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Immunology

Role of the integument in insect immunity: Epicuticular abrasion and induction of cecropin synthesis in cuticular epithelial cells

(epithelia/*Bombyx mori*/*Hyalophora cecropia*/fat body)

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ABSTRACT **When the epicuticle of a silkworm larva, *Bombyx mori*, was lightly abraded in the presence of live *Bacillus licheniformis*, *Enterobacter cloacae*, or bacterial cell wall components, cecropin mRNAs were detected in the underlying epithelial cells and in fat body cells remote from the abraded area. Antibacterial activity due to cecropin was detected in the matrix of the lightly abraded cuticle but not in nonabraded portions of the cuticular matrix or in the hemolymph surrounding the fat body, unless a more severe cuticular abrasion was administered. A light abrasion to a larva of the giant silkworm moth, *Hyalophora cecropia*, in the presence of *E. cloacae* also induced antibacterial activity in the abraded cuticle. These data illustrate that the ectodermally derived lepidopteran larval integument, when challenged by live bacteria or their cell wall components, mounts an immune response. Hence, the insect exoskeleton, which is often considered as an inert protective armor, is indeed actively participating in defense.**

Contributing greatly to successful defense in insects, and arthropods in general, is their outer protective armor, or cuticle. The cuticle is a nonliving matrix of carbohydrate and protein secreted by the underlying monolayer of epithelial cells that cover the quasi-totality of the surface of the insect (1). The cuticle has been considered a mechanical barrier that effectively safeguards against microbial invasion (2, 3).

A second line of defense against microorganisms is the synthesis of specific antibacterial peptides and polypeptides, such as cecropins, defensins, attacins, etc. These molecules have generally been investigated as part of the hemocoelic immune response following an injection of some eliciting substance or after simply puncturing the integument by a pin prick or an incision (4–6). In nature, however, it is probably much more common for the insect cuticle to receive a topical scratch rather than a puncture wound or an injection. In fact, Pasteur (7), when investigating silkworm diseases, noted that the cuticles of silkworms were often marked by topical scratches. Because the integument is the principal interface between the natural external microbial flora and the hemocoel, we chose to investigate whether an immune response would be induced by an epicuticular abrasion in the presence of bacteria.

MATERIALS AND METHODS

Organisms. Silkworms, *Bombyx mori*, were reared on the artificial diet Silkmate 2M (Kyodo Shiryo Ltd., Tokyo) at 24°C under a 14-hr photoperiod. Experiments used fifth instar larvae reared to day 5. Silkworms were also reared under axenic conditions. *B. mori* eggs were surface sterilized with 70% ethanol and 4% formaldehyde then rinsed twice with 100% ethanol and transferred to a sterile container. Hatching larvae were maintained in a sterile environment and fed autoclaved artificial diet. Silkworms reared under these conditions were shown to be germ-free. Larvae of *Hyalophora cecropia* were reared on fresh cherry leaves at 28°C under a 16-hr photoperiod. Fifth instar larvae reared to day 7 were used for experiments. The bacteria used to study induction of an immune response were *Bacillus licheniformis*, isolated from the cuticle of laboratory-reared silkworms, and *Enterobacter cloacae* strain 57-9, obtained from the Institut Pasteur Collection. A streptomycin-resistant strain of *Escherichia coli* MC 4100 was used for antibacterial assays. All strains were maintained in standard nutrient broth.

Abrasion and Bacterial Application. The cuticle can be subdivided in two distinct layers: an outer thin epicuticle and an underlying procuticle made up of obvious horizontal lamellae. The cuticular epithelium lies beneath the procuticle and forms a monolayer of flattened or somewhat columnar cells.

