Haemocytes secrete basement membrane components in embryonic locusts

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Summary

Several monoclonal antibodies raised against a glycoprotein-enriched fraction of adult muscle membranes of Locusta migratoria selectively stain particles within haemocytes and basement membrane in developing locust embryos. Haemocytes containing immunoreactive particles are found associated with areas where basement membrane is being laid down. The underlying ectoderm does not show immunoreactivity. We conclude that haemocytes contribute to basement membrane formation in embryonic locusts.

Key words: Locusta migratoria, haemocyte, basement membrane, monoclonal antibody.

Introduction

There is a long-standing controversy as to whether haemocytes secrete basement membrane components in insects. Lazarenko (1925) was the first to suggest that they do, and Wigglesworth (1933, 1956, 1973, 1979) has published a series of papers presenting increasingly convincing evidence for such a role in Rhodnius. Ashhurst, however, who has done considerable research on insect haemocytes, has written several recent reviews (1979, 1982, 1985) in which she finds the arguments for participation of haemocytes in basement membrane formation unconvincing. Gupta (1985) lists other papers relevant to the controversy and has summarized the evidence for the two opposing points of view.

In the insect literature the term 'basement membrane' has been used for extracellular layers of connective tissue both with and without formed elements such as fibrils. In an attempt to arrive at a more uniform usage Ashhurst (1979, p. 326) has defined a basement membrane as 'a thin amorphous layer, between 50 and 80 nm thick, which is found under the basal surface of the epithelial cells and around muscle cells'. The layer of material that we here refer to as basement membrane fits this description except that it is usually thicker than 80 nm.

We here present immunocytochemical and ultrastructural evidence that haemocytes do contribute directly to basement membrane formation in the embryonic locust, Locusta migratoria.

Materials and methods

A membrane fraction from the metathoracic femoral muscles of adult Locusta migratoria was prepared by sucrose gradient centrifugation and integral membrane components were solubilized by 1% Triton X-100. A glycoprotein-enriched fraction was obtained by affinity chromatography over a Concanavalin A-sepharose column. Material thus purified was injected into BALB-c mice for production of monoclonal antibodies (Koehler & Milstein, 1975). Hybridomas were cloned once by limiting dilution. For screening of hybridoma culture supernatants whole embryos, staged according to the criteria of Bentley, Keshishian, Shankland & Toroian-Raymond (1979), were dissected free of yolk and fixed in 2% paraformaldehyde for approximately 20 min. The supernatant solutions were then screened on these whole-mount embryos, using techniques described in Ball, Ho & Goodman (1985), at 5% intervals from 30 to 60% of embryonic development. Immunoreactivity was detected using secondary antibodies labelled with either fluorescein isothiocyanate (FITC) or horseradish peroxidase (HRP). Control embryos were routinely run with an antibody of known specificity. The monoclonal antibodies were also tested on frozen sections of adult metathoracic femur.

Embryos were fixed for light and electron microscopy for 1–2 h on ice in a mixture of 2% paraformaldehyde, 2.5% glutaraldehyde and 0.0025% CaCl2 in Millonig's phosphate buffer...
Fig. 1. Whole mount of a 30 % embryo of *Locusta migratoria* in which the basement membrane lining the pleuropodia, which are organs lying behind the metathoracic legs, stains intensely with FITC (arrows). Scattered haemocytes (out of focus) are also stained. The embryo has been opened in the dorsal midline (bright longitudinal strips) to facilitate penetration of the antibodies into the coelomic space. Bar, 100 μm.

Fig. 2. In the metathoracic leg of a 40 % whole-mount embryo many haemocytes, containing HRP-labelled darkly staining granules, are associated with a dark-staining basement membrane (arrows). Nomarski optics. ect, ectoderm. Bar, 30 μm.

Fig. 3. In the prothoracic leg of a 35 % whole-mount embryo haemocytes containing immunoreactive particles are present and the basement membrane has begun to stain with FITC. Bar, 30 μm.

Fig. 4. Three haemocytes containing FITC-staining particles are here shown in association with the basement membrane lining the ectoderm of the antenna in a 55 % whole-mount embryo. ect, ectoderm. Bar, 10 μm.

Fig. 5. All of the haemocytes in the pleuropodia of a 40 % whole-mount embryo contain HRP-stained immunoreactive granules. The basement membrane is also stained. Nomarski optics. ect, ectoderm. Bar, 10 μm.
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Fig. 6. Semithin transverse section of a pleuropodium. Three of the four haemocytes in its lumen (arrows) are attached to the basement membrane lining the ectoderm. Bar, 20 μm.

Fig. 7. Electron micrograph of a haemocyte in contact with the ectoderm (ect) of a pleuropodium at 42% of embryonic development. Note abundant basement membrane (bm) between the haemocyte and the ectoderm and the similarity in appearance between one class of intracellular structure within the haemocyte (large arrowheads) and the basement membrane. An example of a second class of inclusion which might be immunoreactive is marked with a small arrow. Bar, 1 μm.

Fig. 8. Basement membrane (bm) apparently being laid down between a haemocyte (h) and the underlying ectoderm (ect). Putative immunoreactive material is enclosed within a cisterna of rough endoplasmic reticulum (large arrowhead). A small vesicle can be seen apparently emptying by exocytosis from the haemocyte onto the basement membrane (small arrowhead). m, mitochondrion. Bar, 0.5 μm.

