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# Birbeck granules, worm-like bodies and "lupus erythematosus virus"

*Background:* The origin of worm-like bodies and their relationship with Birbeck granules is poorly understood. *Objectives:* To clarify the origin of worm-like bodies and their relationship with Birbeck granules. *Materials and methods:* Over 800 electron micrographs of histiocytic disorders and several appendage tumours were reviewed in order to check for worm-like bodies and Birbeck granules. *Results:* Worm-like bodies were most often encountered in mildly- to moderately-proliferative histiocytic tumours. Birbeck granules were observed in more malignant conditions. Narrow endoplasmic reticulum (Nrer), which resembles worm-like bodies, was abundant in worm-like body-rich cells and coexisted with Birbeck rods. Nrer is thought to be one of the candidates that gives rise to worm-like bodies. An ultrastructural similarity exists between worm-like bodies (and octopus body formation) and the so-called "lupus erythematosus virus". *Conclusion:* The presence of Nrer is often concomitant with other organelle markers, and could be a candidate for the origin of worm-like bodies.

**Key words:** worm-like body, Birbeck granule, histiocytic tumor, narrow endoplasmic reticulum, Nrer

The Birbeck granule (BG) is the hallmark organelle of normal Langerhans cells and moderately malignant cells of histiocytic lineage, such as those of Letterer-Siwe's disease. On the other hand, benign histiocytic proliferation usually does not involve BG, but rather so-called "worm-like bodies" (WLB) and comma bodies. Examples are benign cephalic histiocytosis, generalized eruptive histiocytosis, and certain cases of juvenile xanthogranuloma [1] (*table 1*). The organelle marker for the diagnosis of congenital self-healing reticulohistiocytosis (CSHRH) is the BG [2], despite its benign nature (however, CSHRH may sometimes be malignant).

The distinction of histiocytoses as benign or malignant subtypes by means of organelle markers has been feasible because this rule of division has been generally observed and seldom transgressed. During the routine examination of a specimen of desmoplastic trichoepithelioma, we found a large histiocytic cell which contained BG and WLB. Since such hybrid cells are rarely observed, we surveyed their prevalence, origin, and the significance of their association with BG. In contrast with the huge volume of research on Langerhans cells, the ultrastructure and function of WLB have been poorly studied and are not well known. The aim of this study was to present several new aspects of WLB.

## Materials and methods

A firm, skin-coloured, deep-seated nodule on the face of a 35-year-old white man was excised and immediately processed for epon-embedding. Glutalaldehyde and osmium tetroxide solutions were used for fixation, and lead acetate and uranyl acetate for staining the ultrathin sections. In view of the finding of a hybrid cell in this trichoepithelioma, several appendage tumours were examined for the presence of hybrid cells. Standard electron micrographs of various histiocytic tumours and autoimmune connective tissue diseases were also reviewed for comparison with specific organelles.

## Results

### Hybrid cell

The hybrid cell was a large histiocytic cell containing BG, WLB, and/or comma bodies (*figure 1*). These organelles were normal per se; for example, BG were not different in size or shape from those of normal Langerhans cells. The bulb, rod, and the combination of both (*figures 2, 3*) are

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**Table 1.** Frequency of occurrence of specific organelles.

| Diseases | Birbeck granule | Worm-like body | Nrer | LE virus |
|---|---|---|---|---|
| Normal skin | 10/10 | 0/10 | 0/10 | 0/10 |
| Histiocytic proliferation | | | | |
| Benign cephalic histiocytosis | 0/3 | 3/3 | 3/3 | 0/3 |
| Generalized eruptive histiocytosis | 0/3 | 3/3 | 3/3 | 0/3 |
| Eosinophilic granuloma | 0/3 | 3/3 | 3/3 | 0/3 |
| Congenital self-healing reticulohistiocytosis | 3/3 | 1/3 | 3/3 | 0/3 |
| Letterer-Siwe's disease | 3/3 | 0/3 | 3/3 | 0/3 |
| Juvenile xanthogranuloma (rf. 1) | | + | | |
| Inflammatory granulomas | | | | |
| Granuloma annulare | 0/3 | 1/3 | 3/3 | 0/3 |
| Appendageal tumours | | | | |
| Trichoepithelioma | | | | |
| Desmoplastic type | 1/3 | 1/3 | 1/3 | 0/3 |
| Ordinary type | 0/3 | 0/3 | 0/3 | 0/3 |
| Syringoma | 0/3 | 0/3 | 0/3 | 0/3 |
| Tubular apocrine adenoma | 0/3 | 0/3 | 0/3 | 0/3 |
| Basal cell carcinoma | 0/3 | 0/3 | 0/3 | 0/3 |
| Autoimmune connective tissue diseases | | | | |
| Systemic lupus erythematosus | 0/3 | 0/3 | 0/3 | 3/3 |
| Dermatomyositis | 0/3 | 0/3 | 0/3 | 3/3 |
| Systemic sclerosis | 0/3 | 0/3 | 0/3 | 3/3 |
| Sjögren's syndrome | 0/3 | 0/3 | 0/3 | 3/3 |