To obtain an epicuticular abrasion silkworms were gently stroked once on their dorsal and lateral surfaces with ultra-fine emery paper (1500 grit Penguin Brand, Tokyo). A procuticular abrasion was obtained by firmly stroking the dorsal and lateral surfaces 10 times with ultra-fine emery paper. Transmission electron microscopy (TEM) was used to ensure that abrasion did not impair the integrity of the larval cuticle or epithelial cells (8). To obtain a light cuticular abrasion in *H. cecropia*, larvae were stroked with fine emery cloth (Norton K 625) between the tubercules of their dorsal surface. Given that TEM studies were not carried out on *H. cecropia* to evaluate the degree of cuticular abrasion, we qualified this abrasion as "light." Fifty microliters of a given bacterial suspension in early stationary phase or purified microbial cell wall component solution was applied topically to the abraded cuticle. Extracts were prepared from nonabraded and abraded zones of the cuticle of *B. mori* and only the abraded zone of the cuticle of *H. cecropia*.

To prepare microbial cell wall component solutions, dextran T 10 (Pharmacia/LKB Biotechnology) was suspended in apyrogenic water at a concentration of 1 mg/ml and autoclaved. Insoluble peptidoglycan was isolated from *Micrococcus luteus* ATCC 4698 (9). Soluble peptidoglycan was obtained from *Bacillus megaterium* by preparing insoluble peptidoglycan (9) and then by treating the insoluble peptidoglycan with lysozyme (10). Both peptidoglycan preparations were suspended in apyrogenic water at a concentration of 1 mg/ml and autoclaved. Laminarin (Calbiochem) was dis-

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Abbreviations: LPS, lipopolysaccharide; TEM, transmission electron microscopy.

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solved in apyrogenic water and the solution was filtered through a 0.22-μm cellulose acetate filter. The laminarin filtrate was lyophilized and resuspended to a concentration of 1.47 mg/ml. Lipopolysaccharide (LPS) W from *E. coli* 055:B5 (Difco) was mixed with apyrogenic water, sonicated for 10 min, and then filtered through a 0.22-μm filter. The filtrate was lyophilized and subsequently resuspended to a concentration of 1.2 mg/ml.

Collection of Hemolymph. Hemolymph from both species was collected in tubes on ice containing a few crystals of *N*-phenylthiourea and then centrifuged at 5000 × *g* for 5 min at 4°C. Hemolymph plasma samples were used fresh or were frozen at −80°C. Hemolymph plasma samples from *B. mori* (200 μl) were also lyophilized.

Acid Extraction of Antibacterial Peptides from the Abraded and Nonabraded Cuticle. The integument from both insects was dissected in ice-cold sterile Ringer solution (in g/liter: 6.5 NaCl, 0.14 KCl, 0.2 $NaHCO_3$, 0.12 $CaCl_2$, and 0.12 NaH_2PO_4). The integument was then meticulously scraped with a spatula to remove all tissues adhering to the cuticle. The tissue-free cuticle was subsequently rinsed in fresh Ringer solution, cut to separate abraded and nonabraded areas, and then blotted with absorbent tissue paper to remove excess liquid. Each section of cuticle was then extracted on ice for 30 min in 300 μl (*B. mori*) and 700 μl (*H. cecropia*) of 0.2 M sodium acetate buffer (pH 4.0). Abraded cuticles of *B. mori* were also extracted with 10 mM Tris·HCl, at pH 7.2 and pH 8.8, respectively. The cuticular extracts were centrifuged at 10,000 × *g* and either used immediately or frozen (−80°C).

Antibacterial Activity Assay. Antibacterial activity in the cuticular extract and hemolymph plasma of *B. mori* was monitored at 4-hr intervals postabrasion for a period of 12 hr and then at 12-hr intervals up to 96 hr. Antibacterial activity in the cuticular extract and hemolymph plasma of *H. cecropia* was assayed at 0 hr and 24 hr postabrasion. Inhibition zone assay (11) was used to discern antibacterial activity. Each well received 15 μl of hemolymph plasma or acid cuticular extract. It should be noted that the inhibition zones used to assay cecropin activity are not directly proportional to cecropin concentration but to the logarithm of the peptide concentration (11). Lyophilization of hemolymph plasma samples from *B. mori* was used to concentrate weak antibacterial activity to facilitate detection. Hemolymph plasma powder was solubilized in 15 μl of 0.2 M sodium acetate buffer (pH 4.0) and applied to plates. All detection plates were placed at 4°C for 30 min and then incubated at 30°C for 24 hr, at which time inhibition zones were scored. A zone of inhibition (>1 mm from the edge of the well) of bacterial growth in which the agar appeared clear, was considered indicative of antibacterial activity.

