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**Title:** In vivo detection of lamellocytes in Drosophila melanogaster.

**Journal:** Immunology letters

**Year:** 2009

**Vol:** 126 **Page:** 83-84

**DOI:** 10.1016/j.imlet.2009.08.004

**PMID:** 19695290

*In vivo* detection of lamellocytes in *Drosophila melanogaster*

Keywords: hemocyte, *in vivo*, lamellocyte, *Drosophila*, differentiation

Sir,

In both vertebrates and invertebrates the blood cells undergo differentiation in spatially separated organs, in a characteristic time sequence [1-5]. Recently *Drosophila* became a powerful model organism for studying blood cell development and cellular events of innate immunity. The cellular elements of innate immunity, the blood cells or hemocytes in *Drosophila*, have been characterized by morphological criteria and molecular markers, including transcription factors [6,7] and cell-type specific immunological markers [8-10]. In *Drosophila*, at least three main classes of hemocytes can be discerned. The predominant class comprises small round cells with a phagocytic capacity, the plasmatocytes. A second class, the crystal cells, distinguished by pronounced crystal like inclusions in the cytoplasm, are involved in melanin deposition in wounds and around foreign objects. Finally, a class of large flat cells, the lamellocytes, appears when the larvae are infected by parasitoid wasps. Lamellocytes participate in the encapsulation of parasites and differentiate in response to mechanical assault and their development is finely regulated [11, 12, 5].

Hemocytes are distributed in three main hematopoietic compartments in the larva, the lymph gland, the sessile tissue and the circulation. The understanding of molecular events of hemocyte development in these compartments and the definition of signalling pathways that lead to differentiation and the immune response have been facilitated by genetic screens in

combination with functional tests and monitoring of hemocyte subsets. This analysis has been helped by the availability of reporter-gene constructs expressing marker molecules, including fluorescent proteins, *in vivo*. The constructs make possible to analyze and follow the hemocyte development *in vivo* by expressing fluorescent proteins in a cell-type specific manner. Plasmatocytes, lamellocytes and crystal cells can be visualized by appropriate constructs [11, 13-15]; however, lamellocyte specific marker for *in vivo* use has not been described yet.

Recently we have defined a molecular marker for lamellocytes as Atilla-L1, and determined the localization of its gene in the genome [Laurinyecz *et al*., unpublished]. Database analysis revealed that an insertion of a MiET1 element [16] in this gene is available. The element contains a hsp70 promoter controlled *GAL4* gene, and an *EGFP* marker gene driven by the 3xP3 promoter. The *Mi{ET1}CG6579[MB03539]* insertion is located in the *atilla* gene (CG6579) [Laurinyecz *et al*., unpublished] at position 2L:12,177,639 in the 'plus' orientation in the 5' untranslated region. Our analysis revealed that the stock carrying this insertion is suitable for *in vivo* detection of lamellocytes in immune-challenged larvae (Fig. 1A, arrows) as well as in hemocyte overproducing mutants. The use of a panel of lamellocyte specific markers [10] showed that the expression of GFP is specific for/to? lamellocytes. The 3xP3 promoter also shows an expression pattern similar to that of the *eyeless* driver, which appears in the eye discus, brain, gut and anal plate (Fig. 1A, arrowheads) showing up as a background fluorescence appearing in all stocks carrying the MiET1 element [16]. The insertion blocks the expression of the Atilla protein encoded by the *atilla* gene, but does not affect the expression of other, independent lamellocyte-specific markers [10], as L2 and L6 (Fig. 1B,C). These results confirm that the insertion allows *in vivo* investigation of lamellocyte differentiation as well as helps genetic screens with the aim of dissecting signaling pathways

of hemocyte development. We also found that the *GAL4* gene in this insertion is inactive. This however allows the use of this insertion with independent GAL4 drivers in combination with UAS-RFP reporter construct thus making possible double-labelling of hemocyte populations *in vivo* with a clear distinction of lamellocytes.

Acknowledgement: Hungarian National Research Fund Grants, OTKA K-68830 and NK-78024 supported this research and the technical help of Szilvia Tápai is acknowledged.

References

[1] Hultmark D. Insect immunology. Ancient relationships. Nature 1994;367:116-7.

[2] Hoffmann JA, Reichhart JM. *Drosophila* innate immunity: an evolutionary perspective. Nature Immunol 2002;3:121-6.

[3] Hartenstein V. Blood cells and blood cell development in the animal kingdom. Annu Rev Cell Dev Biol 2006;22:677-712. Review.

[4] Lanot R, Zachary D, Holder F, Meister M. Postembryonic hematopoiesis in Drosophila. Dev Biol 2001;230:243-57.

[5] Márkus R, Laurinyecz B, Kurucz E, Honti V, Bajusz I, Sipos B et al. Sessile hemocytes as a hematopoietic compartment in Drosophila melanogaster. Proc Natl Acad Sci USA 2009;106:4805-9.

[6] Evans CJ, Hartenstein V, Banerjee U. Thicker than blood: conserved mechanisms in Drosophila and vertebrate hematopoiesis. Dev Cell 2003;5:673-90. Review.

[7] Evans CJ, Banerjee U. Transcriptional regulation of hematopoiesis in Drosophila. Blood Cells Mol Dis 2003;30:223-8. Review.

