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1. Lyon, M. F., Cattanach, B. M. & Charlton, H. M. in *Mechanisms of Sex Determination in Animals and Man* (eds Austin, C. R. & Edwards, R. G.) 329–386 (Academic, New York, 1981).
2. Eicher, E. M. in *Prospects for Sexing Mammalian Sperm* (eds Amann, R. P. & Seidel, G. E. Jr) 121–135 (Colorado Associated University Press, Boulder, 1982).
3. Dickie, M. M. *Mouse News Lett.* **32**, 43–44 (1965).
4. Bennett, D. B. *Cell* **6**, 441–454 (1975).
5. Johnson, D. R. *Genetics* **76**, 795–805 (1974).
6. Johnson, D. R. *Genet. Res. Camb.* **24**, 207–213 (1975).
7. Eicher, E. M., Beamer, W. G., Washburn, L. L. & Whitten, W. K. *Cytogenet. Cell Genet.* **28**, 104–115 (1980).
8. Eicher, E. M., Washburn, L. L., Whitney, J. B. III & Morrow, K. E. *Science* **217**, 535–537 (1982).
9. Babiarz, B., Garrisi, G. T. & Bennett, D. *Genet. Res. Camb.* **39**, 111–120 (1982).
10. Simpson, J. L. *Disorders of Sexual Differentiation*, 141–155 (Academic, New York, 1976).
11. Ohno, S. *Monogr. Endocr.* **11**, 91 (1979).
12. Forejt, J. & Iványe, P. *Genet. Res. Camb.* **24**, 189–206 (1975).
13. Hammerberg, C. *Genet. Res. Camb.* **27**, 71–77 (1981).
14. Theiler, K. *The House Mouse*, 100–104 (Springer, New York, 1972).
15. Simpson, E., Chandler, P., Washburn, L. L., Bunker, H. P. & Eicher, E. M. *Differentiation* (in the press).
16. Whitten, W. K., Beamer, W. G. & Byskov, A. G. *J. Embryol exp. Morph.* **52**, 63–78 (1979).

Basement membrane polarizes lectin binding sites of *Drosophila* larval fat body cells

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Vertebrate epithelial cells in monolayers are asymmetrical in that their apical and basal membranes differ in morphology and function[1–3]. That this cell polarity depends on the presence of tight junctions can be demonstrated by labelling one surface of a cell monolayer in culture with fluorescent lectins and lipid probes, and subsequently observing whether the labels disperse to the opposite cell surfaces[4,5]. Here we report on a differential distribution of binding sites for the lectin wheat germ agglutinin (WGA) on the cells of *Drosophila melanogaster* larval fat body, and show that the pattern is correlated with the structural association between the cell surfaces and their overlying basement membrane.

The larval fat body of *D. melanogaster* is bilaterally symmetrical, and is organized in a continuous sheet one cell thick. This sheet of adipose cells is covered by extracellular matrix, basement membrane, and is suspended in the haemocoel. Cell shape and its topography with respect to the basement membrane depend on the position of the fat body cell within the sheet[6,7]. Cells located centrally are columnar, with polygonal surfaces apposing the basement membrane, whereas cells from the tissue margins are wedge-shaped with at least three sides covered by basement membrane (Fig. 1*a–c*). In addition to these, the cells forming the commissures that are chains of single cells have a roughly cylindrical shape and only their two ends that are in contact with neighbouring cells in the string are not covered by basement membrane while a cell located at a distal tip of the tissue is toe-shaped, with all its surfaces covered by basement membrane except that surface linking it to its neighbour in the tissue. Thus, each fat body cell has some surface that makes contact with other fat body cells while its remaining surfaces facing the haemocoel are covered by basement membrane.

