Seminars in Immunology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Review

Unraveling tissue repair immune responses in flies

Brian M. Stramer[a,*], Marc S. Dionne[b]

[a] King's College London, Randall Division of Cell and Molecular Biophysics, London SE1 1UL, United Kingdom
[b] King's College London, Centre for the Molecular and Cellular Biology of Inflammation, London SE1 1UL, United Kingdom

ARTICLE INFO

Keywords:
Drosophila
Wound repair

ABSTRACT

Drosophila melanogaster has emerged as a powerful model to understand innate immune responses to infection (note the 2011 Nobel Prize in Physiology or Medicine), and in recent years this system has begun to inform on the role and regulation of immune responses during tissue injury. Due to the speed and complexity of inflammation signals upon damage, a complete understanding of the immune responses during repair requires a combination of live imaging at high temporal resolution and genetic dissection, which is possible in a number of different injury models in the fly. Here we discuss the range of wound-induced immune responses that can be modeled in flies. These wound models have revealed the most immediate signals leading to immune cell activation, and highlighted a number of complex signaling cascades required for subsequent injury-associated inflammatory responses. What has emerged from this system are a host of both local acting signals, and surprisingly, more systemic tissue repair immune responses.

1. Introduction

Most of what we know about immune responses during tissue damage has been gleaned from studies of human tissues and cells, or from mammalian models. However, the complexity of inflammation, and its dynamic nature make it problematic to dissect at a genetic level and difficult to live image. As a result, a number of basic model organisms are starting to be exploited. Ilya Metchnikov was the first to utilize a model organism, starfish larvae, to observe the inflammatory response to wounding [1]. While a simple organism like starfish, does not exhibit the Oxford English Dictionary's definition of inflammation – rubor (redness), calor (heat), tumor (swelling), and dolor (pain) – the central etiology of its wound inflammation (which was highlighted in Ilya's Nobel lecture) is the same: recruitment of professional microbe killing and debris clearing cells. Indeed, *Drosophila*, which is far more genetically tractable than Metchnikov's starfish, has been used as a model of animal immunity for some time. Strikingly, key components of a *Drosophila's* response to pathogenic infection, such as a requirement for Toll signaling, are evolutionarily conserved (and in 2011 a Nobel Prize in Physiology or Medicine was awarded for this discovery). While most of the *Drosophila* work has focused on infection responses, flies are increasingly being utilized to understand the role and regulation of immune responses during wound healing. This review will discuss the physiologically relevant questions that can be addressed in this model, and highlight recent discoveries that have exploited the benefits of this basic model system.

1.1. Drosophila immune cells

Flies have a relatively simple immune system that is composed of hemocytes (insect "blood" cells) that can differentiate into just a few cell-types [2]. The major hemocyte population is the plasmatocyte, which is a macrophage-like phagocytic cell-type that is capable of migratory responses to tissue damage. In contrast, Crystal cells make up a small percentage of the total hemocyte population at embryonic and larval stages and are specifically involved in clotting reactions. Finally, lamellocytes are a special hemocyte cell-type that differentiates in response to infection by parasitic wasps and are required to encapsulate the invading organism to inhibit infection. Flies do not have a lymphocyte-like cell-type and do not show obvious signs of immunological memory, and as a result this model is only used to understand innate immune responses. However, the ability to live image hemocytes within this model, coupled with the evolutionary conservation of a number of immune signaling pathways makes this a powerful model to understand innate mechanisms behind wound-induced inflammation.

* Corresponding author. Tel.: +44 0207 848 6272.
E-mail addresses: Brian.M.Stramer@kcl.ac.uk (B.M. Stramer), Marc.Dionne@kcl.ac.uk (M.S. Dionne).

http://dx.doi.org/10.1016/j.smim.2014.04.004
1044-5323/

2. Modeling epidermal wound recruitment and resolution in flies

Drosophila has an open circulatory system and lacks blood vessels, therefore diapedesis during inflammation cannot be addressed in this model. However, plasmatocytes show very rapid responses to tissue damage and as a result this model has become a powerful system to dissect the innate signals that recruit inflammatory cells. Interestingly, depending on the developmental stage of the animal, the cellular response to damage is different, which has allowed for unique aspects of the response to be dissected. This section along with the next, will discuss *Drosophila* inflammatory responses during "epidermal" damage; however, it should be made clear that flies do not have a classical "epi"dermis as the outer epithelium in flies – which is also not stratified like the mammalian epidermis – lacks an underlying connective tissue layer *i.e.* a dermis. Therefore, tissue fibrosis, which is largely caused by dermal fibroblasts, cannot be modeled in *Drosophila*. Nevertheless, as we will see, a number of evolutionarily conserved innate immune responses during tissue repair can indeed be dissected in this simple organism.

During embryogenesis, *Drosophila* hemocytes develop from precursor cells in the head mesoderm and spread throughout the embryo, appearing to take defined migratory routes [3]. Once they have dispersed they maintain an evenly spaced pattern through contact inhibition of locomotion [4,5] and patrol the hemocoel clearing up apoptotic debris as a result of normal embryogenesis. However, this dispersal pattern can be disrupted by tissue damage: induction of a wound by laser ablation to the embryonic epithelium leads to rapid recruitment of plasmatocytes to the wound site [6–9]. The hemocytes subsequently cease their contact inhibitory behavior and clump within the wound where they engulf cellular debris as a result of the damage. Over the next few hours (depending on the size of the wound), the epithelium will heal and plasmatocytes will return to circulation [8] suggesting that resolution of inflammation can also be modeled in this system (Fig. 1).

