THE BIOLOGICAL INDICATORS OF IMMUNE RESPONSE IN TWO INSECTS

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Abstract

We aim to elucidate the role of eicosanoids in cellular immunity in the squash bug, Anasa tristis. Squash bugs are significant pest insects that cause major crop damage throughout the Midwest each year. Currently we are assessing the immune responses of squash bugs to bacterial challenge by the pathogen Serratia marcescens by documenting hemocyte proliferation, microaggregate formation, and nodulation in comparison to control insects. In addition to a bacterial challenge, we inject the eicosanoid biosynthesis inhibitor indomethacin to observe the potential effects on the immune response. Assays were also performed on the spined soldier bug, Podisus maculiventris, during periods when squash bugs were unavailable due to dispaese. Eleven of 31 challenged squash bugs (35.5%) showed nodulation. The average count per challenged bug was 195 hemocytes and 2.56 microaggregates. This compared to 1 out of the 11 insects with nodulation (9.09%), and an average count of 145 hemocytes and 0.33 microaggregates in the control squash bugs. Of 14 challenged spined soldier bugs, 1 showed nodulation (7.14%) whereas two of 17 control insects showed nodules (11.8%). The hemocyte count was 2820 for challenged spined soldier bugs compared to 1210 in controls. Preliminary experiments with indomethacin showed reduced nodulation, microaggregate formation and hemocytes numbers in spined soldier bugs. More experiments are warranted to determine the overall effect. The bacteria, S. marcescens, induced an immune response in both types of insects. The challenged squash bugs generally showed higher levels of the indicators of immunity that we chose. These results may establish a baseline for subsequent studies.

Materials and Methods

Insects
- Squash bug, Anasa tristis
- Spined soldier bug, Podisus maculiventris

Bacterium
- Serratia marcescens

Drug
- Indomethacin

Solution
- Fast Terrific Broth (TB)
- Phosphate buffer saline (PBS)

Equipment
- Microscope
- Hemocytometer
- Capillary tubes

Culture Bacteria: S. marcescens is grown on a nutrient agar plate at 28.0 °C for three days. On the 4th day, bacteria is grown in Fast TB for 24 hours at 37.0 °C at 220 rpm. Use liquid culture to replate cells.

Injection: Take the optical density of S. marcescens liquid culture at 600 nm. For control insects, we inject Fast TB in abdominal region. For challenged insects, S. marcescens is injected. In next other study, we include an additional injection of indomethacin following the initial injection. Insects are incubated for a specific time period.

Extraction: Punch out opening in thorax of insect to use a capillary tube to withdraw hemolymph. Place hemolymph onto hemocytometer and dilute sample with PBS. Count hemocytes and microaggregate formations under microscope.

Vivisection: Anesthetize the insect in -20 °C. Open and examine insect for nodulation.

Results

Baseline Study: The bacteria, S. marcescens, induced an immune response in both the squash bug and spined soldier bug. When examining for nodulation in the challenged squash bugs, eleven of 31 challenged insects (35.5%) resulted positive. The average count per challenged insect was 195 hemocytes and 2.56 microaggregates. Among the control squash bugs, one out of the 11 insects with nodulation (9.09%), and the insects had an average hemocyte count of 145 along with 0.33 microaggregates. As shown in Figure 3a, the challenged squash bugs generally showed higher levels of the chosen immune response indicators. These indicators being nodulation, hemocyte count, and microaggregation formation.

Squash bugs exhibit diapause during the winter season, thus, we assay a similar insect, the spined soldier bug, that also uses eicosanoids in cellular immunity. Challenging a total of 14 spined soldier bugs with S. marcescens, 1 showed nodulation (7.14%). This is interesting because when compared with the control group, two of 17 control insects showed nodules (11.8%). As for the hemocyte count, the challenged spined soldier bugs averaged 2820 while the control group had 1210. Figure 3b represents the baseline data for the spined soldier bugs. Analysis of the data is difficult with unexpected results, but reasons may be proposed.

Indomethacin Study: We examine the effects of inhibiting the role of eicosanoids in cellular immunity using a biosynthesis inhibitor, indomethacin. With two control squash bugs, we detected no nodule or microaggregate formation and a hemocyte count of 282. This compared to three challenged squash bugs exhibiting no nodule or microaggregate formation and a hemocyte count of 110. We have concluded that S. marcescens induces squash bug immunity, and in Figure 4a, indomethacin does inhibit an immune response. Hemocyte count is lower in challenge insects than control insects as expected.

Three of the 11 control spined soldier bugs (27.3%) showed nodulation, whereas four of the 21 challenged spined soldier bugs (19.0%) showed nodulation. The hemocyte count was 314 with 5.59 microaggregates for challenged spined soldier bugs compared to 502 hemocytes and 0.42 microaggregates in controls. The effects of indomethacin is more evident in spined soldier bugs. In Figure 4b, indomethacin lowered the immune response in nodulation, hemocyte count, and microaggregation formation.

Future Work

1. We will better characterize the biochemical pathways in the squash bug and establish baselines of its immune responses.
2. By elucidating the role of eicosanoids in cellular immunity in squash bugs, we will apply this understanding to other insects alike.
3. In the end, we hope to prevent irreparable damages and potential destruction of the major economic crops found in the Midwest region.

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