

Mammalian and *Drosophila* Blood: JAK of All Trades?

Minireview

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Model organisms such as *Drosophila* have provided insights into mammalian signaling pathways and their importance in development. The biochemical specificities of many molecules have been conserved during evolution, and some have maintained analogous functional roles. In this review, we examine features common to *Drosophila* and human blood systems, focusing on the functions of circulating cells and the signaling pathways involved in hematopoiesis and immune response.

Comparison of *Drosophila* and Human Blood

Red cells, white cells, and platelets are the three cellular constituents of mammalian blood. Their roles are to carry oxygen to all tissues, to confer immunity (both innate and acquired) and scavenge dying cells, and to promote clotting, respectively. Each cell type arises through an ordered and tightly regulated process of self-renewal and differentiation of a pluripotent hematopoietic stem cell. Successive waves of hematopoiesis take place during development. Primitive hematopoiesis consists of embryonic red cells produced by the blood islands of the yolk sac. Definitive hematopoiesis (all lineages) originates from a dorsal (aortic/mesonephros/gonad) compartment; it then switches to the fetal liver and ultimately to the bone marrow. Differentiating stem cells give rise to distinct committed progenitor cells responsible for the lymphopoietic and myelopoietic compartments. In response to both stromal and humoral signals, successive rounds of differentiation generate more mature cells with decreased self-renewal potential, eventually leading to the emergence of mature B and T cells in the lymphoid compartment, and of erythrocytes, macrophage/monocytes, granulocytes and megakaryocytes in the myeloid compartment (Orkin, 1996).

In insects the hemolymph acts as a blood-like carrier system for all materials except respiratory gases. In the *Drosophila* embryo the mesoderm of the procephalon gives rise to hemocytes and their derivatives, the macrophages. The main function of the macrophages, which represent almost 90% of blood cells by the end of embryogenesis, is to discard apoptotic cells. During larval and adult stages, blood cells are involved in innate immune response as well as phagocytosis. In larvae, hematopoiesis occurs in lymph glands located behind the brain which consist of five pairs of lobes associated with the dorsal heart vessel (Figure 1). Many questions concerning the ontogeny, morphology, and function of *Drosophila* blood cells remain unanswered. However, based on phenotypic analyses, a tentative cell lineage has been proposed (Gateff, 1978; Figure 1).

Although, a great deal is known about genes that play

essential roles in lineage commitment and differentiation of mammalian hematopoietic cells, little is known about the genetic control of blood cell lineage in insects. Natural mutations, and mutations induced by targeted homologous recombination in embryonic stem cells, have identified over 20 mammalian genes that act in blood cell differentiation. They include transcription factors, recombinases, signaling molecules, transmembrane receptors and secreted factors (Orkin, 1996). Two factors have been identified in *Drosophila*: Serpent, a GATA ortholog required for the development of hemocytes (Rehorn et al., 1996), and Lozenge (Lz), a transcription factor involved in crystal cell formation (Rizki and Rizki, 1981; Daga et al., 1996). Intriguingly, Lz contains a "RUNT" domain homologous to a portion of human transcription factor AML1. AML1 was originally isolated as a fusion partner in a translocation associated with acute myelogenous leukemia and more recently found to be necessary for definitive hematopoiesis in mice (Orkin, 1996).

***Drosophila* and Human Immune Responses**

Insect and mammalian responses to infection are strikingly similar. In humans, innate immunity provides the first line of defense to protect the organism from infection by foreign pathogens (bacteria, viruses, or parasites). Host recognition is directed against non-self determinants that are invariant among various microorganisms (Medzhitov and Janeway, 1997). This phylogenetically ancient defense mechanism is mediated by activation of a diverse set of receptors found primarily on monocyte/macrophage, granulocytic, and dendritic cells. Invading pathogens are bound, phagocytosed and subsequently killed through production and release of reactive oxygen species. Proteins from inactivated pathogens are degraded and small peptides are displayed on the surfaces of antigen-presenting macrophage and dendritic cells. Antigen presented to T cells triggers adaptive or specific immunity characterized by expansion of killer and helper T cell clones and stimulation of B cell antibody production. In addition, activation of macrophages initiates an acute phase response, characterized by release of inflammatory cytokines (IL-1, IL-8, IL-6, TNF- α), chemotactic factors and other cytokines (such as CSF-1, G-CSF, GM-CSF) that help recruit and activate immune cells (Medzhitov and Janeway, 1997).