After embryogenesis, flies enter larval stages where hemocyte behavior changes dramatically. While plasmatocytes are highly motile in embryos, larval plasmatocytes (which descend from embryonic hemocytes) transform into a completely sessile population and either adhere to tissues or passively circulate throughout the animal within the hemolymph. Plasmatocytes at larval stages, while not migratory, are still capable of recruitment to wounds. For example, damage to the epidermis leads to plasmatocyte recruitment to the wound through a "passive" mechanism whereby circulating cells adhere and become captured at the wound site [10]. Subsequently, as the epidermal wound heals, plasmatocytes return to circulation as the inflammation resolves. However, neither the capture nor resolution of inflammation within larval stages involves active cellular migration. There is speculation that plasmatocytes adhere specifically to the damaged basement membrane [11] and these cells may therefore express receptors that specifically recognize damaged matrix.

Upon completion of larval stages, hemocyte behaviors change yet again. In pupae, the larval tissues histolyse and are replaced by adult populations of cells. Possibly to help clean up the debris as a result of the cell death, plasmatocyte populations become active, disperse throughout the animal, and re-acquire their capacity to actively migrate to wounds [12,13]. Recent work revealed that activation of hemocytes at pupal stages involves ecdysone, a steroid-like hormone in the fly, which drives the expression of plasmatocyte migratory genes [14]. This hormonal signaling is also required for plasmatocyte migratory responses to wounds [14]. It is currently unclear whether similar hormonal changes are responsible for the different behaviors of embryonic and larval hemocytes.

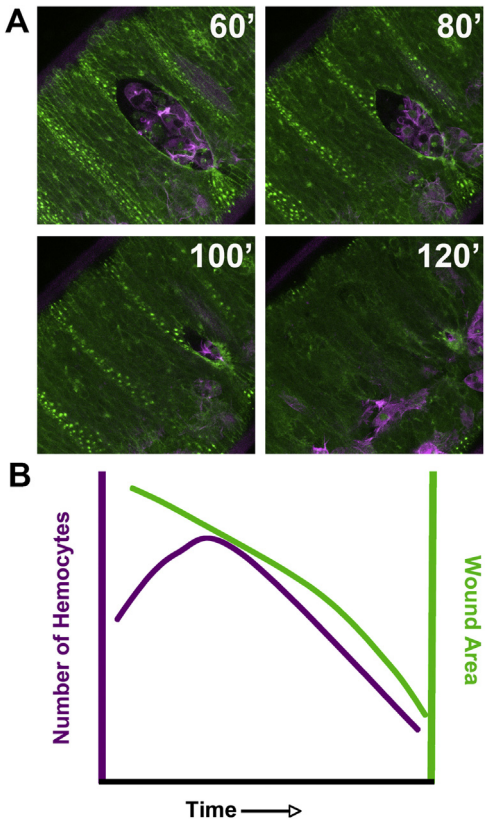

Fig. 1. Timecourse of hemocyte recruitment to embryonic epithelial wounds. (A) *Drosophila* embryo with a labeled epithelium (green) and hemocytes (magenta) wounded by laser ablation. Note that hemocytes accumulate within the epithelial wound site and subsequently disperse during the repair process. (B) Schematic showing the temporal relationship between hemocyte numbers at a wound and size of the damage. Note that upon peak recruitment of hemocytes, numbers at the wound site decrease in relation with the wound size suggesting the inflammation recruitment and resolution can be modeled in this system.

It is interesting that steroid hormones also affect mammalian leukocyte behaviors and inflammatory responses [15,16], although it remains to be determined whether their mechanisms of action are conserved in flies and vertebrates.

Finally, it is intriguing to note that the migratory behaviors of adult hemocytes are completely unknown. The adult population is also immune responsive and capable of responding to infections, however it is unclear whether these cells are capable of actively migrating to sites of damage.

2.1. The role of Drosophila immune cells during epidermal repair

The next question is, what is the function of hemocytes at wound sites? These cells are highly phagocytic, and within wounds – whether embryonic or larval – they will become massively swollen as they engulf large amounts of cellular debris [6,10]. However, similar to PU.1 knockout mice, which lack macrophages and neutrophils, flies lacking professional phagocytes are completely capable of healing epithelial wounds [6,10,17]. Similarly, in a regenerative model of repair in the developing *Drosophila* wing, hemocytes are dispensable [18]. This does not mean that there is no role for professional phagocytes at wound sites. As we will see, wound signals, even from sterile wounds, are capable of inducing anti-bacterial responses, as a major role of inflammation during repair is to protect against an increased risk of infection caused by a break in the epidermal barrier.

Please cite this article in press as: Stramer BM, Dionne MS. Unraveling tissue repair immune responses in flies. Semin Immunol (2014), http://dx.doi.org/10.1016/j.smim.2014.04.004