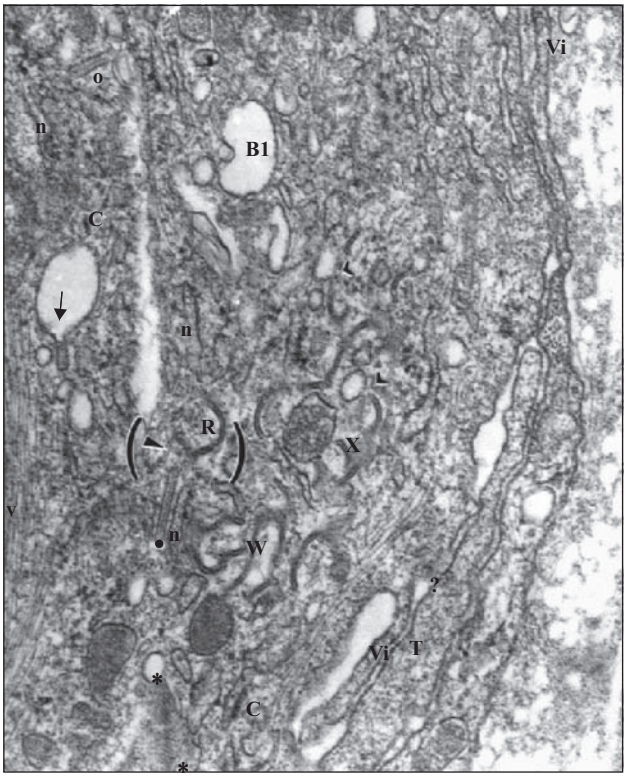


**Figure 1.** A hybrid cell from desmoplastic trichoepithelioma. Several constituents of hybrid cells, such as Birbeck granules and worm-like bodies (W), including comma bodies (C), are observed. The Birbeck granule can present as a simple rod (o), combined with a bulb (arrow), or as a simple bulb (B1). When the rod portion is cut perpendicular to the limiting membranes and through the dense centre layer, it appears grid-like or shows simple parallel striations (*) (*figure 3*). Close positioning of the tubular part of the worm-like body or, more often, the comma body and a vacuole is observed (⌞). Several comma bodies associate to form an octopus body (X) (*figure 4*). Narrow endoplasmic reticula (Nrer) (n) runs alone or parallel (o) with a Birbeck granule rod (•). This hybrid cell contains all three organelles, i.e. Birbeck granules, worm-like bodies, and Nrer. V: vimentin cytoskeleton; Vi: villus; arrowhead within brackets: close positioning of the ring form worm-like body (R) and the rod of a Birbeck granule (•); n: narrow endoplasmic reticulum; W: worm-like body. T: microtubule; ?: formation of Birbeck granule.

common features in normal Langerhans cells. The increased number and the increased activity in proliferative histiocytic tumours leads to other rare granules. In particular, the morphology of the rod part can change. The hybrid cell was not a tumour cell, but a cell in a proliferative environment; it could produce ill-defined sheets of striations (*figures 1-3*). The rod measured 40 nm between two limiting membranes. The size of the bulbs was extremely variable (*figure 1*). These features allowed us to define this organelle as a BG. This hybrid cell also contained WLB and comma bodies (*figure 1*), organelles not observed in normal histiocytes. WLB are curved tubular structures. Comma bodies are short WLB, usually consisting of one single curved tubule (*figure 1*, *figure 4*). The tubular portions are narrower (20-25 nm) than the BG rod (40 nm) (*figure 1*, *table 2*). The bulbs of BG are much larger than those of WLB. Clear attachment of these vacuoles to the tubules of WLB or comma bodies is always dubious (*figure 1*). When a part of a tubular body puffs out, exposing the inside, it appears as a "cotton candy" (*figure 1*, *figure 4*). When a few to several tubules of WLB or comma bodies fuse or anastomose, octopus bodies are created (*figure 1*, *figure 4*). Cotton candy was seen in the rod of BG (*figure 5*), and the octopus body was formed by a "lupus erythematosus virus" (LEV) (*figure 6*).

