Ecdysone induces a termination of encapsulation via GBP-GBPB interaction

Insects have evolved various defense strategies involving both humoral and cellular immune responses to combat invading foreign pathogens. Encapsulation is a conserved cellular immune process targeted against any foreign intruder which is too large to be phagocytosed. Examples include parasitoid wasp eggs, metazoan parasites and artificial chromatography beads. The process requires attachment of hemocytes to the large intruder and subsequent formation of a multilayered capsule. A morphological conversion of plasmatocytes from non-adhesive (unspread) to adhesive (spread) state is critical and essential for the capsule formation.

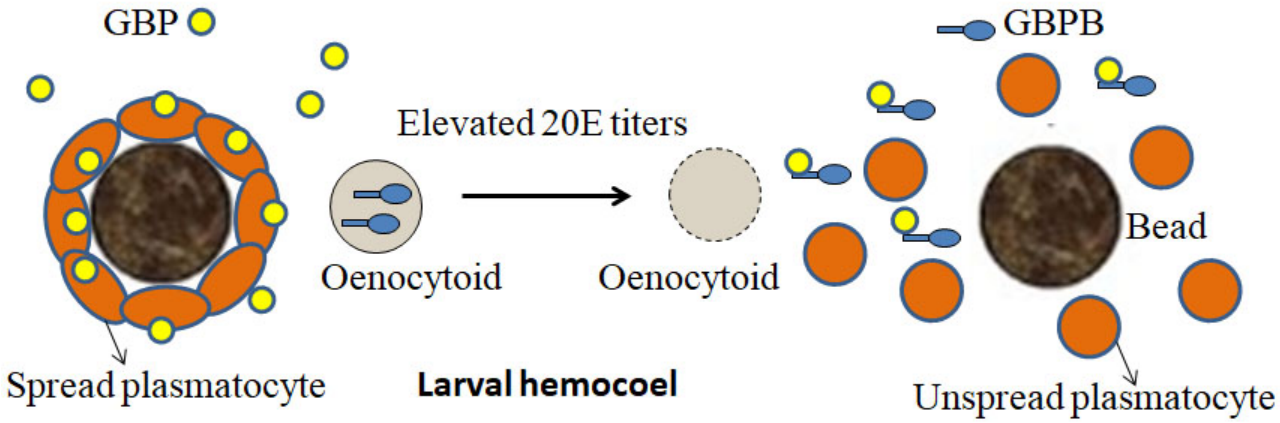

Fig. 1. A proposed model for the release of GBPB from oenocytoids into plasma induced by 20E treatment, thereby suppressing GBP-induced plasmatocyte spreading and encapsulation. The involvement of 20E treatment or bead challenge in promoting the release of GBPB, which binds to GBP through its N-terminus and subsequently scavenges GBP from hemocoel. This leads to the termination of GBP-induced plasmatocyte spreading and encapsulation.

Growth-blocking peptide (GBP) is an insect cytokine that stimulates plasmatocyte adhesion, thereby playing a critical role in encapsulation reaction. The initiation of encapsulation and stimulation of plasmatocytes can be modulated by regulation of cytokine levels. The cytokine titers increase in hemolymph upon immune challenge. Interestingly, the insects must also scavenge the cytokines in hemolymph in a timely fashion in order to avoid excessive stimulation of plasmatocytes. However, the mechanism employed by the insects to inactivate the cytokines remains largely unexplored.

Using the cotton bollworm (*Helicoverpa armigera*) as a model, we have demonstrated the existence of two GBP-binding proteins (namely GBPB1 and GBPB2) primarily found in the

hemocytes and the plasma. Both GBPB1 and GBPB2 show enhanced expression in the plasma during metamorphosis. Oenocytoids easily lose their nuclei and may be prone to lyse during metamorphosis. Given that there is no signal peptide found in either GBPB1 or GBPB2 sequence and the cellular contents tend to be released upon cell lysis, we assume that the released proteins in the plasma are at least partly contributed by oenocytoids during metamorphosis. We found that the steroid hormone 20-hydroxyecdysone (20E) promotes the release of GBPB1 and GBPB2 at least partly from oenocytoids into the plasma.

Plasmatocyte sensitivity to cytokines fluctuates significantly along with larval stages, with 20E enhancing the plasmatocyte sensitivity to cytokines. Since 20E titer significantly increases in hemolymph of *H. armigera* during metamorphosis, the plasmatocytes would be more sensitive to cytokines during this stage. However, continuous maintenance of such high cytokine level could lead to excessive stimulation of plasmatocytes and cause severe cellular damages. This might possibly explain the high levels of GBPB1 and GBPB2 in hemolymph during metamorphosis. *Drosophila* GBP acts to suppress humoral immunity and stimulate cell spreading. Hence, another possible explanation for the need to maintain a low GBP titer during metamorphosis might be that GBP regulates a switch from cellular immunity to humoral immunity when larvae enter metamorphosis from the feeding stage. Therefore, the high levels of GBPB1 and GBPB2 in plasma lead to low titers of GBP in hemolymph during metamorphosis, which inhibits plasmatocyte spreading and activates the expression of antimicrobial peptides.

Further, we confirm the binding of GBPB1 to GBP, and demonstrated that this binding is dependent on N-terminus of GBPB1 but not its C-terminal lipoprotein domain. Given the physiological functions of GBP and GBPB1, we propose that 20E induces the release GBPB1 and GBPB2 at least partly from oenocytoids into plasma. Subsequently, the increased GBPB1 and GBPB2 scavenge GBP in hemocoel by binding via their N-terminus, thereby suppressing GBP-induced plasmatocyte spreading and encapsulation (Fig. 1). Postregulation of insect cytokine activity represents an efficient and effective termination system of cellular immunity. Such a system might be crucial for insect survival, since it avoids excessive stimulation of immune cells and allows the insect to switch quickly from cellular immunity to humoral immunity.

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