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Innate immune cells are dispensable for regenerative growth of imaginal discs

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ARTICLE INFO

Article history:
Received 20 July 2012
Received in revised form
5 November 2012
Accepted 21 November 2012
Available online 10 December 2012

Keywords:
Tissue regeneration
Immune cells
Imaginal discs
Drosophila melanogaster

ABSTRACT

Following tissue damage the immune response, including inflammation, has been considered an inevitable condition to build the host defense against invading pathogens. The recruitment of innate immune leukocytes to injured tissue is observed in both vertebrates and invertebrates. However, it is still not conclusive whether the inflammatory response is also indispensable for the wound healing process by itself, in addition to its role in microbial clearance. In this study we determine the requirement of innate immune cells, both hemocytes and fat body cells, in *Drosophila* imaginal disc regeneration. We investigate wound healing and regenerative cell proliferation of damaged imaginal discs under immunodeficient conditions. To delay development of *Drosophila* at matured third instar larval stage we used a sterol-mutant *erg2* knock-out yeast strain in the medium. This dietary-controlled developmental arrest allowed us to generate larvae free of immune cells without interfering with their larval development. In addition, this approach allowed uncoupling regenerative cell proliferation of damaged discs from their normal developmental growth. We furthermore examined the regenerative cell proliferation of fragmented imaginal discs by transplantation into host flies deficient of immune cells. We demonstrate that the damaged/fragmented discs in immune cells deficient conditions still exhibit regenerative cell proliferation comparable to those of control samples. These results suggest that recruitment of immune cells is not a prerequisite for the regenerative growth of damaged imaginal discs.

1. Introduction

Several systemic and cellular responses to external stimuli are evolutionarily conserved. The immediate immune response to tissue damage is such a well-preserved vital reaction in both vertebrates and invertebrates. Innate immune leukocytes such as neutrophils and monocytes/macrophages play pivotal roles to ensure wound repair by preventing pathological infection (Brancato and Albina, 2011; Dovi et al., 2004). However, it is not yet fully understood whether the inflammatory response is required for the wound healing process by itself, in addition to its role in microbial clearance.

In vertebrates, several studies using different regeneration model systems have been performed to determine the contribution of each immune cell lineage to the wound repair process (reviewed in Eming et al., 2009). However, owing to intricate and dynamic interactions of innate inflammatory cells in concert with adaptive immune lymphocytes, the role of the overall immune response in the outcome of wound healing is still inconclusive. For example, recent studies with transgenic mice undergoing conditional macrophage depletion

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0925-4773/$ - see front matter
http://dx.doi.org/10.1016/j.mod.2012.11.005

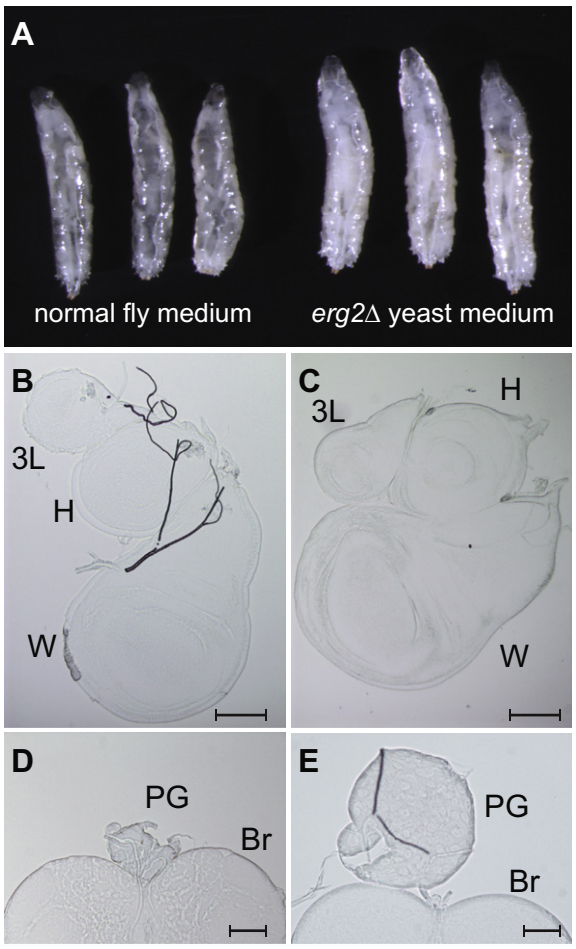

Fig. 1 – Larvae grown on *erg2Δ* yeast medium do not pupate. (A) Five days old third instar larvae reared on normal medium (left), and 12 days old larvae on *erg2Δ* yeast medium (right). Larvae were grown at 25 °C. (B and C) The size of imaginal discs from control 3rd instar larvae (B) and the larvae reared on *erg2Δ* yeast medium (C). (D and E) The size of a prothoracic gland from a control 3rd instar larva (D) and a larva reared on *erg2Δ* yeast showing the enlarged prothoracic gland (E). Abbreviations are; 3L: third leg (metathoracic leg) disc, W: wing disc, H: haltere disc, PG: prothoracic gland, and Br: brain. Scale bar = 100 μm.