## Birbeck granule

The ultrastructure of BG, especially the rod, is more complex than just a rod attached to the bulb. The images may be very different from one section to the next (*figures 2, 3*). This is probably because there are several sheets with a grid pattern, and the sheets roll around the central dense core. The core or central dense layer itself appears to be composed of a grid or lattice structure (*figures 2, 3*). With this basic construction of the rod, the bundled rods (*figure 2*) show a mountain ridge-like structure (*figures 1, 2*), where the number of rods can still be counted, e.g. four rods in bundle 1 and two in bundle 2. These are cut through the plane near the surface. Bundles 3 and 4 appear to show a deeper section plane profile of striations. Bundle 5 is cut

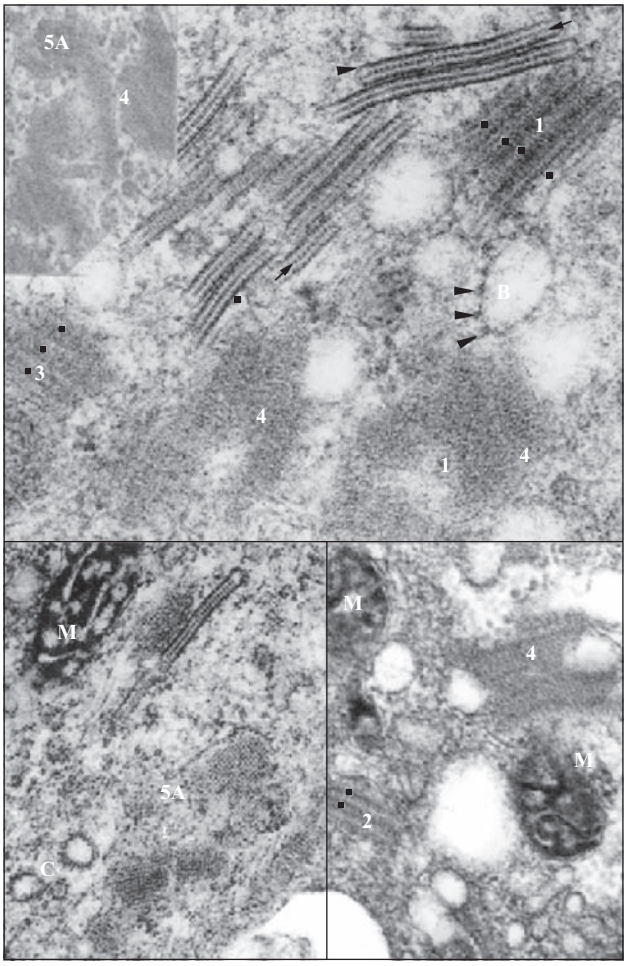


**Figure 2.** Langerhans cell histiocytosis. Birbeck granules show different profiles depending on the planes of section. When the section plane is parallel to the long axis of the rod and through the limiting membrane, i.e. the wall of bundled rods, the number of rods in the bundle can be counted (four rods in group 1, two rods in group 2, and three rods in group 3). Deeper sections expose striations in group 4 and a lattice or a grid pattern in 5A. The best known profile that allows Langerhans cells to be recognized is a rod with a central chain of dots (in a "zip-like" pattern), as in group 5 (arrows). This pattern is from a section plane that has been moved parallel to the limiting membranes (arrowheads) and through the middle of the central chain of dots (*figure 3*). One bulb (B) bears ribosomes (■) on its membrane. C: coated vesicle; M: mitochondria.

