Sample Scientific Paper

Tracking the Changes in Lipid Bodies Induced by Bee Venom in Rat Mast Cells

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Personal Reflection

During the past 2 semesters I worked in the lab, I was able to get experience developing and planning out experiments with the help of my mentors in the lab. During this time, I gained experience creating experiments, understanding the goals in an experiment, troubleshooting problems before, during and after an experiment and thinking critically during this process. I was also introduced to new techniques including cell culture, sterile laboratory techniques, DIC microscopy and several new features of confocal microscopy.

Introductions

Lipid bodies (LB) are intracellular protrusions containing lipids such as triacylglycerols and cholesteryl esters surrounded by a phospholipid monolayer [1]. LBs are found in multiple cell types, and have been studied in several cell types including adipocytes and mast cells. LBs have a role in lipid metabolism, including those linked to inflammatory processes [2]. Thus, LBs can be important factors in things such as obesity, diabetes and inflammatory diseases like Crohn’s disease, as well as inflammatory responses in toxins and allergies [2-4]. The goal of the experiment is to determine the morphological responses of lipid bodies in inflammatory responses simulated by the addition of bee venom.

Methods and Materials

Fixed Cells

RBL2H3 cells grown on a coverslip (overnight, 37°C) and stimulated with bee venom (100ng/mL, 1ug/mL and 10ug/mL for 1h, 6h and 24h). A pseudonegative response was also recorded, in which the venom was replaced with normal media after 10 minutes at the specified time of simulation. After the treatment, the cells were washed in 1X PBS, fixed (0.4% PFA, 1h, RT) and stained with DAPI (1uM, 15’, RT) and Oil Red O (2.1% w/v, 15’, RT).

Live Cells

RBL2H3 cells were seeded in 3cm coverslip dishes (overnight, 37°C). The cells were then washed with 1mM Calcium Assay Buffer for DIC microscopy or stained with LTOX green neutral lipid stain (15X) and counterstained with SiR Actin (1uM) or WGA (5ug/mL) for confocal microscopy. The cells were stimulated with bee venom (1ug/mL and 250ng/mL) or Ionomycin (1uM).

Imaging

All cells were imaged using a Nikon Ti Eclipse microscope with Elements and EZ-C1 software.

Results

In the bee venom stimulated fixed cells, there were no noticeable correlations between any of the bee venom concentrations or stimulation times. Psuedonegative responses also yielded approximately the same responses. However, in live cells, a profound degranulation response was noticed in the bee venom stimulations (1uM) within a few minutes. DIC microscopy revealed the formation of giant plasma membrane vesicles (GPMVs) around the cell membrane

during the response to the venom. The LTOX neutral green stain also showed a slight signal around the GPMVs formed.

SiR Actin and WGA counterstains were used to track cell membrane location and morphology. WGA stains showed the loss of membrane structure, while SiR Actin revealed that the F-actin was scarsely located around the GPMV membrane. Instead, it appeared around the membrane of the cell and junction between the cell and the GPMV.

Discussion

Although a response was seen in both the bee venom and ionomycin stimulations, there was a difference in the degranulation. For ionomycin, the vesicles formed appeared as droplets around the cell membrane. This is a standard degranulation response. However, the vesicle formation in response to the bee venom had also formed GPMVs. The difference was hypothesized to be due to the complexity of the components in bee venom—which has the potential to activate more inflammatory pathways than a single component.

In addition, the stains and counterstains show that the membrane morphology of the GPMV is different than a normal membrane. There is very little F-actin in the GPMVs after the junction between it and the cell, and the signal of the LTOX in the membrane is small compared to the membrane from the cell it buds from. This shows that the GPMV formed may be created by stretching the available membrane, rather than using up the lipid resources in the cell to create new phospholipids for it.

References

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