When freshly dissected larval fat bodies in Ringer solution are treated with fluorescein isothiocyanate-conjugated WGA (FITC-WGA), the tissue surfaces light up with fluorescent specks (Fig. 2*a*). If the FITC-WGA is pretreated with chitobiose, its haptenic sugar, it fails to bind to the fat body surfaces. To determine whether FITC-WGA was bound to the basement membrane, to the cell surfaces beneath it, or to both, we treated fat bodies on microscope slides with FITC-WGA, rinsed them with distilled water, placed a coverglass over the tissues, and applied gentle pressure, squeezing the cell contents from the lipid-laden cells to free the basement membrane sheets. The coverglass was then lifted slightly and the membranes thoroughly flushed with several changes of distilled water to remove cellular debris. Such preparations showed intense fluorescence in the areas overlying cell surfaces and only slight fluorescence in the regions corresponding to intercellular spaces (Fig. 2*b*). This differential distribution of WGA binding suggests either that basement membrane overlying cell surfaces contains more lectin binding sites than intercellular regions or that the cell membranes which also contain WGA binding sites remain adherent to the basement membrane during preparation of the samples. The latter possibility is the more likely because osmotic disruption of the cells obviously releases their internal contents but may not be effective in dislodging the cell membranes from the basement membrane.

To examine the distribution of lectin binding sites on fat body cell surfaces free of basement membrane, we first reacted the fat bodies with FITC-WGA and then dissociated the cells using collagenase[8]. Each dissociated cell retained the shape that it originally had in the intact tissue. We presume this result to be due to the high concentration of lectin used, as we previously found[9] that 50–200 $\mu g\ ml^{-1}$ of WGA immobilizes (fixes) surface structures of *Drosophila* cells in culture whereas low (5–10 $\mu g\ ml^{-1}$) concentrations distort cell shape. The surfaces of the dissociated fat body cells that had been covered by basement membrane remained intensely fluorescent while the lateral surfaces that had been in contact with neighbouring cells lacked fluorescence (Fig. 2*c*). These dissociated cells were re-treated with FITC-WGA to determine whether the lateral surfaces contain binding sites that were inaccessible to the lectin in the intact tissue. Second lectin treatment did not alter the fluorescence patterns; no additional WGA binding was noticeable at

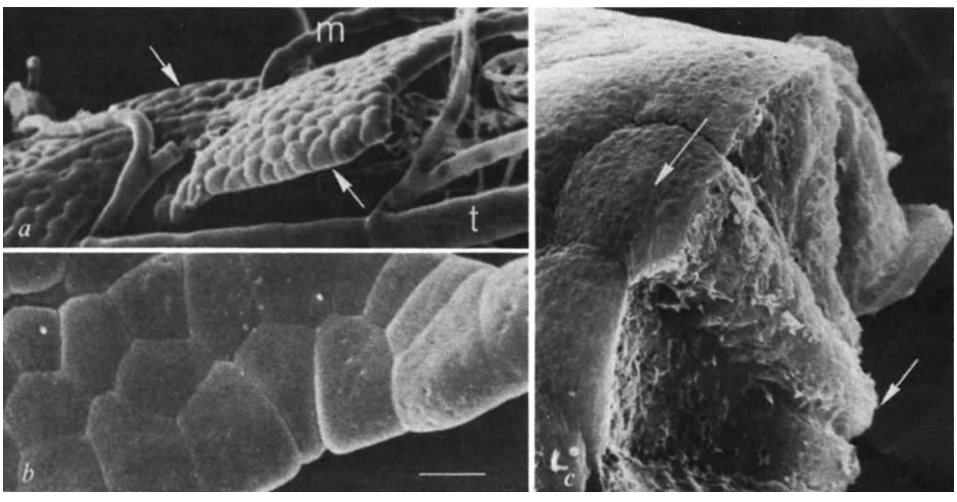

Fig. 1 *a*, A scanning electron micrograph of a portion of larval fat body illustrating a sheet of adipose cells (arrows); trachea, t; malpighian tube, m. *b*, A slightly higher magnification of another region of fat body showing the shape of cells in the centre and at the margins of the tissue. Scale bar, 50 μm. *c*, View of an intercellular face of fat body fractured by a micromanipulator interfaced with the scanning electron microscope[7]. The arrows indicate the two cell surfaces covered by basement membrane.