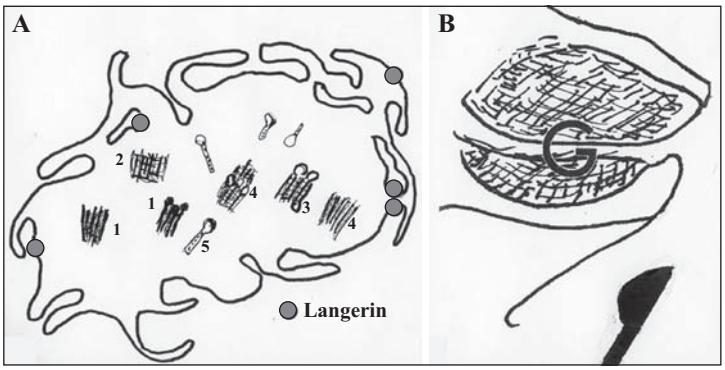


**Figure 3.** Congenital self-healing reticulohistiocytosis (CSHRH). **A)** Diagramatic drawing of various aspects of Birbeck granules. When this organelle is present, the cell is recognised as a Langerhans cell. The numbers correspond to those in *figure 2*. **B)** A grid pattern (G) of 5A, as shown in *figure 2*.

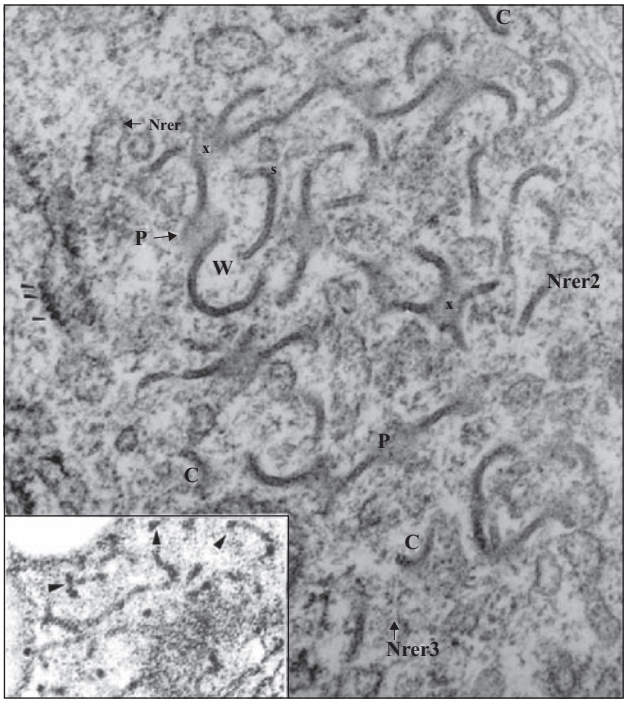


**Figure 4.** Worm-like bodies (W), comma bodies (C), puffy cotton candy-like swelling (P), octopus body (x), and narrow rough endoplasmic reticula (Nrer 1, 2, 3) with many (⌐) or only a few ribosomes. Arrowheads in the inset refer to the cross-section profile of tubules of worm-like bodies, revealing round "tubules" which are mostly not hollow. Ribosomes (20-25 nm) (⌐) serve as a size reference.

**Table 2.** The appearance of key organelles.

| Organelle | Diameter | Configuration | Length |
|---|---|---|---|
| Birbeck granule | 40 nm (rod) | Rod and bulb (tennis racket) | Variable |
| Worm-liked body | 20 nm | Coiled worm | Short to medium |
| Nrer | 20-80 nm | Thin RER or SER | Medium to long |
| LE virus | 20-25 nm | Anastomosing tubules | Short to medium |
| | Common ultrastructure | | |
| Collagen banding | 68 nm | Fibre | |
| Glycogen particles | 10-40 nm | Electron-dense round granule | |
| Ribosomes | 20-25 nm | Same as glycogen | |
| Plasma membrane | 8-10 nm | Sheet | |

perpendicular to the limiting membrane and through the centre; this pattern is the most frequent rod profile of BG. Bundle 5A is sectioned through the central dense layer and grid-like structure. These uncommon profiles are more frequently encountered in histiocytic tumours. In tumoural histiocytes, the production of Birbeck rods is probably increased, such that these rare structures are more likely to be observed.