Insects have also developed efficient host defense mechanisms against microorganisms including phagocytosis by individual plasmatocytes and the rapid transient synthesis of antimicrobial peptides. In addition, encapsulation can occur in response to bacterial infection through production of lamellocytes that aggregate around the pathogen. The aggregate somehow signals crystal cells to lyse and release components of the phenoloxidase cascade resulting in melanization of the capsular material (Ip and Levine, 1994).

Boman et al. (1972) demonstrated that adult flies injected with attenuated bacteria were protected against subsequent infection by normally lethal doses of the same bacterial strains. This immunity is mediated by

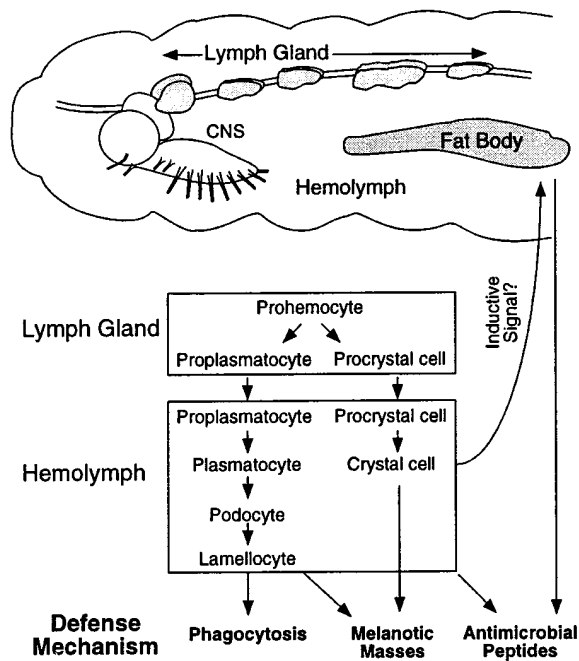


Figure 1. Biology of the *Drosophila* Blood Cell System and Immune Response
Modified from Gateff, 1978. See text for details.

induction of antimicrobial proteins in the fat body (reviewed by Meister et al., 1997). Circulating hemocytes, when activated by the invading agent, trigger a cellular response in the fat body, a functional homolog of the liver, presumably by release of a cytokine-like factor. This priming of the fat body, and to a lesser extent of plasmatocytes, results in the synthesis and release of antimicrobial peptides. These peptides fall into two classes: antibacterial (Cecropin, Diptericin, Attacin, Defensin) and antifungal (Drosomycin) peptides. One peptide, Metchnikowin, exhibits both antibacterial and antifungal activities. Due to a lack of mutations in genes encoding these peptides, it is not known whether loss of individual peptides increases susceptibility to infection. However, a mutation in the *immune deficiency* (*imd*) gene leads to loss of inducibility of a subset of antibacterial genes and causes hypersensitivity to infection (reviewed in Meister et al., 1997).

Thus, *Drosophila* and vertebrates have similar innate immune responses and phagocytic defense mechanisms. **Signaling Pathways Mediating the Innate Immune Response in Humans**

In vertebrates, the innate immune response is initiated by recognition of determinants specific to invading microorganisms and production of inflammatory cytokines. Released cytokines activate cognate receptors and mobilize two major signaling pathways: the NF- κ B and Janus kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathways. Cooperativity between these pathways is essential for the integrity of the response; suppression or inactivation of either dramatically reduces the ability of the organism to combat infection. Although the NF- κ B and JAK/STAT pathways are recruited via different cytokines during the acute

phase response, these pathways cooperate and converge at the level of transcription to induce expression of inflammatory genes (Ohmori et al., 1997).