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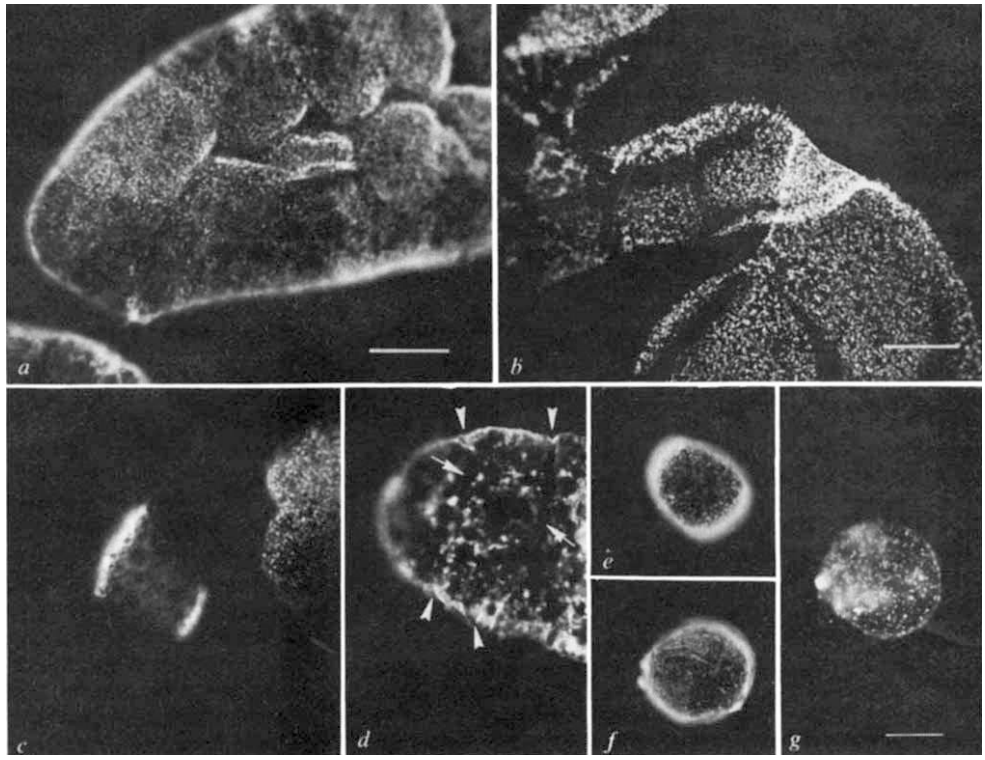

Fig. 2 *a*, FITC-WGA binding at the surfaces of an intact region of fat body, comparable to that in Fig. 1*b*. Freshly dissected tissue was rinsed with Ringer solution and treated with 100 $\mu g\,ml^{-1}$ FITC-WGA in Ringer at room temperature for 3 min. The outlines of the individual cells are apparent. Scale bar, 50 μm. Same magnification for *b–d*. *b*, FITC-WGA binding to a basement membrane preparation of a commissure region obtained by squashing and rinsing freshly dissected tissue to remove intracellular contents. WGA binding is concentrated over cellular regions and sparse at intercellular regions. *c*, Lateral view of a cell isolated from fat body that had been treated with FITC-WGA before incubation with collagenase. WGA binding is limited to the cell surfaces formerly covered by basement membrane and facing the haemocoel. The orientation of this cell corresponds to that of the indicated cell in Fig. 1*c*. The polar view of three cells, to the right, compares with the surface view of cells in tissue in *a*. *d*, Frozen section of a fat body cell (arrowheads) showing FITC-WGA binding at its two external surfaces and not its internal surfaces (arrows). This cell, unlike the bulk of the cells in the frozen sections, is sufficiently separated from its neighbours for the intercellular margins to be clearly visible. Intracellular binding also occurs in sectioned material. *e*, A fat body cell which was first isolated by treating the tissue with collagenase and then reacted with FITC-WGA. The fluorescent halo is an optical effect which is not so pronounced in *f* which shows the same cell after it has been rolled over and slightly flattened. FITC-WGA is dispersed over the cell surfaces and not limited to some surfaces as in *c* and *d*. *g*, A fat body cell from a pupa shows FITC-WGA binding sites as small specks over its surface. Large intracellular protein globules are autofluorescent. Scale bar, 50 μm; same magnification for *e* and *f*.