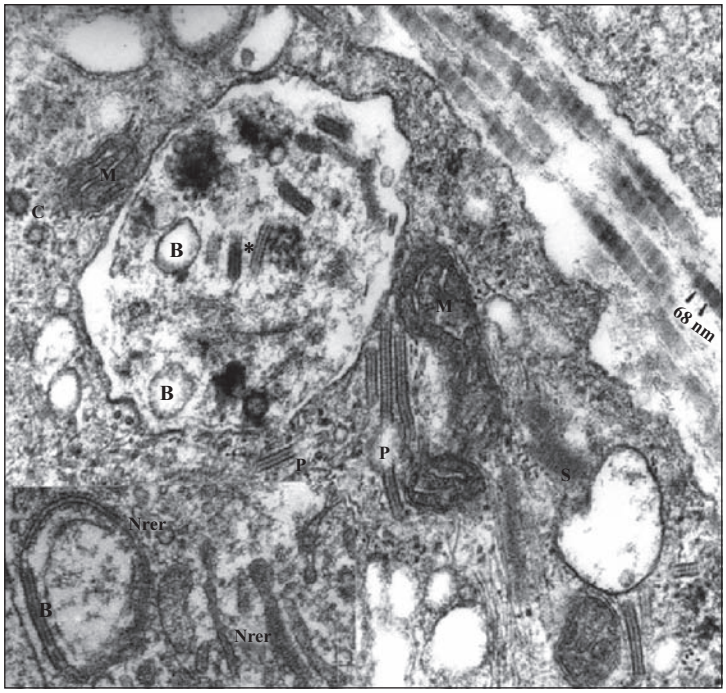


**Figure 5.** Congenital self-healing reticulohistiocytosis. Arrangement of Birbeck granules. In the centre, there is a large phagosome containing degenerated rods (*) and bulbs (B) of Birbeck granules. In the inset, a combination of a rod of Birbeck granules (B) and Nrer is shown. P: puffy swelling; s: striation pattern (*figure 2*, group 4). Collagen fibril shows 68-nm periodical banding, which serves as a reference. C: coated vesicle. M: mitochondria.

