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Developmental changes in haemocyte morphology in response to *Staphylococcus aureus* and latex beads in the beetle *Tenebrio molitor* L.

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ABSTRACT

The evolutionary success of insects is undoubtedly related to a well-functioning immune system. This is especially apparent during insect development by the adaptation of individuals to the changing risk of infection. In addition, current studies show that the insect immune system is characterized by some specificity in response to natural pathogens (for example, bacteria, viruses or fungi) and artificial challengers (for example, latex beads or nylon filaments). However, developmental changes and the specificity of immune system reactions simultaneously have not been analysed. Thus, the aim of the present research was to determine changes in haemocyte morphology in response to attenuated *Staphylococcus aureus* and latex beads across each developmental stage of the beetle *Tenebrio molitor*.

The results of the present research clearly showed differences in the morphology of *T. molitor* haemocytes during development. The haemocytes of larvae and 4-day-old adult males were characterized by the highest adhesion ability, which was expressed as the largest average surface area, filopodia length and number of filopodia. In contrast, the haemocytes of pupae and 30-day-old adult males had a significantly lower value for these morphological parameters, which was probably related to metamorphosis (pupae) and immunosenescence (30-day-old adults).

The haemocytes of the tested individuals reacted differently to the presence of *S. aureus* and latex beads. The presence of *S. aureus* led to a significant decrease in all previously mentioned morphological parameters in larvae and in both groups of adult individuals. In these groups, incubation of haemocytes with latex beads caused only a slight decrease in surface area and filopodia length and number. This morphological response of haemocytes to biotic and artificial challengers might be related to an increase in the migration abilities of haemocytes during infection. However, the differences in haemocyte reactivity towards *S. aureus* and latex beads might be explained by differences in pathogen recognition. Conversely, increased adhesive abilities of pupal haemocytes were also observed, which might be related to the specificity of metamorphosis and the hormonal titre during this developmental stage.

1. Introduction

Insects represent the largest animal order in the world. Their evolutionary success is related to a huge adaptive ability of insects to different environmental conditions, which has allowed them to inhabit ecological niches that are not available for other arthropods (Stork et al., 2015). Undoubtedly, this phenomenon is also related to their well-functioning immune system (Urbański et al., 2016b).

The insect immune system is based on innate immune mechanisms, which are usually divided into the cellular and humoral immune response (Rolff and Siva-Jothy, 2003; Carton et al., 2008; Govind, 2008; Strand, 2008). The cellular response includes most immunological processes in which haemocytes participate. They have three main cellular mechanisms: phagocytosis, nodulation, and encapsulation (Strand, 2008; Urbański et al., 2014). Humoral responses include immune-related molecules such as lysozyme, antimicrobial peptides (AMPs) or phenoloxidase (PO) (Urbański et al., 2014, 2016a). However, it should be highlighted that the border between the humoral and cellular response is artificial because cellular mechanisms very often depend on the humoral response, and the synthesis and activity of immune-related molecules are the result of haemocytes activity (Strand, 2008; Urbański et al., 2013, 2016b).

Research examining the function of the insect immune system has been conducted for many insect species. In addition, in immunological