Binding of IL-1 to the IL-1 receptor type I (IL-1RI) and to IL-1R accessory protein (IL-1R-AcP) recruits the IL-1 receptor-associated kinase IRAK, a serine/threonine kinase, to the signaling complex. Interestingly, IL-1RI and IRAK show considerable homology to *Drosophila* Toll (TI) and Pelle, respectively. Final steps in NF- κ B activation involve phosphorylation, proteasome targeting, and degradation of I κ B, the cytoplasmic inhibitor of NF- κ B. NF- κ B is then free to move to the nucleus to activate expression of genes involved in the immune function (Stancovski and Baltimore, 1997).

JAK/STAT activation occurs in parallel in the innate immune response. Binding of IL-6 to its receptor (IL-6R α /gp130), brings receptor-associated JAK proteins (JAK1, JAK2, or Tyk2) into close apposition. This results in JAK activation and tyrosine phosphorylation of gp130 at several residues, including a STAT-binding site. Bound STATs are then phosphorylated by activated JAK, leading to homo- or heterodimerization and nuclear translocation. Nuclear STAT complexes then activate gene transcription by binding to DNA recognition sites in target genes.

Other transcription factors have been implicated in the inflammatory response in addition to NF- κ B and STATs. AP-1 and C/EBP β (NF-IL6) are activated by IL-6, IL-1, LPS, and other inflammatory mediators. For example, C/EBP β null animals are defective in killing *Listeria* and tumor cells. Interestingly, promoters of many proinflammatory genes contain NF-IL6 sites interspersed with NF- κ B and AP-1 sites, a situation also found in the promoters of anti-bacterial genes in *Drosophila* (Meister et al., 1997). Finally, in mammals and *Drosophila*, p38/JNK has been implicated in a variety of immune-related pathways (Sluss et al., 1996).

Toll and 18W Regulate *Drosophila* Immune Responses

As in mammals, the *Drosophila* immune response involves molecules homologous to components of the NF- κ B pathway. To date, three NF- κ B-like Rel proteins have been identified in flies: DI (dorsal), which also plays a role in dorsal/ventral embryonic patterning, Dif (dorsal-related immune factor), and Relish (Ip and Levine, 1994; Meister et al., 1997). Although all three proteins are strongly induced upon bacterial infection and can activate transcription from promoters of antimicrobial genes in tissue culture, only DI and Dif have been implicated in the immune response in vivo.

Lemaitre et al. (1996) have shown distinct regulation of induction of antibacterial and antifungal peptides. TI signaling is specifically involved in the regulation of the antifungal pathway. TI and IL-1/NF- κ B cascades exhibit striking structural and functional homology. Adult flies carrying a mutation affecting the TI signaling cascade have abnormal expression of Drosomycin (an antifungal agent), and, to a lesser extent, Cecropin A (an antibacterial agent) when challenged with bacteria. Loss-of-function (LOF) mutations in *spätzle* (encoding the TI ligand), *Ti*, *tube*, and *pelle* abolish the strong induction of Drosomycin and Cecropin A in infected adult flies. Conversely, gain-of-function (GOF) mutations in *Ti* or LOF mutation

in *cactus* (*cact*, homologous to I κ B) result in constitutive nuclear localization of DI and Dif, as well as constitutive induction of Drosomycin. Interestingly, there is no increase in the expression of antibacterial genes in these mutants, suggesting that DI and Dif nuclear localization is not sufficient for their activation. Recently, Wu and Anderson (1998) have found that the TI pathway is not required for nuclear import of Dif, as it occurs normally during infection of either *Tl* or *pelle* mutant larvae. Thus, separate pathways exist to discriminate between Cact-DI and Cact-Dif complexes in order to promote nuclear localization of the appropriate Rel protein.

A second IL-1R-related *Drosophila* receptor, 18W, has been shown to participate in the immune response (Williams et al., 1997). 18W is found primarily in fat body cells, lymph glands, and garland cells. Like all immunity genes, *18w* expression is up-regulated during infectious challenge, leading to the appearance of several new transcripts. Some encode secreted forms of 18W, analogous to mammalian IL-1Ra, a secreted IL-1R antagonist that serves to titrate free IL-1. Larvae of *18w* null mutants have decreased viability and are more susceptible to bacterial infection. This susceptibility correlates with a failure to produce a subset of antibacterial peptides at wild-type levels. Decreased expression of antibacterial genes appears to result from reduced nuclear translocation of Dif during infection. Interestingly, nuclear translocation of DI remains unaffected. Induction of the antibacterial agent Attacin is dramatically reduced in *18w* mutants, but unaffected in mutants of the TI pathway.