Fig. 9. A large vesicle, containing many smaller vesicles, apparently emerging from a haemocyte (h) into the space where basement membrane is being laid down adjacent to the ectoderm (ect). Bar, 0.25 μm.
were cut using glass knives and stained with toluidine blue. The association occurs throughout the body.

were able to examine thin sections with the assurance that any cells found in the lumen of the pleuropodia were viewed clearly using differential interference contrast optics and all appear to contain immunoreactive granules (Fig. 5).

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Results

Four hybridoma cell lines that produced monoclonal antibodies (MAb) specific for particles within haemocytes and for basement membrane (Figs 1–5) of embryonic locusts were isolated. In addition to their embryonic specificity the antibodies also strongly and specifically bound to the margins of muscle fibres (presumably to basement membrane) and nerve (presumably to neural lamella) in frozen sections of adult femoral tissue, providing further evidence that the antigens are localized in connective tissue.

At 30% of embryonic development two of the monoclonal antibodies (HM1B3 and HM2C9) stain only the inside surface of the pleuropodia and a few haemocytes scattered throughout the coelomic cavity (Fig. 1), while MAb HM3A12 stains only haemocytes. At this same stage MAb Co3H12 stains both haemocytes and adjacent areas of basement membrane which are now appearing on the inner wall of the ectoderm.

By 40% of embryonic development immunoreactive haemocytes are abundant and many are located against the inner wall of the ectoderm where basement membrane is now staining more intensely. The association occurs throughout the body (Figs 2–5) but we chose to study it ultrastructurally in the pleuropodia (embryonic organs located posterior to the metathoracic legs) for three reasons. First, judging by immunoreactivity, the amount of basement membrane lining the pleuropodia appears to be greater than anywhere else in the embryo at 40–45% of embryonic development. Second, haemocytes are abundant within the pleuropodia during this same interval, and third, the haemocytes there can be viewed clearly using differential interference contrast optics and all appear to contain immunoreactive granules (Fig. 5).

Because the pleuropodia appear to contain a uniform population of haemocytes at this stage we were able to examine thin sections with the assurance that any cells found in the lumen of the pleuropodia would contain immunoreactive granules. Fig. 6 shows a transverse section of a pleuropodium with haemocytes in its lumen. As shown in Fig. 7, such cells contain only two types of intracellular structures that appear to be of the correct size and distribution to correspond to the immunoreactive particles seen in whole-mount preparations. The more common of these inclusions (Fig. 7) is located within cisternae of rough endoplasmic reticulum (Fig. 8) and appears identical to the basement membrane in morphological appearance and staining properties. The other type of inclusion stains more darkly and contains granular matrix material (Fig. 7). Fig. 8 shows a small vesicle apparently emptying by exocytosis from a haemocyte into the area where basement membrane is being laid down between the haemocyte and the ectoderm. In Fig. 9 a large vesicle, containing many smaller vesicles, is shown apparently emerging from a haemocyte into the area of basement membrane deposition.

Discussion

Several investigators have applied histochemical methods to study the composition and development of the extracellular matrix in insects. Although our antibodies are presumably more specific than the periodic acid-Schiff (PAS) stain used by Wigglesworth (1956) our findings are basically similar to his (1956, 1973, 1979): i.e. (a) haemocytes are found adjacent to the basement membrane at times and locations where it is apparently being laid down, (b) particles within the haemocytes stain the same as the basement membrane, and (c) the haemocytes can be seen apparently releasing material onto areas where basement membrane is forming.

Ashhurst (personal communication) has pointed out that histochemical studies on adult locust haemocytes (Costin, 1975) failed to detect any of the substances that are present in the connective tissue matrix surrounding the ejaculatory duct of the adult locust. Although we have not biochemically characterized the antigens against which our antibodies were raised it is likely from the way they were prepared that they represent glycoconjugates that are commonly found in basement membranes of both adult and embryonic tissue. Detergents were employed throughout the staining procedures to ensure that the antibodies penetrated cell membranes, but the only intracellular sites exhibiting immunoreactivity were the particles within the embryonic haemocytes. No binding of antibodies was observed in the ectoderm and it thus appears that this specific component of the basement membrane is exclusively produced by the haemocytes. It is, however, quite possible either that this class of haemocytes or the particles within them are only found in embryonic locusts.

Ashhurst (personal communication) has read our paper and concludes that...either the antibodies are
recognizing the same epitope on different molecules or that they are recognizing an epitope on a molecule secreted by the blood cell which is being transferred to the underlying epithelial cell, via the basement membrane. In answer to the first point, the epitope is present on molecules in haemocytes and basement membrane but not in the epithelial cells, which, aside from the haemocytes, would be the other likely source of immunoreactive material in the basement membrane. This combination of coincidences appears to us unlikely, especially in view of the fact that, judged by differences in the time and pattern of onset of staining by the monoclonal antibodies, we are probably dealing with more than one immunoreactive epitope. The evidence against Ashhurst's second suggestion is that we have never seen any immunoreactivity within the epithelial cells.

We conclude that although much of the basement membrane is clearly secreted by adjacent epithelial cells (Ashhurst, 1979, 1982, 1985) the haemocytes do make a significant direct contribution to at least certain areas of basement membrane during embryonic development of Locusta migratoria.

Although Dr Doreen Ashhurst does not agree with our conclusions we are grateful for her comments, which have resulted in a better paper. We thank Aleksandra Plazinska for help in raising the monoclonal antibodies.

References


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