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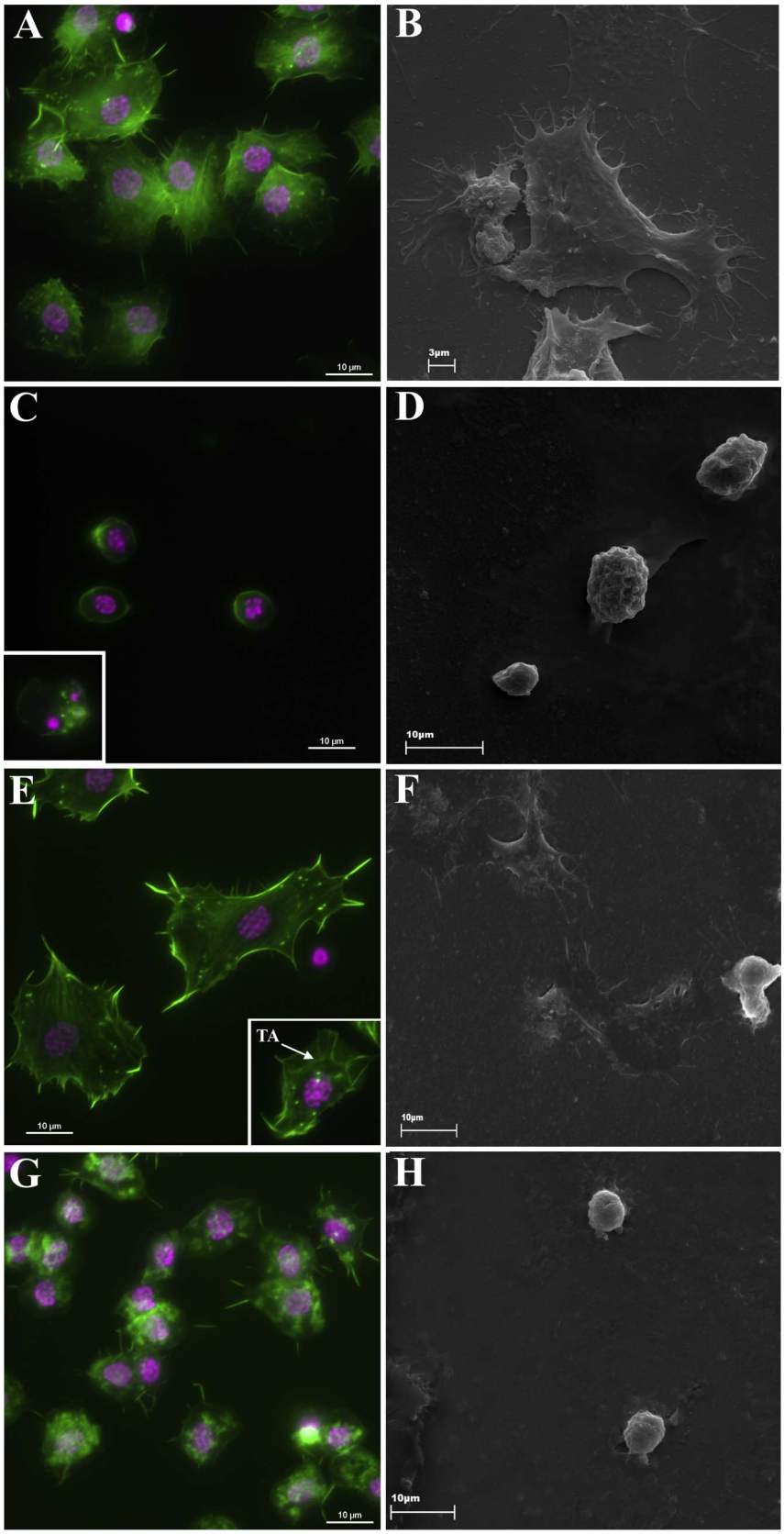

Fig. 1. Representative images of haemocytes of larvae (A, B), pupae (C, D), 4-day-old adult males (E, F) and 30-day-old adult males (G, H) of *Tenebrio molitor*. In figures A, C, E and G, F-actin cytoskeleton of haemocytes (green) was stained with Oregon Green® 488 phalloidin. DAPI (pink) was used for DNA of the haemocyte nuclei detection. For visualization of haemocytes in figures B, D, F and H, scanning electron microscopy technique was used. Figure C, in frame – haemocyte with nucleus and membrane defragmentation. Figure E, in frame – haemocytes with visible transverse arc (TA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bioassays, individuals in different developmental stages have been commonly used. Moreover, the activity of the insect immune system was determined by various methods based on the use of different pathogens or synthetic structures that may imitate pathogen infection (for example, latex and Sephadex beads or nylon filaments), all of which influence the ambiguity of the obtained results and impair their interpretation. Thus, the aim of the present research was to evaluate changes in haemocyte morphology in response to biotic (attenuated