Protein Quantification. The protein concentration was measured by using the Pierce bicinchoninic acid protein assay reagent.

Native Polyacrylamide Gel Electrophoresis (PAGE). Electrophoresis of native proteins from acid cuticular extracts and from hemolymph plasma of *B. mori* was carried out in a 15% gel at pH 4.3, with the stacking gel omitted (12). Antibacterial proteins and peptides were located by the technique of Hultmark *et al.* (13). Synthetic cecropin A (Peninsula Laboratories), derived from sequence analysis of cecropin A purified from *H. cecropia*, was used as an antibacterial peptide control and molecular weight standard.

Tissue Dissection for RNA Isolation. All dissections were performed microscopically with sterile dissecting tools. Randomly selected abraded silkworms were dissected individually in sterile ice-cold Ringer solution. Tissues from each insect were then rinsed in fresh Ringer solution, pooled, and snap-frozen in liquid nitrogen. Fat body lobes were carefully removed from the peripheral fat body layer beneath the cuticular epithelium. Great care was taken to avoid contact with the epithelial cells and to avoid contamination with other tissues. Remaining peripheral fat body and muscle adhering to the body wall was carefully dissected to minimize contamination of epithelial cells. Following the majority of tissue removal from the body wall, the fine pearly layer of epithelium was delicately peeled from the cuticle.

RNA Extraction and Northern Blot Hybridization. Total cellular RNA was prepared from pooled frozen peripheral fat body and pooled epithelial cells using the LiCl/urea method (14). Northern blotting was carried out using standard techniques (15). The cecropin hybridization probe consisted of a ^{32}P-labeled random-primed 400-base-pair *B. mori* cecropin B24 cDNA clone (16). The ^{32}P-labeled random-primed α-tubulin probe was used as a constitutively expressed internal standard (17).

RESULTS

Epicuticular and Procuticular Abrasion. Silkworms that had received an epicuticular abrasion gave no noticeable signs of abrasion (Fig. 1*A*). Abraded epicuticle was further examined at the ultrastructural level with TEM to confirm that the procuticle and epithelial cells had received no injury (Fig. 1*B*). During examination of numerous integumental sections, hemocytes were neither observed within the epithelium nor adhering to the basement membrane beneath the abraded zone of the cuticle. If, however, *B. mori* cuticle was more intensely abraded, the majority of the procuticle was removed (Fig. 1*C*). Such abrasion occasionally exposed the epidermis to the external environment but did not physically damage the epidermis, as judged by TEM observation (Fig. 1*D*).

Induction of Antibacterial Activity in the Cuticle and Hemolymph. Epicuticular abrasion in the presence of the naturally occurring *B. licheniformis* induced antibacterial activity in the abraded area of the cuticle as evidenced by inhibitory zones surrounding cuticular extracts in antibacterial assays (Fig. 2*A*). It should be noted that antibacterial activity detected in cuticle following an epicuticular abrasion was localized specifically in the abraded areas. We could not detect antibacterial activity in nonabraded areas of the cuticle at a distance of a few millimeters from the site of abrasion or in the hemolymph plasma (Fig. 2*A*), even when the plasma was concentrated 13-fold (data not shown). Because of fluctuations in the number of bacteria colonizing the cuticle, which depended on the relative humidity in the rearing containers, the degree of steaming of the artificial diet, the number of larvae reared together, etc., we applied another bacterium, *E. cloacae*, in a homogeneous fashion to each of the abraded cuticles. The results were the same as with *B. licheniformis* (Fig. 2*A*). *H. cecropia* larvae that received a light cuticular abrasion in the presence of *E. cloacae* showed, like *B. mori*, activity in the abraded cuticle but not in the hemolymph plasma (Fig. 2*B*). The cuticular antibacterial activity of *H. cecropia* was not characterized in this study.

Procuticular abrasion of *B. mori* in the presence of *B. licheniformis* and *E. cloacae* resulted in antibacterial activity in the abraded cuticle and in the hemolymph plasma. Activity was particularly intense in the latter; however, nonabraded cuticle showed no activity (Fig. 2*C*).