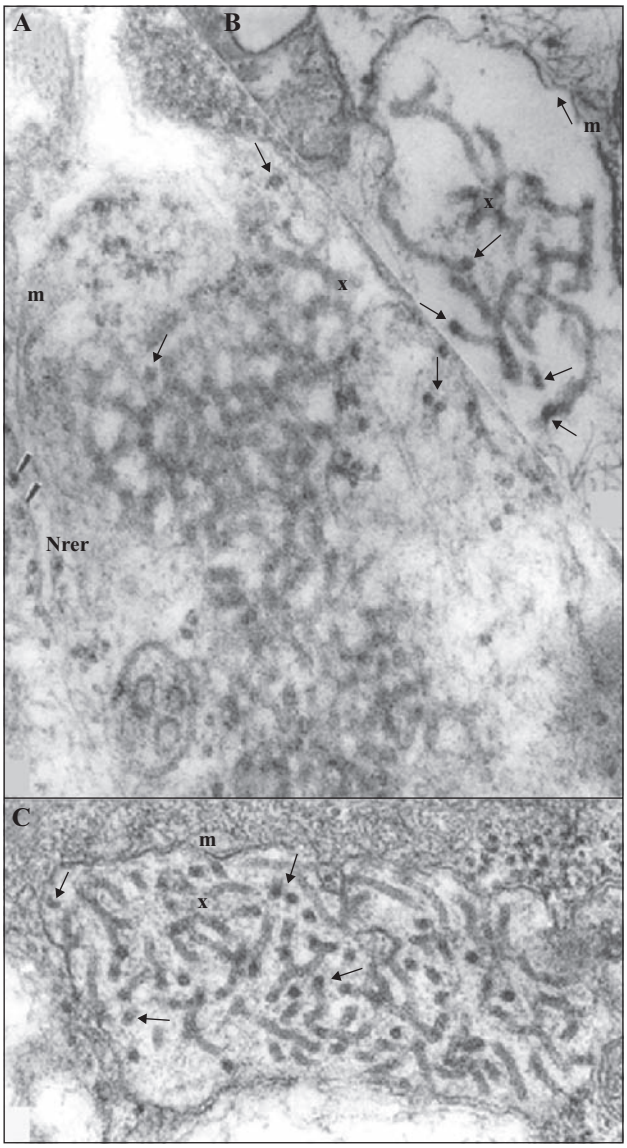


**Figure 6.** Systemic lupus erythematosus. "LE virus" in three different profiles. A) Mostly composed of tightly bound, anastomosing aggregations with a tubuloreticular aspect, surrounded by rough endoplasmic reticulum which is in continuity with Nrer (➚). B) Loosely aggregated tubular structures resemble worm-like bodies, as shown in *figure 4*; an octopus body is observed (x). The cross-section reveals that these "tubular" structures are mostly not hollow tubes but solid rods (arrows). C) Loosely aggregated tubular structures most closely resembling worm-like bodies (*figure 1*, inset of *figure 4*). The tubule diameters are 20-25 nm, the size of a ribosome (➚ in [A]) (*table 2*), and on cross-section, the tubules appear round and solid. m: mitochondria.

## Narrow endoplasmic reticulum (Nrer)

Straight linear, curved, comma-shaped and small ring-form endoplasmic reticula with narrow cisterna are abundant in some benign histiocytic tumours (*table 1*, *figures 7, 8*). Cisterna membranes are attached to a variable number of ribosomes, usually not abundant (*figures 1, 7, 8*). This special type of endoplasmic reticulum looks like WLB, particularly when the attached ribosomes are scant (*figures 7, 8*). The width between delimiting membranes is widely variable (20-80 nm), and some are thinner than BG rods (40 nm).

## The relationship between abnormal cytoplasmic structures

Nrer most often coexists with other organelles, such as Birbeck rods (*figures 7, 8*). Nrer may also be continuous

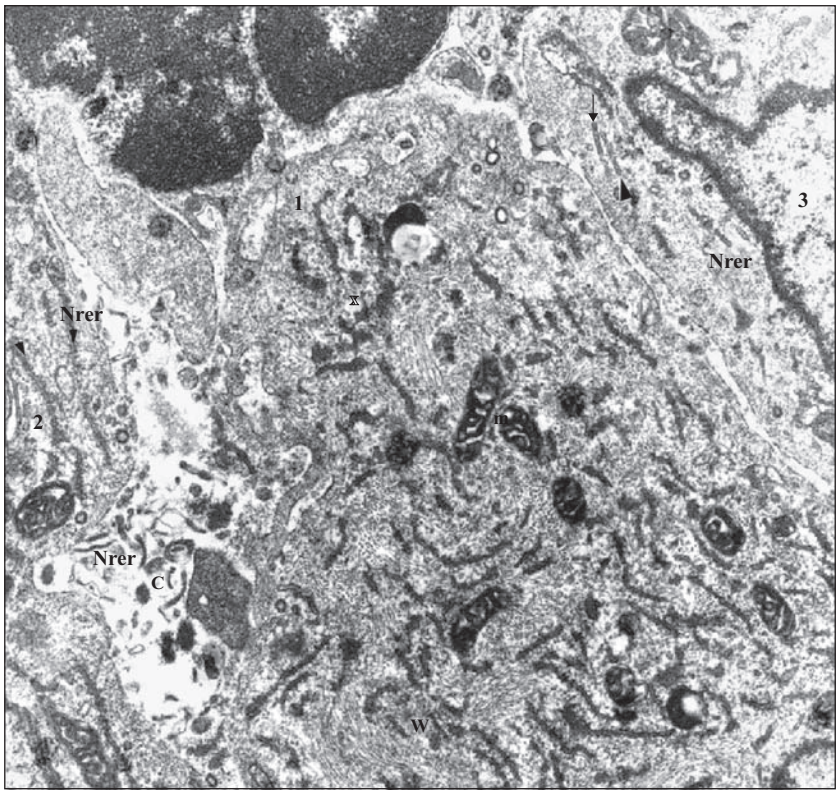


**Figure 7.** Congenital self-healing reticulohistiocytosis. Narrow rough-surface endoplasmic reticula (Nrer) are observed. There are three large histiocytic cells. Cell 1 is the largest and has a number of Nrer, similar to worm-like bodies and comma bodies (*figure 1*). The space between cells 1 and 2 contains cellular debris, comma bodies (c), and released Nrer. Cell 3 contains Nrer (arrowheads) and a structure similar to the rod portion of a Birbeck granule (arrow). The structure marked by x in cell 1 is similar to an octopus body shown in *figure 4*. m: mitochondria. w: worm-like body.

with the rods (*figure 5*). If we consider WLB as Nrer-related structures, the combination of Nrer and Birbeck rods (*figure 5*; insert) is not surprising.