the intercellular surfaces exposed by dissociating previously labelled fat body.

The preceding observations suggest that most of the lectin binding sites of the cell surfaces are concentrated at those surfaces facing basement membrane. It is, however, possible that exposure to collagenase and the subsequent washing required to remove the enzyme modifies WGA binding sites. Therefore, frozen sections of fat body were first lightly fixed in 2% buffered paraformaldehyde and then treated with FITC-WGA to assure that lateral surfaces and exterior surfaces were simultaneously exposed to the lectin. Figure 2*d* confirms that WGA binding sites are more heavily concentrated at the cell surfaces in apposition to basement membrane.

To visualize the distribution of WGA binding sites over the cell surfaces and basement membrane as well as to establish that WGA penetrates the basement membrane and binds to the underlying cell surfaces, we treated fat bodies with peroxidase-conjugated WGA (P-WGA) or ferritin-conjugated WGA (F-WGA), and examined epon sections in the transmission electron microscope. As a control for the specificity of WGA binding, fat bodies were incubated with P-WGA or F-WGA that had been pretreated with chitobiose. The peroxidase reaction was intense at the cell surfaces and basement membrane but negative in the controls (Fig. 3*a*, *b*). Likewise, ferritin particles were distributed in the basement membrane and the underlying cell surfaces, but none were found in the controls, excluding the possibility of nonspecific entrapment of ferritin in fat body basement membrane. An additional control was omission of osmium post-fixation and staining of sections with vanadatomolybdate[10] rather than uranyl acetate–lead citrate. No apparent differences in basement membrane ultrastructure were detected among the groups, but fixation of intracellular components was poor when osmic acid was omitted.

The basement membrane surrounding the fat body of a mid-third instar larva is 40–60 nm thick. At low magnification it appears amorphous, but at higher magnifications (×50,000–100,000) a fibrillar network can be seen. Its mesh-like nature is best viewed in tangential sections which also reveal that fine fibrillar attachments extend from the fat body cell surfaces into the basement membrane net (Fig. 3*c*). Figure 3*c*, *d* also illustrates the disposition of F-WGA in the basement membrane network and on the surfaces of the underlying fat body cell membranes. The surfaces of fat body cells beneath basement membrane are highly convoluted[11], and ferritin particles are distributed along all these surfaces.

During the pupal period, basement membrane of the larval fat body is dissolved and the dissociated individual adipose cells round up and disperse within the pupal haemocoel[12]. When these dissociated cells are washed with Ringer solution and treated with FITC-WGA, the binding sites appear as minute fluorescent specks scattered over the surfaces (Fig. 2*g*). None of the cells from the pupa show an asymmetrical distribution of binding sites, suggesting that reorganization of the fat body cell surface occurs when the cells are freed from the tissue masses. This reorganization, which involves cell shape, must also include redistribution of the lectin binding sites at the cell surface.

Dissolution of fat body basement membrane and rounding of the free cells can be mimicked *in vitro* by incubating freshly dissected larval fat bodies with collagenase and thoroughly rinsing the freed cells with Ringer solution[8]. When experimentally rounded fat body cells are treated with FITC-WGA, their surfaces light up in a pattern resembling that of pupal cells (Fig. 2*e*, *f*). Fluorescent specks are distributed over the cell surfaces rather than asymmetrically concentrated in some areas.