Time Course of the Appearance of Antibacterial Activity. Antibacterial activity was detected in the lightly abraded cuticle of *B. mori* as early as 8 hr after abrasion in some instances and consistently at 12 h postabrasion (Fig. 3*A*). This activity persisted in the abraded cuticle for up to 96 hr postabrasion. At each time interval, immediately prior to cuticular dissection, hemolymph plasma was collected from each silkworm and was subsequently assayed for antibacterial activity along with the corresponding cuticular extract. Again, no antibacterial activity was detected in the he-

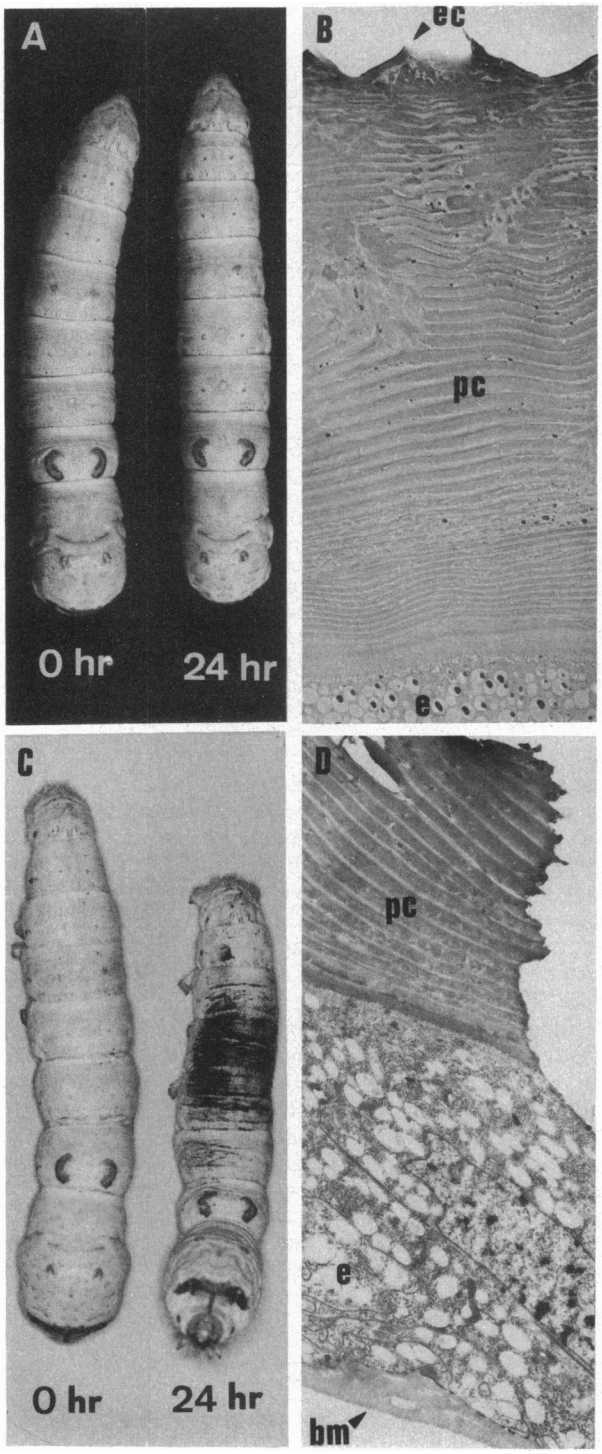

FIG. 1. (*A*) Silkworm having received an epicuticular abrasion, 0 hr postabrasion and 24 hr postabrasion. (*B*) Micrograph of the silkworm integument following an epicuticular abrasion. (×3200.) (*C*) Silkworm having received a procuticular abrasion, 0 hr postabrasion and 24 hr postabrasion. (*D*) Micrograph of the silkworm integument following a procuticular abrasion. (×5400.) ec, Epicuticle; pc, procuticle; e, epithelial cell; bm, basement membrane.

molymph plasma of silkworms that had been subjected to an epicuticular abrasion in the presence of bacteria (Fig. 3*A*).

