CHAPTER 6

Inducible Humoral Antibacterial Immunity in Insects

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Introduction

Insects have effective immune systems composed of both cellular (Boman et al., 1978; Boman and Hultmark, 1981; Salt, 1970) and humoral components (Boman, 1982; Boman et al., 1986; Boman and Hultmark, 1987; Faye et al., 1975; Götz and Boman, 1985). Cellular immune reactions include phagocytosis, nodule formation and encapsulation (Götz and Boman, 1985; Ratcliffe and Rowley, 1979; Ratcliffe et al., 1985), whereas humoral immune reactions involve synthesis and release of several antibacterial immune proteins (Boman and Hultmark, 1987; Götz and Boman, 1985), some capable of killing both gram-positive and gram-negative bacteria. The expression of this multicomponent humoral immune system requires *de novo* synthesis of RNA and proteins with broad antibacterial activity. There are at least three families of antibacterial proteins: lysozyme, cecropins and attacins (Boman et al., 1986; Boman and Hultmark, 1987; Boman and Steiner, 1981).

Humoral immunity studies in insects have been conducted mostly in lepidopterans (Briggs, 1958; Chadwick, 1967; Hoffmann et al., 1981; Stephens and Marshall, 1962) and in diapausing pupae of silkmoths, e.g. *Anthaerea pernyi*, *Samia cynthia* and *Hyalophora cecropia* (Boman and Hultmark, 1987; Boman et al., 1974; Faye et al., 1975; Hultmark et al., 1980). Pupae of *H. cecropia* constitute a particularly good model for studying humoral immunity as they are large (5-10 g) and contain 1-2 ml of haemolymph. They also undergo a long diapause (6-9 months) that allows immune proteins to be synthesized without much interference from other biosynthetic processes (Boman et al., 1986; Boman and Hultmark, 1981).

This paper is concerned exclusively with the inducible humoral antibacterial factors of insects and explains the techniques used to immunize insects for detection of immune proteins and antibacterial
activity. Hopefully, it will provide students with the basic knowledge required for conducting experiments on those insect species which have not yet been studied.

Bacteria Used in Assays

The bacteria commonly used in bacteriolysis assyas are dried *Micrococcus luteus* (for lysozyme) from Sigma Chemicals and log-phase *Escherichia coli* K-12, strain D31, an ampicillin- and streptomycin-resistant mutant with a defective lipopolysaccharide. *Enterobacter cloacae*, strain B12, resistant to nalidixic acid, as well as *E. coli* D31 have been used for induction of immunity (Boman *et al.*, 1974; Flyg *et al.*, 1987; Hultmark *et al.*, 1982; Hultmark *et al.*, 1980; Kaaya *et al.*, 1987). Heat-killed *Pseudomonas aeruginosa* has been reported to induce immunity in insects (Chadwick, 1970; Ginsrich, 1964) but we now know that live bacteria are much more effective (Boman *et al.*, 1978; Boman and Steiner, 1981; Kaaya and Darji, 1988).

Insects Immunized

Among the insects reported to have been successfully immunized with bacteria are *Galleria mellonella* and some other lepidopterans (Briggs, 1958; Chadwick, 1967; Hoffmann *et al.*, 1981; Stephens and Marshall, 1962); a hemipteran (Ginsrich, 1964); silkworms (Boman *et al.*, 1974; Faye *et al.*, 1975; Hultmark *et al.*, 1980; Qu *et al.*, 1982); dipterans, e.g. *Drosophila* (Boman *et al.*, 1972; Flyg *et al.*, 1987; Robertson and Postlethwait, 1986), tsetse flies (Kaaya and Darji, 1988; Kaaya *et al.*, 1987), *Phormia terranovae* (Keppi *et al.*, 1986; Keppi *et al.*, 1989), *Sarcophaga peregrina* (Ando *et al.*, 1987; Okada and Natori, 1983); *Manduca sexta* (Dunn and Drake, 1983; Hurlbert *et al.*, 1985); the darkling beetle (Spies *et al.*, 1986b); locusts (Lambert and Hoffmann, 1985) and *Rhodnius prolixus* (De Azambuja *et al.*, 1986). However, much of the available data on insect immunity have been obtained from diapausing pupae of silkworms, especially *Hyalophora cecropia*.

Immunization Procedure

Diapausing pupae of *S. cynthia* and *H. cecropia* can be stored in a refrigerator at 8°C. During experiments they are usually stored at 25°C, with a relative humidity of 60-80% and a 15.5-hour light: 8.5-hour dark photoperiod. They are vaccinated with viable cells of *E. cloaca* B12 or *E. coli* D31 injected into the thorax; for *S. cynthia* about 5 x 10⁵ and for *H. cecropia* 10⁶ cells in normal saline using an ‘AGLA’ micrometer syringe (Wellcome Reagents, London) (Boman *et al.*, 1974; Faye *et al.*, 1975; Hultmark *et al.*, 1980) or an Arnold hand micro-applicator (Burkard Scientific (Sales) Ltd., Rickmansworth, England) fitted with a 1-ml glass syringe and a 31-gauge needle. Control pupae are injected with an equal volume of normal saline. To stop excessive bleeding, the injection wound