In conclusion, our studies demonstrate that *Drosophila* larval fat body cell membranes are polarized, as indicated by a heavy concentration of WGA binding sites on those surfaces covered by basement membrane and facing the haemocoel. We suggest

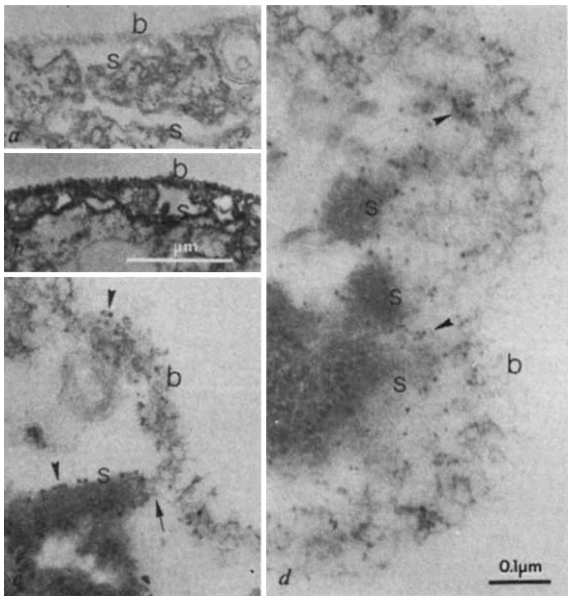

Fig. 3 *a*, A transmission electron micrograph (TEM) of fat body treated with P-WGA that had been preincubated with chitobiose. This is the control for *b*; basement membrane, b; fat body cell surface, s. Scale given in *b*. *b*, TEM of fat body treated with P-WGA showing the intense peroxidase reaction in the basement membrane and the cell surface. Note also that the fat body cell surfaces are highly convoluted, a factor that may account for the speckled appearance of the cells treated with FITC-WGA, because at some points, such as the surfaces marked s in *a*, there will be a triple layer of FITC-WGA excited by UV light and viewed from above. *c*, TEM of a small region of fat body surface treated with F-WGA. This selected area illustrates fine fibrous connections (arrow) between the fat body cell surface, s, and the overlying basement membrane, b. The arrowheads indicate some of the ferritin particles in the basement membrane and on the cell surface. Magnification same as *d*. *d*, A tangential section of basement membrane treated with F-WGA showing the fibrillar network constituting an open structure. The arrowheads point to some of the ferritin particles. The electron-dense material to the left is through the surface, s, of the fat body cell. Tissue fixed in buffered formaldehyde–glutaraldehyde; section stained in vanadatomolybdate.

that a structural association of the cell membrane and the overlying basement membrane stabilizes this orientation of cell-surface molecules. When the basement membrane is lost during the pupal stage, the cytoskeleton must rearrange as the cells assume a spherical shape. Concomitant with this reorganization is the loss of cell-surface polarity as it exists in the intact tissue and a redistribution of lectin binding sites of the cell surface. Note also that pretreatment of fat body tissue with WGA must immobilize cytoskeletal elements underlying the cell surfaces so that the fat body cells dissociated subsequently retain their original shapes.