Following a procuticular abrasion, the activity in the hemolymph plasma was detectable 3–4 hr later than that in the abraded procuticle of the same insect (Fig. 3*B*). In all native acid PAGE experiments, the antibacterial activity detected in cuticular extracts and hemolymph plasma samples was found to migrate to a position that corresponded only to that of the cecropin family of antibacterial peptides.

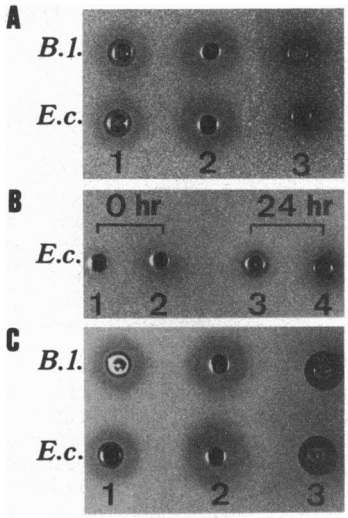

FIG. 2. (*A*) Antibacterial inhibition zone assay 24 hr following an epicuticular abrasion in the presence of *B. licheniformis* (*B.l.*) and *E. cloacae* (*E.c.*) of cuticular extracts from abraded (column 1) and nonabraded (column 2) areas of the cuticle and hemolymph plasma (column 3). (*B*) Antibacterial inhibition zone assay following a light abrasion of the cuticle of *H. cecropia* larvae in the presence of *E. cloacae* (*E.c.*). Cuticular extract and hemolymph plasma 0 hr postabrasion (wells 1 and 2, respectively) and of cuticular extract and hemolymph plasma 24 hr postabrasion (wells 3 and 4, respectively). (*C*) Antibacterial inhibition zone assay 24 hr following a procuticular abrasion in the presence of *B. licheniformis* (*B.l.*) and *E. cloacae* (*E.c.*) of cuticular extracts from abraded (column 1) and nonabraded (column 2) areas of the cuticle and hemolymph plasma (column 3). Note that the large opaque zone annuli around cuticular extract wells are due to the acid (pH 4.0) effect of cuticular extracts; they should not be confused with antibacterial zones of the cuticular extracts, which are transparent and less diffuse.

Induction of Antibacterial Activity in Axenic Silkworms. Limitations on the number of axenic larvae forced the restriction of this experiment to procuticular abrasion. Purified microbial cell wall components such as LPS and insoluble and soluble peptidoglycan consistently induced antibacterial activity in the abraded cuticle and hemolymph plasma, whereas laminarin rarely induced any antibacterial activity and dextran T 10 induced no antibacterial activity (Table 1). Axenic silkworms that had received only an application of apyrogenic water following procuticular abrasion showed no response. These results demonstrate that purified bacterial cell wall components applied to the sterile abraded procuticle can induce antibacterial activity in the procuticle and in the hemolymph.

Identification of Molecules Responsible for Cuticular Antibacterial Activity. Because the antibacterial activity was limited to the cuticle after epicuticular abrasion, we next determined the chemical nature of these antibacterial molecules in the cuticle of *B. mori* in order to develop molecular probes and test whether the epithelial cells were involved in the induction of molecules conferring the cuticular antibacterial activity.

High-pressure liquid chromatography, peptide mapping, and amino acid sequencing of cuticular antibacterial molecules confirmed that they were indeed cecropins (W.-J.L. and P.T.B., to be reported elsewhere). No antibacterial activity was detected in cuticular extracts when neutral or basic extraction buffers were employed.

Northern Blot Hybridization. We tested whether the epithelial cells were involved in an immune response by looking for cecropin mRNA following epicuticular abrasion. North-

