

Hemocyte Density and Differentiation in *Apis mellifera* Worker Bees Infected with *E. Coli*

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Introduction

The domesticated honey bee, *Apis mellifera* plays a vital role in the pollination of crops globally. In recent years, a notable decline in pollinators, including *A. mellifera*, has led scientists to investigate the causes, one of which includes the ability of these pollinators to resist disease. Hemolymph is the circulatory fluid within insects that acts as a transport system for immune cells. These immune cells range in function from phagocytosis to fat storage to coagulation. Since hemolymph flows openly in the bee interior, it is crucial to look at the cellular changes to see the impact of infection on single bees and better grasp how to approach insect disease.

Objective:

Data suggest that hemocyte types are actually stages of singular cell differentiation in response to stimuli. This study looks to see if infection is a trigger for cellular proliferation and differentiation.

Material and Methods

Bee Selection and Injection

Newly hatched worker bees were captured from four separate hives and chilled on ice for 15 minutes. Then 31 g sterile insulin syringes were prepared with 10 μ L of either insect ringer or *E. coli* in PBS. Bees were then immediately incubated at 34 $^{\circ}$ C for 2 hours



Two hatching worker bees

E. coli Preparation

Use of a stock of *E.coli* cultured in LB broth was grown to 0.51-0.53 absorbency at 550nm according to Gatchenberger et al. In order to achieve an injection of about 10⁵ CFUs, 1 mL of culture was centrifuged, resuspended, and diluted in 1x PBS.



Dorsal needle injection under the 4th abdominal turga

Cell Count Preparation

After incubation bees were chilled on ice for 15 min. Using sterile needles we removed 10 μ L of hemolymph dorsally from the abdomen. Sample was diluted 1:1 in incubation fluid outlined by Stoepler et al. Trypan blue was then added in a 1:1 ratio and mixed thoroughly with a pipette. A BioRad-TC20 cell counter measured total cell count and viability in 10 μ L samples.

Slide Preparation and Analysis

In the same manner of extraction for cell count preparation, 10 μ L samples of hemolymph were extracted and systematically smeared onto clean glass microscope slides. These slides were fixed in 10% formalin solution for 30 minutes, washed in 1x PBS, and stained with 5% Giemsa dye for 20 minutes, and rinsed with 1x PBS. Hemocytes on the fixed slides were counted under a light microscope at 40x over a randomly selected 2cm x .01cm square area.

Results

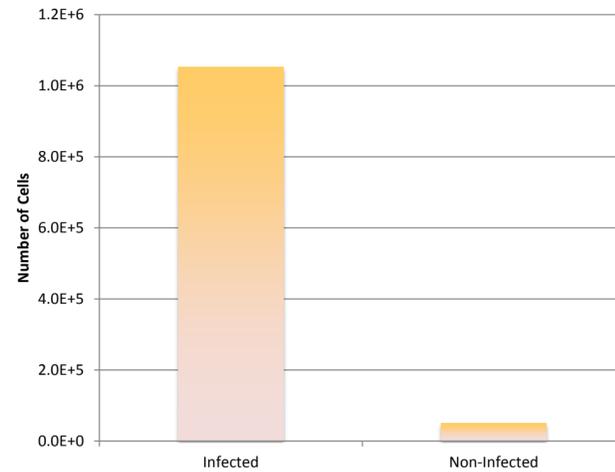


Fig. 1: Average Number of Cells per mL. Graph shows a stark difference to the total amount of hemocytes in a sample of hemolymph between experimental and control averages (n=28). Unpaired T-test results show that the difference is significant ($p=0.0365$). Amounts calculated by BioRad.

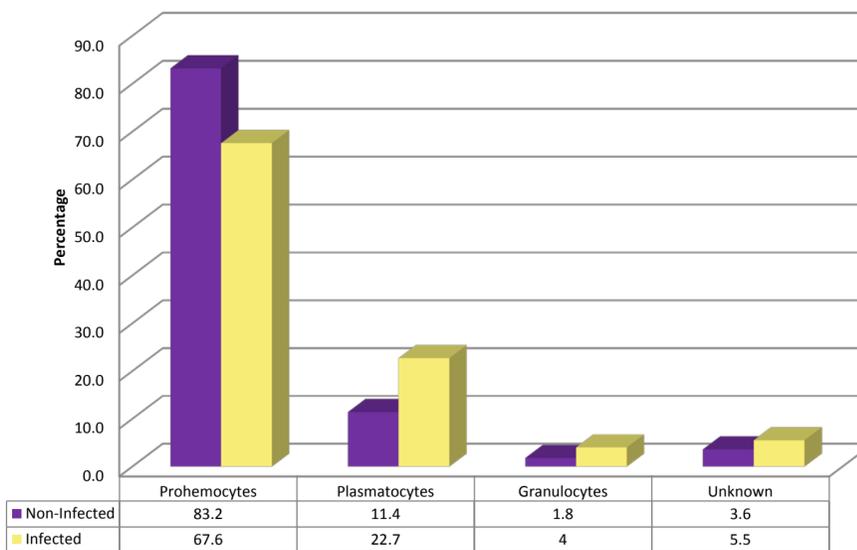


Fig. 2: Average Percentage of Hemocyte Composition. Graph shows greatest number of cell types in both groups are the prohemocytes and plasmatocytes. There is no significant difference between each type of cell in the two groups (n=18), nor is there a significant difference between prohemocytes and plasmatocyte percent composition within either group. Cell types counted using light microscope.



Fig. 3 Differentiated hemocytes under a light microscope. Different hemocytes with stained nuclei and membrane at 40x

A: Prohemocyte B:Plasmatocyte C: Granulocyte D: Unidentified

Discussion

Eliciting an infection in the honey bees creates a significant increase in total hemocyte count of cells larger than 4 μ m (Fig 1). There is no significant difference in the types of hemocytes present in bees infected with *E. coli* and not (Fig 2). In a fully developed bee, hemocytes are fully differentiated in the circulating hemolymph; and bacterial infections trigger the mobilization of hemocytes, but not necessarily induce a specific hemocyte type. Past studies suggest prohemocytes may give rise to the other types of hemocytes, and certainly the number of prohemocytes in newly hatched bees compared to other cell types does hint at the importance of prohemocytes in the hemolymph. Given the lack of difference in cell types with and without infection, though, differentiation likely does not occur in circulating hemolymph. Examining the hemopoietic organs during infection may give more insight into the significant rise in cells.

Future Directions

Understanding the typical cellular immune response at a particular age for *A. mellifera* is crucial for understanding the pathology of certain diseases that occur in colonies. Although bacterial infections and viral infections affect the immune response differently, knowing the typical response to a bacterial infection can aid as a proxy for understanding the effect viruses may have on the immune cells. These results are particularly crucial for understanding how Deformed Wing Virus (DWV) may not only affect the ability of *A. mellifera* to launch a typical immune response, but also may answer the question of what cells the virus targets over the course of an infection, or if the virus does in fact discriminate between cells at all.



A healthy worker bee beside two bees infected with DWV

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References are available upon request