IN VIVO IMMUNOSTAINING OF HEMOCYTE COMPARTMENTS IN *DROSOPHILA* FOR LIVE IMAGING

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INTRODUCTION: The immune cells (hemocytes) of the *Drosophila melanogaster* larva show surprising similarities to the blood cells of mammals. The different types of effector hemocytes form hematopoietic compartments, in which their division and differentiation take place. Although previous studies exist about the structure of these anatomical sites, only recently did it become possible to characterize them *in vivo*, with the combined use of fluorescent microscopy and transgenic reporter constructs. However, detailed investigation of these hematopoietic tissues required the dissection of the larvae, which compromised structural definition. Therefore, we sought to establish a method by which the composition of these tissues can be studied *in vivo*.

METHODS: We immobilized *Drosophila* larvae, which expressed hemocyte-specific *in vivo* reporter (*Hml>GFP*), using an acetylcholinesterase inhibitor (Dichlorvos). By mounting these larvae on prepared Petri dishes, we could investigate the hemocyte compartments and perform time-lapse videomicroscopy using a confocal microscope on live larvae. To complement the *in vivo* reporters with our existing molecular markers, we created mixtures of hemocyte-specific primary antibodies and fluorescently labeled secondary antibodies, and injected these mixtures into live animals. The injected larvae were immobilized and microscopic analysis was carried out.

RESULTS: Microscopic analysis of the immobilized *Hml>GFP* larvae showed that the use of Dichlorvos does not affect the structure of the hematopoietic compartments, and that immobilized larvae can be studied in time lapse experiments for over 45 minutes. By injecting live *Hml>GFP* larvae with the mixture of hemocytes-specific primary and fluorescently labeled secondary antibodies, we identified the phagocytes within the sessile compartment. We also characterized the sessile compartment of tumorous larvae, and found that it is vastly expanded when compared to that of the wild type larvae.

CONCLUSIONS: We developed a method that can be used to study the structure and composition of hematopoietic tissues in the *Drosophila* larva, and possibly, in other insect species (Csordás et al., 2014).

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