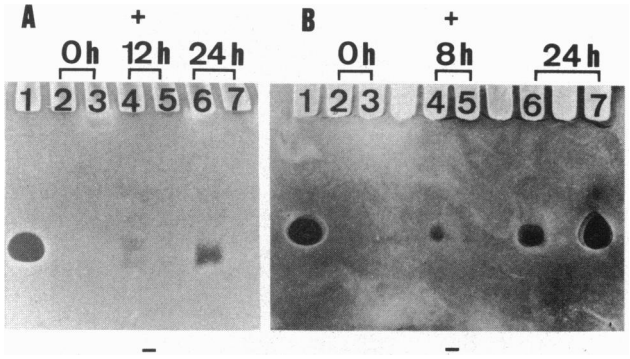

FIG. 3. Time course of the appearance of antibacterial activity in cuticular extract and hemolymph following an epicuticular abrasion in the presence of *E. cloacae* (*A*) or a procuticular abrasion in the presence of *E. cloacae* (*B*). (*A*) Synthetic cecropin A (2.5 μg) (lane 1), cuticular extract (lane 2) and hemolymph (lane 3), 0 hr postabrasion; cuticular extract (lane 4) and hemolymph (lane 5), 12 hr postabrasion; cuticular extract (lane 6) and hemolymph (lane 7), 24 hr postabrasion. One-hundred and fifty micrograms of protein per lane was used for cuticular extract and hemolymph samples. (*B*) Synthetic cecropin A (2.0 μg) (lane 1); cuticular extract (lane 2) and hemolymph (lane 3), 0 hr postabrasion; cuticular extract (lane 4) and hemolymph (lane 5), 8 hr postabrasion; cuticular extract (lane 6) and hemolymph (lane 7), 24 hr postabrasion. Sixty micrograms of protein per lane was used for cuticular extract and hemolymph samples. Native samples were run at 200 V toward the cathode (−) in a 15% polyacrylamide gel (pH 4.3).

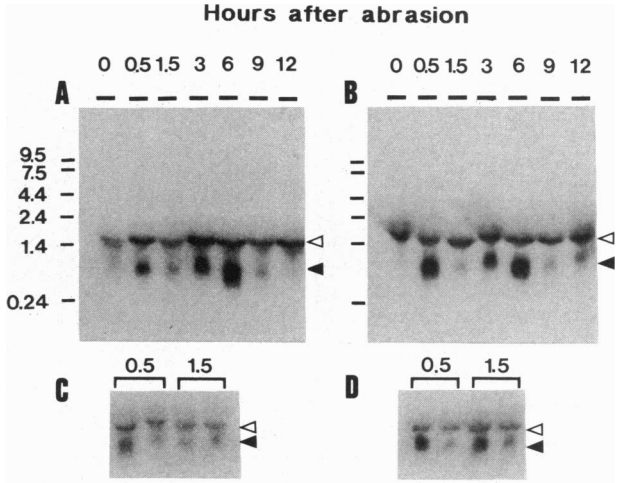

FIG. 4. Kinetics of the induction of cecropin transcripts following an epicuticular abrasion in the presence of *E. cloacae*. Total cellular RNA (50 μg) from pooled epithelial cells (*A* and *C*) and from pooled fat body tissue (*B* and *D*) was analyzed by Northern hybridization. The blots were probed simultaneously with random-primed cecropin Bm cec B24 and α-tubulin cDNAs as described in the text. The constitutively expressed α-tubulin mRNA served as an internal standard. An RNA ladder (Bethesda Research Laboratories) was used for the size marker (in kilobases).

ern blots, in which Bm cec B24 cDNA was used to probe mRNA extracted from pooled epithelial cells and pooled fat body tissue, showed that both tissues synthesized cecropin mRNA as early as 0.5 hr postabrasion and for a duration of 9–12 hr, with the highest number of transcripts at 6 hr postabrasion (Fig. 4 *A* and *B*). The early induction response (0.5 and 1.5 hr postabrasion) in both tissues was more or less intense (Fig. 4 *C* and *D*). Some silkworms seemingly respond more rapidly than others. However, no variation among pools was observed after 1.5 hr postabrasion.

Even though the fat body contained numerous cecropin transcripts, the absence of cecropin antibacterial activity in the hemolymph of the lightly abraded larvae is not clear at present.

