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Phagocytosis in the hibernated queens of Bombus agrorum – a preliminary observation

Fagocytoza u matek *Bombus agrorum* w hibernacji – badania wstępne

K e y w o r d s: bumble bee, hibernation, immunity, phagocytosis
S ł o w a k l u c z o w e: trzmiel, hibernacja, odporność, fagocytoza

The concept that the insects elaborated various defense strategies against microbial infections, attacks from parasites and predators is now commonly accepted (9). The immune system of holometabolous insects depends on two main categories of defense reactions: haemocyte mediated processes such as phagocytosis and encapsulation of foreign objects (7, 8) and cell-free defense mechanisms represented by the antimicrobial immune proteins (1, 2, 3, 4). The antibacterial activity of insect haemolymph is attributed to innate and inducible immune peptides and small proteins.

Phagocytosis is a defense reaction of insects against invading pathogens. This cellular immune reaction is accompanied by changes both in the number of circulating haemocytes and in the relative proportions of different haemocyte types in the blood. In general, the infection of the hemocoel initiates a premature differentiation of haemocytes and their migration towards chemotactic stimulus. In the final stage of the phagocytic process, the engulfed bacteria, spores or small fragments of the fungal mycelium are digested in a phagolysosome that is formed by the combination of a lysosome with a phagosome. The lysosomal hydrolytic enzymes that destroy bacteria in some instances act against engulfed fungous material. Most probably, plasmatocytes and granular cells active in phagocytosis of bacterial cells can ingest and destroy foreign objects in phagocytic process (3, 6). The role of the phenoloxidase system and melanins cannot be excluded in phagocytosis of bacteria and insect pathogenic fungi (3).

The suggestion that in the bumble bees, like in other insect species, lysozyme together with phagocytosis constitute the second line of defense against invasion of bacteria initiated the presented here preliminary studies on phagocytosis in the hibernating queens of the bumble bee, *Bombus agrorum*.

MATERIAL AND METHODS

Bumble bees. Twenty-seven hibernating *B. agrorum* queens were used as a source of blood lysozyme. The bumbles were preserved at a refrigerator at 4°C for 4 months. Samples of haemolyph were taken by the use of microsyringe (5 μL/insect) from a dorsal vessel into the Eppendorf tube on ice-bath and mixed with 10 μL of insect physiological saline.

Bioassay. Ten-microliter sample of hemolymph was centrifuged (200 x g, 10 min, 6°C), supernatant was withdrawer and 10 μL of *Staphylococcus aureus* 209P (10^8 cells/ml) was added and after mixing the sample was incubated at 32°C for 30 min. After careful washing with the insect physiological saline, monolayers of haemocytes on microscopic slides were prepared. One hundred phagocytosed bacteria cells were counted under the light microscope after staining with methylene blue and the phagocytic index (PI) was calculated from the equation:

PI = number of bacterial cells inside the phagocytic haemocyte x 100/number of phagocytosing haemocytes

Counting of 400 haemocytes per monolayer and determination of the percentage of phagocytically active cells measured the activity of haemocytes.

RESULTS

Live cells of *S. aureus* have been attached to phagocytic cells of *B. agrorum*, engulfed and then subjected to a variety of intracellular immune defense processes. In hibernating naive insects the percentage of haemocytes active in phagocytosis was below 3% and the value of phagocytic index was 1.2 (0.5–1.3) cells/haemocyte. These results are based on triplicate examinations, and here are presented the mean values.

DISCUSSION

The hibernating bumble bees possess a number of mechanisms that inhibit or even destroy bacteria invading the hemocoel. One set of mechanisms is represented by humoral entities, among them mainly by bacteriolytic activity of hemolymph lysozyme. The second set of mechanisms represent cellular defense reactions, mainly phagocytosis (3, 4).

Up to now it can be assumed that in the hibernating bumble bees, cellular defense mechanisms have dominated in the antibacterial protection of the hemocoel. Most probable is the situation that phagocytic activity of haemocytes and bacteriolytic activity of lysozyme together can clear hemocoel of the hibernating bumble bee queens from bacterial invaders (5). Most probably, these two mechanisms, like in the bees, form the very effective line of defense in the hemocoel of hibernating bumbles (6).

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STRESZCZENIE

Ziarniste hemocyty i plazmatocyty trzmiela wychwytują i niszczą obce ciała, głównie bakterie, w procesie fagocytozy. U matek trzmiela *Bombus agrorum* poddanych hibernacji odsetek hemocytów zaangażowanych w fagocytozie wynosił poniżej 3%, zaś wartość indeksu fagocytarnego wyniosła 1,2 (0,5–1,3) komórek bakteryjnych/hemocyt. Chociaż znane są komórki zaangażowane w fagocytozie hibernujących trzmieli, to jednak mechanizmy i czynniki odgrywające rolę w tym procesie nie są w pełni poznane. Można przypuszczać, że u *B. agrorum* podobnie jak i u innych przedstawicieli *Apoidea* u hibernujących matek trzmiela lizozym ściśle współdziała z fagocytozą w likwidacji bakterii wnikających do jamy ciała owada.