

## Abstract

In present studies, the cellular and humoral immune response of the greater wax moth *Galleria mellonella* to *Pseudomonas aeruginosa* exotoxin A was investigated. A decrease in the total number of hemocytes, mainly granulocytes and plasmatocytes, was detected in the hemolymph of insects injected with exoA. Fluorescent staining showed that the cells exhibited features of apoptotic and autophagic cell death, e.g. cytoplasm vacuolization and chromatin condensation. The flow cytometry revealed a significant increase in the number of phosphatidylserine- and active caspase 3-positive hemocytes, which indicates induction of apoptosis by exoA. The parameters of the humoral response in the hemolymph of insects injected with exoA indicated inhibition of phenoloxidase activity (8-15 hours after injection) and a significant decrease in lysozyme-type activity. In addition, the hemolymph showed no anti-*E. coli* activity, and no peptide bands with molecular mass below 6.5 kDa were observed on electrophoretic gels, which indicated the absence of antimicrobial peptides. A decrease in the apoLp-III level in the hemolymph was observed, especially 4 to 15 hours after the administration of exoA to the insects. Afterwards, the amount of the protein gradually increased, reaching the level observed in the control group after 24 hours. A correlation in time between changes in the apoLp-III amount in the fat body and the hemocytes of exoA-challenged larvae was demonstrated. An initial increase in apoLp-III (1-8 hours) and a decrease in its level after 15-18 hours were detected in both tissues after exoA injection. In turn, after 24 hours, the level of apoLp-III increased approximately 2-fold in the hemocytes but remained at a reduced level in the fat body. Two-dimensional electrophoresis and immunoblotting with anti-apoLp-III antibodies revealed changes in the profile of apoLp-III isoforms in the examined tissues. Two isoforms of apoLp-III with isoelectric points of  $pI \sim 6.5$  and  $\sim 6.1$  in the hemolymph and with  $pI \sim 6.5$  and  $\sim 5.9$  in the hemocytes as well as one isoform with  $pI \sim 6.5$  in the fat body with an additional polypeptide with a  $pI \sim 6.9$  were detected. After exoA injection, an approx. 30 % decrease in the level of both forms apoLp-III in the hemolymph was observed. In hemocytes, only a 65% decrease in the amount of the  $pI \sim 5.9$  isoform was detected, while an additional polypeptide of  $pI \sim 5.2$  appeared. No significant differences were observed in the amount of the major isoform in the fat body, while the polypeptide with  $pI \sim 6.9$  disappeared completely.

**Keywords:** *Galleria mellonella*, *Pseudomonas aeruginosa*, exotoxin A, host-pathogen interactions, immune response

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