the development of the growing thrombus, parasitisation of *Drosophila* larvae also gives rise to an inflammatory state, which leads to the recruitment of additional haemocytes into the circulation (Lanot et al., 2001; Markus et al., 2009; Zettervall et al., 2004).

After attaching to the wasp egg, plasmatocytes change from a rounded to a more spread morphology. In a process similar to platelets during thrombosis, once plasmatocytes are attached and start to spread on the wasp egg, they extend filopodia from their cell periphery towards other plasmatocytes (Williams, 2009). After spreading around the wasp egg, plasmatocytes form cellular junctions, effectively separating the wasp egg from the larval haemoceol (Russo et al., 1996; Williams et al., 2005). Next, lamellocytes, the second class of circulating haemocytes, introduced above, recognise the plasmatocytes surrounding the wasp egg. Although it is evident that adhesion and change in cell shape are an essential part of the cellular immune response against parasitoid wasp eggs, we still do not fully understand the spatiotemporal regulation of the signalling events that are involved in this response.

Towards this goal, Wertheim and colleagues performed a microarray analysis of *Drosophila* genes that are upregulated after parasitisation by the parasitoid wasp *Asobara tabida* (Wertheim et al., 2005). The analysis revealed a number of genes that are involved in cell adhesion and cytoskeleton regulation, including genes encoding Hemolectin, a protein that contains a von Willebrand factor domain and known to be involved in coagulation (Lesch et al., 2007), two fibrinogen-like proteins, integrins and tubulins, all of which are also centrally involved in mammalian thrombosis (Jennings, 2009), further highlighting that both processes are related. In addition, Irving and colleagues reported that the *Drosophila* β-integrin Myospheroid, and possibly the α-integrin αPS4, is necessary for lamellocytes to correctly encapsulate parasitoid wasp eggs (Irving et al., 2005). Furthermore, our work has highlighted the importance of another adhesion molecule, the *Drosophila* L1CAM homologue Neuroglian (Nrg), in the encapsulation response (Williams, 2009). L1CAM was previously shown to be necessary for platelet aggregation during thrombus formation, where it interacts with integrins (Prevost et al., 2002). The Rho-family GTPases Rac1 and Rac2 were shown to be involved in the *Drosophila* cellular immune response against the parasitoid wasp egg from *Leptopilina boulardi* (Williams et al., 2005; Williams et al., 2006). Intriguingly, mammalian Rac1 is known to be necessary for platelets to form a proper thrombus (Akbar et al., 2007; McCarty et al., 2005). Gaining a thorough understanding of the *Drosophila* anti-parasitoid response thus might lead to it being used as an in vivo model to identify new anti-thrombotic drugs.

Concluding remarks

Phagocytic cells must be capable of migration by chemotaxis, as well as internalisation and digestion of external dead or live material, regardless of whether they act as a unicellular organism, such as free-living amoebae, or inside a multicellular organism. The functions of phagocytic cells serve many diverse biological processes, such as nutrition, tissue remodelling, pathogen recognition and clearance, antigen presentation, cytokine secretion and the elimination of altered cells or body parts from the organism itself (Desjardins et al., 2005). This panel of functions relies on protein-sensing complexes, adhesion molecules, signalling pathways, membrane dynamics and cytoskeleton modifications that are mostly evolutionarily conserved (Abedin and King, 2010; Wang, 2009; Pollard and Cooper, 2009; Orlando and Guo, 2009; Insall and Machesky, 2009). Although amoebae are extensively used as the simplest genetic model to provide new insight into molecular mechanisms of adhesion, chemotaxis and phagocytosis (Abedin and King, 2010; Jin et al., 2009), the use of more complex non-mammalian animals, such as nematodes (D'mello and Birge, 2010), *Drosophila* (Irving et al., 2005; Kocks et al., 2005; Avet-Rochex et al., 2007; Williams et al., 2006; Stuart and Ezekowitz, 2008) and zebrafish (Herbomel et al., 1999; Lieschke et al., 2001; Mathias et al., 2009), has provided novel tools for the investigation of phagocyte function in developmental tissue remodelling, tissue repair and host defence. Nematodes principally increased our understanding of apoptotic cell clearance (Zhou and Yu, 2008; Kinchen and Ravichandran, 2010), whereas *Drosophila* and zebrafish additionally seem to be relevant models for the study of innate immunity (in both cases) or adaptive immunity (in the case of zebrafish). A main achievement of the past decade is undoubtedly the development of live imaging in complex organisms, such as nematodes, *Drosophila* (Stramer et al., 2005: Wood et al., 2006), zebrafish (Levraud et al., 2009) and even mammalian models (Coombes and Robey, 2010), for the study of macrophage behaviour in various wild-type or mutant genetic contexts.

The field of *Drosophila* cellular immunity, in particular, has witnessed significant developments over the past two decades, expanding our general understanding of innate immunity. A number of recent studies have demonstrated that the *Drosophila* cellular immune response can help in our understanding of the mechanisms involved in haematopoiesis, wound healing, thrombosis,

Journal of Cell Science

immunosurveillance cell migration and immune activation. Results obtained in *Drosophila* not only support data obtained in mammalian cells, reinforcing our knowledge of immune cell function, but have also helped us to gain knowledge regarding the molecular mode of action of known proteins, as well as their roles in vivo during development or in response to infection. Recently, researchers have started to use the *Drosophila* cellular immune response as a tool to define how innate circulating immunosurveillance cells interact with tumours to restrict tumour growth (Pastor-Pareja et al., 2008). These findings demonstrate that, in the coming decades, *Drosophila* will continue to be a powerful model for increasing our understanding of the multifunctional innate cellular immune response.

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Journal of Cell Science

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