DISCUSSION

The prime function of any immune system is to offer protection against the natural flora of microorganisms, especially bacteria of the host organism (4). Endogenous antibacterial peptides, such as cecropins, defensins, magainins, etc., are essential components of the immune systems of invertebrates and vertebrates (4, 6, 18). In insects, the injection of bacteria induces the synthesis of such antibacterial peptides in fat body and hemocytes (5). However, the responses that have been elicited by the injection of bacteria may not reflect a naturally occurring phenomenon. An epicuticular abrasion, a deep cuticular scratch, or the loss of an appendage is much more likely to occur during the insect's short life-span. Furthermore, the outer surface of the insect cuticle is constantly populated with an abundant bacterial flora (19, 20). If the epicuticle is compromised as in our experiments, this bacterial flora comes into contact with the cuticular matrix. Bacterial cell wall components presumably infiltrate the cuticular matrix and reach putative receptors on the epithelial cell surface, thus initiating a sequence of events leading to the appearance of antibacterial activity in the cuticle.

Janeway (21) emphasizes that primitive organisms, which have few lymphoid cells, consist mainly of surface epithelia

Table 1. Induction of bactericidal activity in the hemolymph plasma and cuticle of axenic silkworms

Treatment	No. of larvae	Antibacterial activity (zone of inhibition >1 mm)			
		Detected		Not detected	
		Plasma	Cuticle	Plasma	Cuticle
NA	16	0	0	16	16
A	24	0	0	24	24
NA + LPS	8	0	0	8	8
A + LPS	8	8	8	0	0
NA + insoluble peptidoglycan	24	0	0	24	24
A + insoluble peptidoglycan	24	24*	24*	0	0
NA + soluble peptidoglycan	8	2*	0	6	8
A + soluble peptidoglycan	8	8*	8*	0	0
NA + laminarin	8	0	0	8	8
A + laminarin	16	3*	0	13	16
NA + dextran T 10	8	0	0	8	8
A + dextran T 10	16	0	0	16	16

NA, nonabraded; A, abraded.
*Inhibition zones indicating bactericidal activity were not entirely transparent.

and that it is necessary for each of their cells to be capable of generating immune effector mechanisms. Such cells must possess three characteristics: polyspecificity, self–nonself discrimination, and rapid-response kinetics (21). The rapid induction of cecropin mRNA in the cuticular epithelial cells and the presence of active peptide in the abraded cuticle of silkworm larvae show that these cells possess these three characteristics, supporting Janeway's hypothesis. Furthermore, our observations indicate a highly localized antibacterial response rather than one involving the entire organism.

Our results further demonstrate that the degree of abrasion provokes a differential response in epithelial cells and fat body in terms of antibacterial activity due to cecropin, whereas the amount of cecropin mRNA is quasi-identical in both tissues. These results may suggest that the immune effector response, in this case cecropin antibacterial activity, is determined by whether the insect cells receive a specific bacterial signal, such as LPS/peptidoglycan or some other unknown component. Trauma—i.e., epicuticular abrasion—may be sufficient to induce cecropin mRNA transcription, but translation into an active peptide may necessitate an LPS or peptidoglycan signal. One could also speculate that no cecropin activity can be detected in the hemolymph following an epicuticular abrasion due to posttranscriptional regulation of cecropin synthesis or transport from the fat body, or perhaps a modification of mature cecropin by inhibition or degradation in the hemolymph, which would mask or destroy its antibacterial activity. A decade ago, Boman *et al.* (22) made observations along these lines when comparing the response to an immune challenge (live bacterial injection) versus that to an injury (sterile salt solution injection) in *H. cecropia* pupae. Not only did immune and injured pupae produce similar amounts of total RNA but also, when translated *in vitro*, "injury" RNA gave rise to protein patterns similar to those obtained with "immune" RNA. However, no P9 (= cecropin) activity was detected *in vitro*. On the contrary, P9 antibacterial activity was high *in vivo* in the immune pupae but was lacking in the injured pupae (22). No explicit explanation was given for the absence of P9 antibacterial activity in injured larvae (22). More recently, using Northern blot analysis following the injection of bacteria into *Manduca sexta*, Dickinson *et al.* (23) demonstrated the presence of cecropin D-like mRNA transcripts not only in fat body but also in several other tissues, including epidermal cells. However, these authors did not detect antibacterial activity in tissues other than fat body. It remains to be elucidated whether the mRNA for the cecropin D-like peptides in the various tissues was translated into active peptide. Dimarcq *et al.* (24) found that a sterile injury given to maggots of *Phormia terranovae* reared under strictly axenic conditions can effectively induce the transcription of defensin and diptericin genes, suggesting that local disruption of the integument aspecifically initiates a signaling mechanism that fat body and hemocytes can interpret. They did not mention, however, whether the mRNA transcripts were translated into active peptides after sterile injury.

