Michał Sułek

Streszczenie w języku angielskim

Studies of host-pathogen interactions are an essential part of immunobiological sciences. Precise analyses of the mutual interaction in the insect-microorganism system may result in e.g. (i) discoveries of new bioactive compounds, (ii) elucidation of insect immune strategies and pathogen infection strategies, (iii) identification of unknown pathogenic microorganisms, and (iv) development of new bioinsecticides. Moreover, since the innate immune mechanisms in insects and mammals have many common elements, some of the discoveries may reveal new properties of the human immune system.

As part of this study, certain aspects of the humoral immune response of the greater wax moth *Galleria mellonella* to infection with *Pseudomonas entomophila* bacteria through such infection routes as e.g. damaged body cover, were analyzed. The analysis of insect survival after injection of various doses of bacteria showed a correlation between the amount of the injected bacteria and insect mortality. Based on the results obtained, three infectious doses were distinguished: 10 CFU, 50 CFU, and 500 CFU per caterpillar, hereinafter conventionally referred to as low, high, and proper doses, which were used for further research.

The histological analysis of cross-sections of infected larvae revealed destruction of the fat body. Furthermore, it has been documented that although the bacteria was injected directly into the hemocoel, the microorganisms migrated into the intestinal lumen and destroyed this organ. The analyses of the activity of humoral effectors after infection with the doses of 10 CFU and 50 CFU demonstrated induction of the expression of selected genes encoding immune proteins and peptides as well as appearance and enhancement of the antibacterial activity of insect hemolymph. This was correlated with the induction and antibacterial activity of low molecular weight (<10 kDa) components of the hemolymph of the infected insects. The value of the aforementioned immune parameters was directly proportional to the injection dose.

The research also demonstrated a phenomenon of specific immunological priming of *G. mellonella* larvae with *P. entomophila* bacteria. The hemolymph of primed larvae had higher antibacterial potential, which was correlated with a greater number of transcripts of genes encoding protein immune effectors. Consequently, the primed insects exhibited a slower progression of the infection and a higher survival rate.

The analysis of the low-molecular-weight proteome of non-primed and primed insects infected with (i) the low (10 CFU) or high (50 CFU) dose of bacteria and (ii) the specific dose

(500 CFU) facilitated the identification of proteins and peptides whose level was modulated in the experimental conditions. These included known compounds: galiomycin, defensin-like peptide, proline peptides -1 and -2, anionic peptide -1, cecropin D-like peptide, and lysozyme as well as three previously unknown proteins. These were dipetalogastin-like serine protease inhibitor (IPSD), whose amount increased after the bacterial infection, homeobox 5-like protein, whose presence in the hemolymph was determined by the infection, and kazal domain peptide Pr13a, whose amount increased in the hemolymph of insects in repeated contact with the pathogen. The identified proteins exhibited antibacterial activity against *P. entomophila* accompanied by changes in the biophysical and topographic parameters of the bacterial surface. Moreover, the IPSD protein had serine protease inhibitor activity, as it was found to inhibit the action of trypsin and elastase.

The discovery of the three new bioactive compounds in *G. mellonella* hemolymph expands the knowledge of the immunity of this insect and opens new possibilities for basic and applied research.