[8] Kurucz E, Zettervall CJ, Sinka R, Vilmos P, Pivarcsi A, Ekengren S et al. Hemese, a hemocyte-specific transmembrane protein, affects the cellular immune response in Drosophila. Proc Natl Acad Sci USA 2003;100:2622-7.

[9] Kurucz E, Márkus R, Zsámboki J, Folkl-Medzihradszky K, Darula Z, Vilmos P et al. Nimrod, a putative phagocytosis receptor with EGF repeats in Drosophila plasmatocytes. Curr Biol 2007;17:649-54.

[10] Kurucz E, Váczi B, Márkus R, Laurinyecz B, Vilmos P, Zsámboki J et al. Definition of Drosophila hemocyte subsets by cell-type specific antigens. Acta Biol Hung 2007;58 Suppl:95-111.

[11] Zettervall CJ, Anderl I, Williams MJ, Palmer R, Kurucz E, Ando I et al. A directed screen for genes involved in Drosophila blood cell activation. Proc Natl Acad Sci U S A 2004;101:14192-7.

[12] Márkus R, Kurucz E, Rus F, Andó I. Sterile wounding is a minimal and sufficient trigger for a cellular immune response in Drosophila melanogaster. Immunol Lett 2005;101:108-11.

[13] Goto A, Kadowaki T, Kitagawa Y. Drosophila hemolectin gene is expressed in embryonic and larval hemocytes and its knock down causes bleeding defects. Dev Biol 2003;264:582-91.

[14] Braun A, Lemaitre B, Lanot R, Zachary D, Meister M. Drosophila immunity: analysis of larval hemocytes by P-element-mediated enhancer trap. Genetics 1997;147:623-34.

[15] Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 1993;118:401-15.

[16] Metaxakis A, Oehler S, Klinakis A, Savakis C. Minos as a genetic and genomic tool in Drosophila melanogaster. Genetics 2005;171:571-81.

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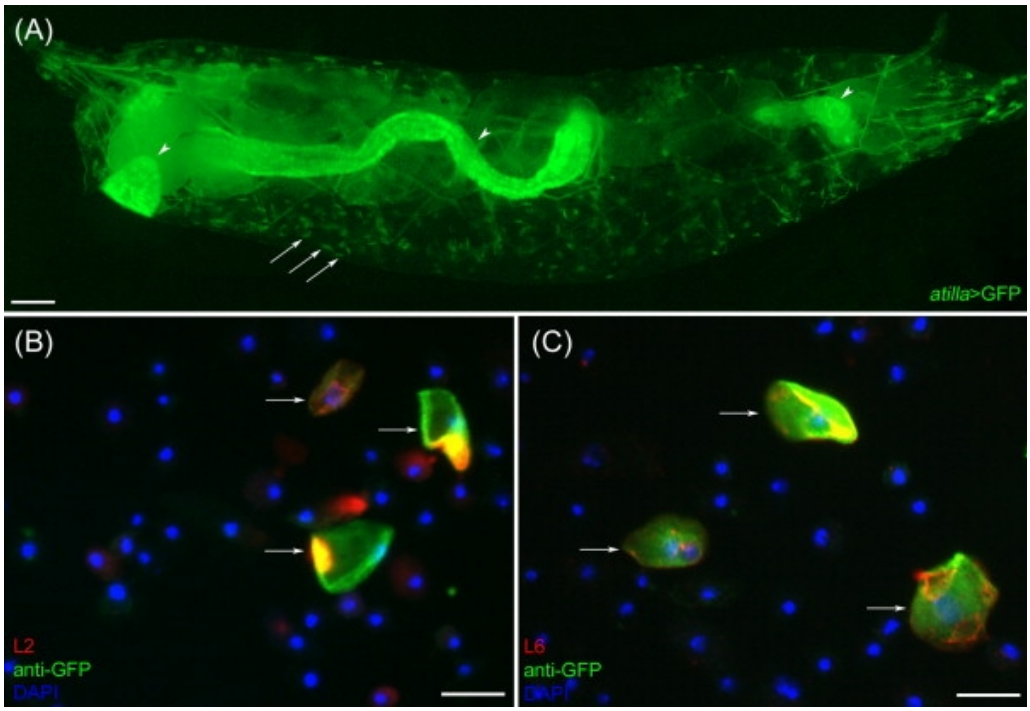


Legend to Fig. 1

Lamellocytes in the *Mi{ET1}CG6579[MB03539]* larva

(A) GFP expressing lamellocytes are visible through the cuticule of the wasp infested *Mi{ET1}CG6579[MB03539]* larva. Arrows are pointing at lamellocytes, arrowheads point to organs showing background fluorescence characteristic for the MiET1 element [16] (scale bar: 100µm). (B,C) The GFP expression overlaps with the L2 (B) and L6 (C) antigen expression in the hemocytes of homozygous *Mi{ET1}CG6579[MB03539]; l(3)mbn*$^{1}$ larvae. Lamellocytes are visualized by indirect immunofluorescence. The GFP expression in lamellocytes is enhanced by rabbit anti-GFP staining and anti-rabbit Alexa-488 (green). The expression of lamellocyte specific antigens is detected with mouse anti-L2, anti-L6 and anti-mouse Alexa-568 (red). Arrows are pointing at/to ? double labelled lamellocytes (scale bars : 20µm).