Evidence that a third, complementary mechanism modulates the immune response in *Drosophila* comes from analysis of fly stocks carrying the recessive mutation immune deficiency gene (*imd*) (reviewed in Meister et al., 1997). These flies exhibit decreased viability upon bacterial infection due to a striking inability to up-regulate antibacterial genes during the immune response. In this mutant background, however, the antifungal peptide Drosomycin remains fully inducible.

A human protein has been recently identified that is more homologous to TI than IL-1RI. Although its ligand is unknown, ectopic expression of a constitutively active mutant form resulted in NF- κ B activation and expression of inflammatory cytokines including IL-1, IL-6, and IL-8 in human cell lines (Medzhitov et al., 1997).

Leukemia in Humans and *Drosophila*

In addition to sharing similar defense response mechanisms, insects and mammals are both susceptible to disorders resulting from aberrant blood cell proliferation. Human leukemias are characterized by uncontrolled proliferation of lineage-specific blood cells blocked in their differentiation. A diverse array of proteins have been implicated in leukemogenesis, including transcription factors, tyrosine kinases, effector proteins, and growth factor receptors (Orkin, 1996). Recently, a fusion protein was identified in pediatric leukemia patients that combines a portion of the *ets*-related *TEL* transcription factor and the kinase region of JAK2 (Lacronique et al., 1997). In the *TEL*/JAK2 fusion protein, the *TEL* portion promotes dimerization, leading to constitutive kinase activity and unregulated proliferation of the mutant blood cells.

Mutations have been identified that cause similar overproliferation of *Drosophila* blood cells. Almost half a

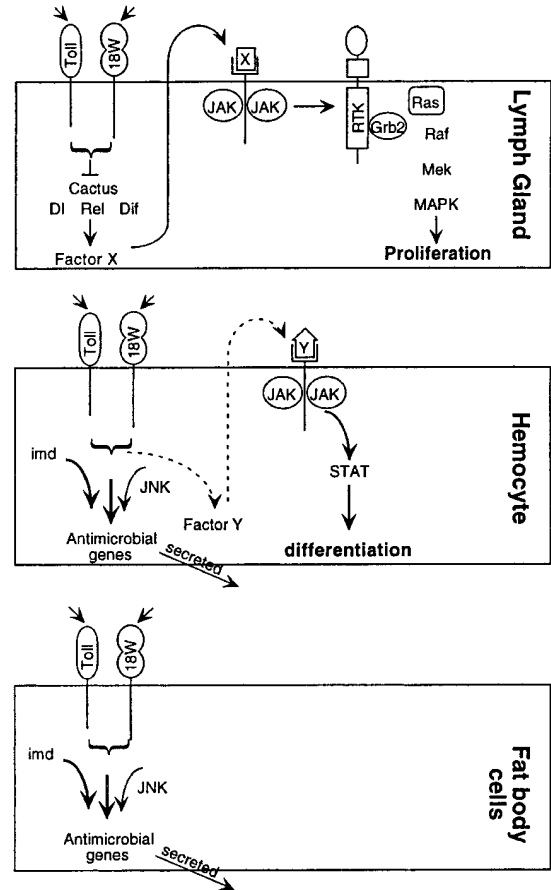


Figure 2. Model
See text for details.

dozen mutants have been found to have high incidence of melanotic masses. These can be benign or malignant neoplasms, and some are lethal to the organism (Gateff, 1978). The masses appear around the second larval stage, and are termed melanotic pseudotumors; they appear as large black growths within the body cavity. These masses may either be free floating or attached to internal organs. They are composed of two types of cells: round or polygonal cells and lamellocytes. Growth of melanotic tumors is a consequence of the constitutive proliferation of prohemocytes in the lymph gland, and the encapsulation of spherical cells by precociously formed lamellocytes. The spherical cells are thought to correspond to abnormal blood cells because some can be found free floating in hemolymph. However, this remains to be rigorously demonstrated. By the third instar stage, crystal cells contribute to melanization of these tumors, and once the tumor is fully melanized, its growth stops.

