Insects are the largest class of the animals. They owe the evolutionary success to inter alia their efficiently functioning immune system. Its role is to protect the insect from the proliferation therein of pathogenic bacteria, viruses, or fungi. On the other hand, pathogens have evolved mechanisms that allow them to force insects' defence barriers and intense proliferation in the body of animals. Both insects and microorganisms constantly improve their defence and virulence mechanisms, respectively. Therefore, a constant "arms race" occurs and the survival is at stake. Host-pathogen interaction does not take place in a vacuum, but under conditions of the external environment. Both abiotic (e.g. changes in the temperature) and biotic (previous infection or parallel infection with another organism) factors can significantly affect the mutual interaction between host and its pathogen. The aim of our research is to understand how both of these groups of factors modulate the course of the infection. The presented research plan relates to an immune response of insects that previously in their lives were exposed to the same pathogen, so it concerns the biotic factors. This is particularly interesting due to the fact that insects have not developed so-called acquired immunity that humans have. It is associated with lymphocytes and the production of specific antibodies after the first exposure to a given pathogen, which allows more efficient recognition of the same pathogen during the next contact. This fact is used in the protective vaccination. As already mentioned, the immune system of insects does not have lymphocytes nor the capacity to produce antibodies. In the response to infection, it produces and secretes so-called defence peptides, which possess antimicrobial properties, to the hemolymph (insect blood). Moreover, the hemolymph cells, called hemocytes, can phagocytise (engulf) infecting microorganisms. Insect hemolymph is very rich in various proteins and peptides that have antimicrobial properties and therefore is a source of research of substances that may be an alternative to antibiotics. Antimicrobial compounds contained in the hemolymph cooperate with each other in fighting infection. Their synthesis and activity can be induced and changed during infection. Despite the absence of antibodies, recent studies have shown that insects have some sort of immunological memory. It is manifested by the fact that animals that had previously defeated infection are more resistant to another one. Moreover, this increased resistance can be passed on to next generations. This phenomenon is referred to as immune priming. Understanding the mechanisms that modulate the immune response of insects is the subject of our scientific interests and research themes of this proposal. Our insect model is the greater wax moth Galleria mellonella, which is the pest of apiaries, causing a disease called "bee galleriose". It will be infected with the bacteria Bacillus thuringiensis, which is widespread in nature and, after getting into the moth's body, multiply intensively in the hemolymph causing so-called septicaemia.

We intend to compare the course of the humoral immune response of insects infected for the first time and of these that have already come into contact with the same pathogen.

Hemolymph from both groups of animals will be collected at particular time-points after infection and tested for its antimicrobial properties. Additionally, the protein profiles of hemolymph from both groups of animals will be compared and the level of apolipophorin III will be detected. This protein is involved in the infection recognition, stimulates the activity of antimicrobial peptides, and possesses antimicrobial activity itself. An additional consideration is the expression of genes encoding immune-related proteins and peptides in the insect fat body. This organ is an analogue of the mammalian liver and the main source of hemolymph proteins and peptides. Proteins, including those to be secreted into the hemolymph are produced there. The first stage is called transcription, which involves rewriting the genetic information contained in DNA into messenger RNA (mRNA). Then, after attachment of the ribosomes, the protein is being synthesized in the process called translation (protein biosynthesis). The measure of gene expression is then the number of the corresponding messenger RNA. We intend to compare the changes in the amount of mRNA for particular defence proteins and peptides of Galleria mellonella infected for the first time and those that were previously in contact with the pathogen. The research results will be analysed in the context of the survival curves of the first time and repeatedly infected insects.

The effects of the implementation of the proposed project will bring us closer to understanding how insects that do not have antibodies remember previous contact with the infection and, in the case next infection, fight the intruder more efficiently.