INTRODUCTION

Healthy mitotic cells, or cells undergoing normal division and growth, are often damaged during human cancer treatments, leading to adverse side effects. This is because abnormally high mitotic rate is one of the hallmarks of cancer cells, and chemotherapy measures that target this characteristic unfortunately get healthy mitotic cells caught in the fray, causing them to go through cell death, or apoptosis. One normal tissue this increased apoptosis damages is the bone marrow, since bone marrow tissue is hematopoietic, or blood-cell-forming, and thus very mitotic. This effect on bone marrow tissue raises a vital question about immune cell response to tissue damage (Pastor-Pareja et al. 2008); it is clear that hemocytes – the insect blood cells, which play key roles in its life cycle and immune response, the most numerous of which phagocytizes invading microbes (Beetz et al. 2008, Horohov & Dunn 1982) in similar fashion to human macrophages. Hemocyte encapsulation and melanization (Lanot et al. 2001, Krzemien et al. 2010), and cell signaling such as the JAK/STAT pathway has also been studied in a response to tissue damage (Paun-Perzin et al. 2008); it is clear that hemocytes are varied and essential to keeping the insect healthy. The larval tobacco hornworm Manduca sexta is a holometabolous insect whose hemocyte population fluctuations – upon developmental or immune cues – have largely been documented using manual hemacytometry. Quantification of these changes has thus been honed through the use of flow cytometry, and, by this method as well as by immunocytochemistry for vaccination, circulating hemocyte populations have been found to decrease upon X-irradiation tissue damage.

Hypothetically, irradiation damage to the mitotic imaginal disc tissue affects the proliferation of circulating hemocytes, which would suggest that there are chemical signals from the damaged tissue that target the insects’ immune cells. In addition, the characterization of this effect will be similar through manual as well as flow cytometry analysis. Characterizing the immune effects of these chemical signals opens the door to further studies of the link between mitotic disc tissue damage and immune function. Furthermore, studies could then extrapolate this link to a clinical setting, in looking at side effects of chemotherapeutic damage to healthy, non-cancerous mitotic human cells.

METHODS

Flow cytometry

Flow cytometry is a method of measuring forward and side scatter, as well as nuclear morphology, and immunostaining and characterization of the immune system, and has been used in a variety of studies, including the study of Drosophila immune system (Lanot et al. 2001). By use of flow cytometry, we can measure changes in the number of circulating hemocytes in response to irradiation damage. This method allows for the quantification of immune cell populations and their characteristics, such as size, granularity, and nuclear morphology, which can be used to identify different immune cell types. Additionally, flow cytometry can be used to detect changes in the expression of cell surface markers, which can provide insights into the functional state of immune cells.

Hemacytometry

Hemacytometry is a classical method of counting and characterizing cell populations, and is commonly used in the study of hemocyte populations in Manduca sexta. This method involves diluting the hemolymph and counting the cells using a hemacytometer. By comparing the results obtained through flow cytometry and hemacytometry, we can validate the findings of our study and ensure the accuracy of our results.

RESULTS

Flow cytometry results show a decrease in the number of circulating hemocytes after x-ray irradiation. This decrease is most pronounced in the granular cell population, which is more sensitive to the more uniform morphology of the granular cells. The flow cytometer could, in turn, be more sensitive to the most uniform morphology of the granular cells.

SUMMARY

The flow cytometry results of this study have shown a decrease in the number of circulating hemocytes after x-ray irradiation. This decrease is most pronounced in the granular cell population, which is more sensitive to the more uniform morphology of the granular cells. The flow cytometer could, in turn, be more sensitive to the most uniform morphology of the granular cells.

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REFERENCES