revealed their essential role in the wound repair process (Goren et al., 2009; Lucas et al., 2010; Mirza et al., 2009). But in two of these studies, macrophage-depletion caused prolonged accumulation of neutrophils at wound sites (Goren et al., 2009; Lucas et al., 2010). Neutrophils and their proteases have been implicated in mediating the tissue damage associated with chronic inflammatory diseases (Yager and Nwomeh, 1999). In inflammatory reaction macrophages are responsible for the clearance of neutrophils (Meszaros et al., 2000). Indeed, the repair of epidermal wound in the neonatal PU.1 null mouse that lacks both functioning neutrophils and macrophages exhibited more efficient repair without leaving fibrosis and scar at wounded site (Martin et al., 2003). Furthermore, the wound closure is accelerated by solely depleting neutrophils in young mice (Dovi et al., 2003), while it is delayed in aged mice (Nishio et al., 2008).

Similar to the vertebrate models, studies using the fruit fly *Drosophila melanogaster* have recognized the conserved interrelation between the immune response and wound repair (reviewed in Belacortu and Paricio, 2011; Razzell et al., 2011). Given the absence of the adaptive immune system in insects, the innate immune system exclusively conducts the host defense (Lemaitre and Hoffmann, 2007). In *Drosophila*, the hemocyte lineages comprising plasmatocyte, crystal cell, and lamellocyte are responsible for the cellular innate immunity (Fauvarque and Williams, 2011; Meister, 2004). The plasmatocyte, accounting for more than 95% of hemocytes, is a dedicated phagocyte displaying functions similar to the mammalian monocyte/macrophage lineage. In contrast, crystal cells and lamellocytes have no obvious counterparts in mammals. The crystal cells secrete the components necessary for melanization of invading pathogens as well as for wound repair. The lamellocytes encapsulate invading large parasites, but are rarely present in healthy larvae. The absence of adaptive lymphocytes in *Drosophila* makes the study of the roles of the innate immune responses easier. Additionally, advanced technical and genetic manipulations in *Drosophila* facilitate aseptic tissue damaging *in vivo*. Thus, *Drosophila* provides a useful model system to study the contribution of innate immune cells to wound healing and tissue regeneration.

Live imaging of hemocytes in *Drosophila* embryonic and larval skin wound repair models revealed the dynamics of migration and phagocytosis of wound cellular debris by migrating hemocytes (Babcock et al., 2008; Pastor-Pareja et al., 2008; Stramer et al., 2005). The healing of aseptic wound in hemocyte-depleted *serpent* mutant embryos showed an entirely normal time course of wound re-epithelialization (Stramer et al., 2005). Moreover, the genetic ablation of hemocytes in larvae prior to epidermal wounding did not impair epithelial closure (Babcock et al., 2008). These results suggest that wound healing of epidermis does not require hemocytes under aseptic wound conditions.

Hemocytes were also found to adhere to the disrupted basal membrane of imaginal discs upon wounding or during tumorigenesis (Pastor-Pareja et al., 2008). A tumor suppressor activity by the recruited hemocytes was reported in disc-derived malignant tumor. However, their function in imaginal disc regeneration has not been determined. Indeed, imaginal discs exhibit a remarkable regeneration ability (reviewed in (Bergantinos et al., 2010b)). Unlike *Drosophila* epidermal wounds, in which the epidermal cells do not proliferate during healing, the regeneration of imaginal discs is accompanied by a localized massive cell proliferation at the wound site forming the so called regeneration blastema (Abbott et al., 1981). Interestingly, in disc regeneration the cell proliferation at the wound site begins concomitantly with a peaking in the number of adherent hemocytes around 24 h after wounding (Bryant and Fraser, 1988; McClure et al., 2008; Pastor-Pareja et al., 2008). This is also the case in vertebrate wound healing, where the number of attached macrophages peaks during the phase of tissue formation (Martin and Leibovich, 2005).

Furthermore, in classical transplantation experiments of fragmented imaginal discs not only hemocytes but also fat body cells frequently adhere to the wound site of disc