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1. Pisam, M. & Ripoche, P. *J. Cell Biol.* **71**, 907–920 (1976).
2. Cereijido, M., Ehrenfeld, J., Meza, I. & Martines-Palomo, A. *J. Membrane Biol.* **52**, 147–159 (1980).
3. Sang, Ü. H., Saier, M. H. & Ellisman, M. H. *Expl Cell Res.* **122**, 384–391 (1979).
4. Dragsten, P. R., Blumenthal, R. & Handler, J. S. *Nature* **294**, 718–722 (1981).
5. Dragsten, P. R., Handler, J. S. & Blumenthal, R. *Fedn Proc.* **41**, 48–53 (1982).
6. Rizki, T. M. in *The Genetics and Biology of Drosophila* Vol. 2b (eds Ashburner, M. & Wright, T. R. F.) 561–601 (Academic, London, 1978).
7. Rizki, T. M., Rizki, R. M., Allard, L. F. & Bigelow, W. C. in *Scanning Electron Microscopy/1976/II* (eds Johari, O. & Becker, R. P.) 611–618 (IIT Research Institute, Chicago, 1976).
8. Rizki, R. M. & Rizki, T. M. *Wilhelm Roux Arch. dev. Biol.* **189**, 207–213 (1980).
9. Rizki, R. M., Rizki, T. M. & Andrews, C. A. *J. Cell Sci.* **18**, 113–121 (1975).
10. Callahan, W. P. & Horner, J. A. *J. Cell Biol.* **20**, 350–356 (1964).
11. Gaudecker, B. von *Z. Zellforsch.* **61**, 56–95 (1963).
12. Rizki, R. M., Rizki, T. M., Bebbington, C. R. & Roberts, D. B. *Wilhelm Roux Arch. dev. Biol.* **192**, 1–7 (1983).

T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy

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In some subjects the infective agent of leprosy, *Mycobacterium leprae*, causes disseminated (lepromatous) disease. Such subjects have a major role in the transmission of the disease and show deficient T-cell responses both *in vivo* and *in vitro* to *M. leprae*, but not to other antigens[1–4]. Numerous studies have recently shown that T cells with functional capabilities after initial triggering with antigen can be maintained in a state of continuous proliferation *in vitro* when cultured in medium containing interleukin 2 (IL-2)[5–8]. Here we have studied the effect of IL-2 rich T-cell conditioned medium on lepromatous peripheral blood mononuclear cells. Our results show that although lepromatous T cells fail to produce IL-2 after exposure to *M. leprae* they can respond by proliferation to *M. leprae* in the presence of T-cell conditioned medium, suggesting that the unresponsiveness in lepromatous leprosy results from a deficiency in the production of IL-2 or related factors and not a lack of *M. leprae*-reactive T cells.

As shown in Table 1, peripheral blood mononuclear cells (PBMC) of lepromatous patients [4 with the borderline form (BL) of the disease, 13 with the lepromatous (LL) form], responded poorly, as expected, to *M. leprae* in culture. The mean stimulation index (SI) was 1.13 ± 0.15. However, the specificity of this unresponsiveness is shown by their response to purified protein derivative (PPD) (SI mean 5.41 ± 1.47). The background ^{3}H-thymidine incorporation varied and was very high in some patients, for example patients 8 and 9. The reason for this remains unclear, but may be related to bacterial load, as these two patients had the highest bacteriological index (BI) in the group. T-cell conditioned medium (TCM) alone failed to stimulate DNA synthesis in most of the patients. On the otherhand, when TCM was added with *M. leprae*, the SI increased in all the patients (mean 8.95 ± 2.28). In patients 3, 8, 9 and 11, this increase was due to a decrease in background and is therefore of questionable significance, but in the others it was due to an increased response to TCM+*M. leprae*. Moreover, in 13 patients (3 BL, 10 LL) TCM also clearly increased the response to *M. leprae* as compared with *M. leprae* alone.

These observations do not appear to be due to phytohaemagglutinin (PHA) present in TCM, because control supernatants prepared from PBMC in the absence of PHA, but reconstituted with PHA in concentrations analogous to that of TCM, were found to be without effect.

As shown in Table 2, lepromatous patients also failed to produce IL-2 in response to *M. leprae*. The effect of TCM and *M. leprae* on PBMC from subjects not exposed and not responsive to *M. leprae* alone was also studied. As shown in Table 3, such subjects (Norwegian blood donors) remained unresponsive to *M. leprae* in the presence of TCM. Thus, the effect observed

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