The best characterized *Drosophila* mutation associated with a leukemia-like phenotype is *Tum-I*, a dominant GOF mutation in the JAK kinase Hopscotch (*hop*, see review by Hou and Perrimon, 1997). The *Tum-I* tumor phenotype is caused by a single amino acid substitution (Gly341Glu) that results in a hyperactive kinase. *Tum-I* causes overabundance of *Drosophila* blood cells, leading to formation of melanotic masses and hypertrophy

of the larval lymph glands. Interestingly, transplantation of the lymph gland cells of affected animals (but not of the melanotic masses) leads to new melanotic tumors and death of the recipient. Thus, as in human leukemias, *Drosophila* can develop lethal tumors as a result of uncontrolled blood cell proliferation.

JAK/STAT and NF- κ B Pathways Control Hemocyte Proliferation in *Drosophila*

Analysis of *Tum-I* provided the first evidence that, similar to mammals, the JAK/STAT pathway is required for *Drosophila* blood cell proliferation. Interestingly, reduction in the activity of the *Drosophila* STAT (*stat92E*) gene suppresses the incidence of melanotic tumors associated with *Tum-I* (Hou and Perrimon, 1997), but does not affect the blood cell overproliferation phenotype of dominant GOF *hop* alleles (Luo et al., 1997). Luo et al. proposed that the STAT branch of the JAK/STAT pathway is not required for hemocyte proliferation, but instead regulates expression of genes involved in other hematopoietic functions such as the differentiation of hemocytes into lamellocytes.

Until recently, the *Drosophila* NF- κ B pathway appeared to be involved in immunity but not blood cell proliferation. However, recent observations suggest a tighter link between the *Drosophila* NF- κ B and JAK/STAT pathways. In addition to its role in immune response, the *Ti* pathway has been implicated in controlling hemocyte density (Qui et al., 1998). Hemocyte counts are decreased in *Ti*, *pelle*, and *tube* mutants and increased in *cact* mutants. Furthermore, melanotic tumors develop in both LOF *cact* mutants and GOF *Ti* mutants, in a fashion reminiscent of *hop* GOF mutants.

In Figure 2, we present a speculative model that attempts to integrate available information on signaling pathways in hemocytes and fat body cells. We propose that the JAK/STAT pathway in *Drosophila* is directly regulated by input from the *Ti/18W* and *imd* pathways, probably through secretion of a factor(s) by activated hemocytes. This model is based on findings in the mammalian system that the NF- κ B pathway controls expression of many of the cytokines that signal through the JAK/STAT pathway.

Perspectives

Although still in its infancy, study of *Drosophila* hematopoiesis shows great promise. Striking parallels with human hematopoiesis underscore the value of flies as a genetic system to gain insights into the innate immune response and blood cell development. Recently, Wu and Anderson (1998) have identified in a systematic screen more than 40 loci that affect *Drosophila* immunity. Further, the availability of fly mutations in molecules involved in various signaling pathways makes *Drosophila* a powerful system in which to characterize functional cross-talk between these pathways. Already, genetic evidence in *Drosophila* suggests that hemocyte proliferation and differentiation do not depend upon the same subset of signaling molecules (Luo et al., 1997). Thus, Hop may exert its effect on blood cell proliferation by activating the Ras/Raf/MEK/MAPK pathway instead of using a STAT protein to transduce signal. Possibly, proliferation of hemocytes in *Drosophila* is specifically achieved through the combined activation of Hop and a tyrosine kinase receptor (such as the EGFR), leading

to the mobilization of the Ras pathway. In accord with this model, Yamauchi et al. (1997) have shown that mammalian JAK can phosphorylate the EGFR and activate Ras. Lastly, as our understanding of *Drosophila* hematopoiesis gets more sophisticated, it will be of interest to determine whether cytokines and transcription factors that specify *Drosophila* blood cell lineages have also been conserved during evolution.

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