Insect protection against viruses

SIR — Insects exhibit both humoral and cellular immune responses that are effective against various pathogens and parasites including fungi, protozoa, bacteria, nematodes and parasitic hymenoptera[1]. No insect immune response effective against viral infections has yet been described. Using a recombinant of the baculovirus *Autographa californica* M nuclear polyhedrosis virus containing the *lacZ* reporter gene (AcMNPV-hsp70/lacZ)[2], we investigated the physiological basis of resistance in *Helicoverpa zea*, an important agricultural pest and a highly refractory host[3]. We discovered that the larvae of this insect are actually very susceptible to infection by AcMNPV, but infected cells are encapsulated by haemocytes and subsequently cleared. These results demonstrate that the insect immune response can be effective against viral pathogens.

We orally inoculated newly moulted third-instar *H. zea* with 2,200 polyhedra of AcMNPV-hsp70/lacZ using a microapplicator (ISCO) and maintained the insects individually at 28 ± 1 °C on an artificial diet. Larvae were killed at various hours post-inoculation (h.p.i.), and larval tissues were processed for elucidation of the blue lacZ signal and examined to determine the extent of viral infection[2]. During the first 20 h.p.i., the proportion of lacZ-expressing insects increased to 96%, and early pathogenesis was nearly identical to that described for *H. virescens*, a closely related species highly susceptible to fatal AcMNPV infections[4]. Between 48 and 72 h.p.i., however, the proportions of *H. zea* that were positive for lacZ decreased to 40%, suggesting that some larvae had cleared their infections. In many insects killed at 30 h.p.i. and at later times, we observed small brown patches colocalized with blue lacZ signals in the epidermis of tracheae associated with the midgut (*a*, *b* in the figure). These infected tracheae are surrounded by aggregations of haemocytes, often containing capsules encompassing the host cells expressing lacZ (*c* in the figure). We never encountered a temporal decline in lacZ-positive larvae nor observed a haemocyte encapsulation response while examining thousands of dissected susceptible hosts challenged with the same viral construct[2,4,5]. Morphologically, the capsules in *H. zea* are identical to those attributed to the insect cellular immune response in which circulating haemocytes surround, immobilize and kill invading pathogens and parasites[1].

To assess the extent to which the immune response of *H. zea* is responsible for halting the spread of AcMNPV infection, we monitored the distribution of lacZ expression within tissues following application of chemical and biological agents known to immunosuppress larval lepidopterans. Chemically, we compromized the immune responses of fourth-instar *H. zea* with intrahaemocoelic injections of diethyldithio-carbamic acid (DDCA)[6]. In these experiments, 24 h after injection of DDCA, we observed more widespread lacZ expression and larger individual viral plaques compared to sham-injected controls (*d*, *e* in the figure). Biologically, we used parasitization by the wasp *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to immunosuppress *H. zea* larvae. The female reproductive tract of this endoparasitoid contains a symbiotic polydnavirus that is injected during oviposition. In combination with other factors from the wasp, polydnaviral gene products prevent recognition, encapsulation and destruction of parasitoid eggs and larvae[7–9]. In our experiments, we allowed *C. sonorensis* to oviposit into *H. zea* larvae immediately before we orally inoculated them with AcMNPV-hsp70/lacZ. We subsequently compared lacZ expression in these caterpillars with unparasitized control insects and found that the distribution of the reporter gene signal was greater in parasitized *H. zea* than in control insects (*f* in the figure), indicating that the cellular immune response is a significant factor in preventing the spread of infection within *H. zea* larvae.

Our observations on baculovirus infections in the resistant host, *H. zea*, indicate that the immune response defines, in part, the functional host range of AcMNPV and suggests a possible strategy whereby this baculovirus can be genetically manipulated to become a more efficacious pesticide. Specifically, the incorporation of polydnavirus genes with immunosuppressant activity into the genome of AcMNPV might lower resistance in *H. zea* and other pest species and enable these pests to be controlled with a recombinant of AcMNPV.

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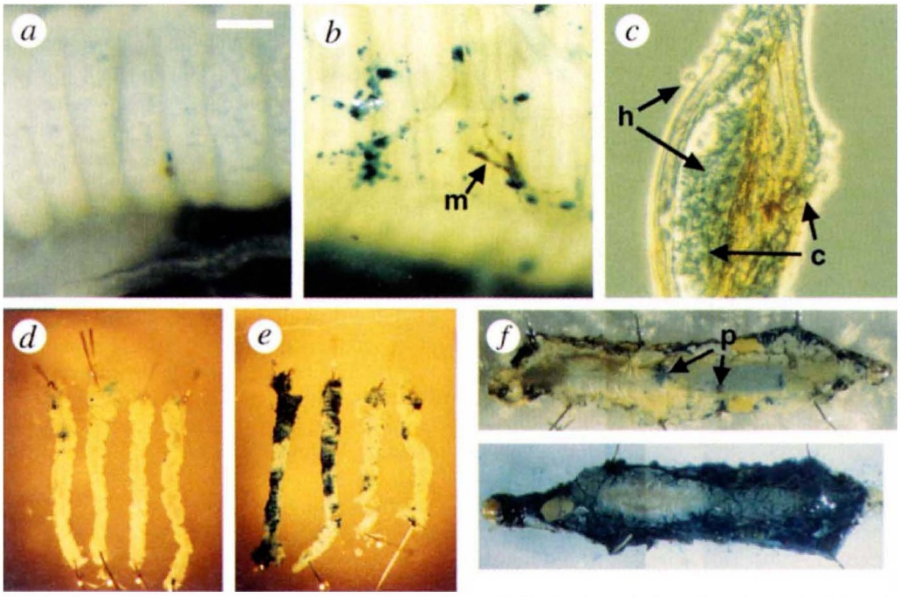

Cellular immune response and lacZ expression in *H. zea* following inoculation of newly moulted fourth-instar larvae with 44 (*a*–*e*) or 34 (*f*) polyhedra of AcMNPV-hsp70/lacZ. *a*, Colocalization of lacZ (blue) and melanization (brown) on the larval midgut at 48 h.p.i. (bar in *a*, 0.37 mm); *b*, melanization (m) along tracheal branches expressing lacZ at 48 h.p.i. (bar, 0.18 mm); *c*, aggregated haemocytes (h) surrounding a melanized capsule (c) on a tracheal branch (bar, 100 μm); *d*, maximum distribution of lacZ on midgut tracheae from a larval cohort sampled 24 h after intrahaemocoelic inoculation of 1 μl ddH_2O at 18–20 h.p.i. (bar, 0.43 mm); *e*, maximum distribution of lacZ on midgut tracheae from a larval cohort sampled 24 h after intrahaemocoelic inoculation of a 1-μl aqueous solution containing 0.1 mg DDCA (Sigma) at 18–20 h.p.i. (bar, 0.43 mm); *f*, maximum distribution at 96 h.p.i. of lacZ from a larval cohort parasitized by *C. sonorensis* (below) and a control cohort (above) (bar, 60 mm). In the control larva, blue coloration along the dorsal aorta (upper and lower margin of the whole mount) is an endogenous signal and does not reflect viral infection[4]. In this specimen, viral lacZ signals are restricted to much smaller plaques (p) in the central region of the midgut.

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