From the observations of Boman *et al.* (22) and Dickinson *et al.* (23), together with the data presented in this study, we can speculate that the mechanisms and molecules involved in the induction of insect cecropin mRNAs may not necessarily be the same as those required for the synthesis of the mature peptide or for the appearance of antibacterial activity. Epicuticular abrasion in the presence of bacteria rather than bacterial injection may provide a model for elucidating the sequence of events involved in the defense reaction. This in turn would give us a better understanding of the mechanisms and molecules that are responsible for cecropin gene transcription and those responsible for posttranslational events leading to the mature peptide and antibacterial activity.

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1. Wigglesworth, V. B. (1974) *The Principles of Insect Physiology* (Chapman & Hall, London).
2. Metchnikoff, E. (1892) *Leçons sur la Pathologie Comparée de l'Inflammation* (Masson, Paris).
3. Boman, H. G. (1986) *Immune Mechanisms in Invertebrate Vectors* (Oxford Science, Oxford).
4. Boman, H. G. (1991) *Cell* **65**, 205–207.
5. Boman, H. G., Faye, I., Gudmundsson, G. H., Lee, J.-Y. & Lidholm, D.-A. (1991) *Eur. J. Biochem.* **201**, 23–31.
6. Hoffman, J. A. & Hetru, C. (1992) *Immunol. Today* **13**, 411–415.
7. Pasteur, L. (1870) *Etudes sur la Maladie des Vers à Soie* (Gauthier-Villars, Paris).
8. Brey, P. T., Ohayon, H., Lesourd, M., Castex, H., Roucaché, J. & Latgé, J.-P. (1985) *Comp. Biochem. Physiol.* **82A**, 401–411.
9. Akari, Y., Nakatani, T., Nakayama, K. & Ito, E. (1972) *J. Biol. Chem.* **247**, 6312–6322.
10. Yoshida, H., Ochiai, M. & Ashida, M. (1986) *Biochem. Biophys. Res. Commun.* **141**, 1171–1184.
11. Hultmark, D., Engström, A., Bennich, H., Kapur, R. & Boman, H. G. (1982) *Eur. J. Biochem.* **127**, 207–217.
12. Gabriel, O. (1971) *Methods Enzymol.* **22**, 565–578.
13. Hultmark, D., Steiner, H., Rasmuson, T. & Boman, H. G. (1980) *Eur. J. Biochem.* **106**, 7–16.
14. Auffray, C. & Rougeon, F. (1980) *Eur. J. Biochem.* **107**, 303–314.
15. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
16. Taniai, K., Kato, Y., Hirochika, H. & Yamakawa, M. (1992) *Biochim. Biophys. Acta* **1132**, 203–206.
17. Kalfayan, L. & Wensink, P. C. (1981) *Cell* **24**, 97–106.
18. Lehrer, R. I., Ganz, T. & Selsted, M. E. (1991) *Cell* **64**, 229–230.
19. Brey, P. T., Latgé, J.-P. & Prevost, M. C. (1986) *J. Invertebr. Pathol.* **48**, 34–41.
20. Latgé, J.-P., Sampedro, L., Brey, P. T. & Diaquin, M. (1987) *J. Gen. Microbiol.* **133**, 1987–1997.
21. Janeway, C. A., Jr. (1992) *Immunol. Today* **13**, 11–16.
22. Boman, H. G., Boman, A. & Pigon, A. (1981) *Insect Biochem.* **11**, 33–42.
23. Dickinson, L., Russell, V. & Dunn, P. E. (1988) *J. Biol. Chem.* **263**, 19424–19429.
24. Dimarcq, J.-L., Zachary, D., Hoffmann, J. A., Hoffmann, D. & Reichhart, J.-M. (1990) *EMBO J.* **9**, 2507–2515.