### "Lupus erythematosus virus"

The so-called "lupus erythematosus virus" (LEV), also known as tubuloreticular inclusion bodies, is found in all autoimmune connective tissue diseases (*table 1*, *figure 6*). When the LEV is packed tightly within the endoplasmic reticula of small-to-medium-sized blood vessel endothelial cells, the resemblance is not immediately obvious. However, if they are loosely packed and anastomosing, even making octopus bodies (*figure 6*), the typical figure is recognised easily (compare *figure 6B* and *figure 4*). The cross sections show a solid dense profile (*figure 6*), like most WLB (*figure 4*).
The frequency of occurrence of specific organelles is summarised in *table 1*. The dimensions of key organelles are summarised in *table 2* in order to demonstrate their similarities.

## Discussion

The prevalence of the hybrid cell within histiocytic proliferation appears to be low, as we could not find another cell carrying typical BG or WLB, although we found many tumour cells with narrow, rough-surfaced endoplasmic reticula (Nrer) and WLB. This finding may warrant further investigation of Nrer as the origin of WLB. The genesis, ultrastructural morphology, and functions of WLB are poorly known, in contrast to that of BG, for which every aspect has been intensively investigated. The discovery of langerin [3, 4], a mannose-binding lectin which captures antigens falling onto the Langerhans cell membrane [5] and channels them to the antigen-processing pathway, confirmed our original proposal that BG are endocytotic organelles and not the product of the Golgi apparatus [6]. In this present study, we did not find WLB formation at the cell membrane, suggesting that these structures are not generated by cell membrane apposition. We can only speculate on several possibilities regarding their formation based on the following:

– (1) WLB are old, effete or damaged BG. Senescent BG are usually shed through the stratum corneum or dropped off into the dermis together with the host Langerhans cell. The damaged granules do not transform into WLB, but undergo autophagocytosis (*figure 5*).
– (2) WLB are derived from narrow endoplasmic reticula. The data in *figures 7 and 8* support this hypothesis which was already proposed by Caputo [1].
– (3) The LEV is a variant of a paramyxovirus. Certainly, the LEV closely resembles the paramyxoviridae family of

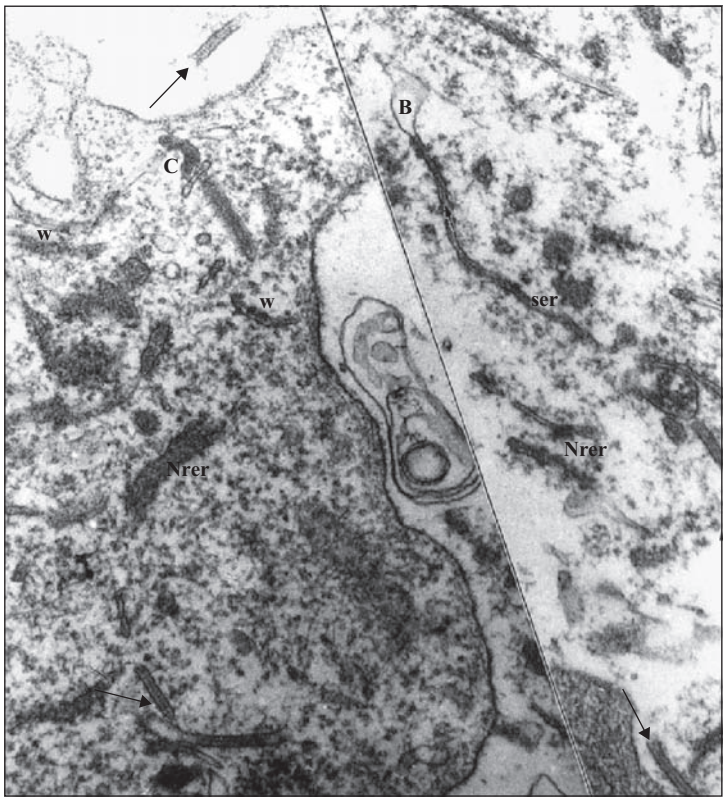


**Figure 8.** Congenital self-healing reticulohistiocytosis. This composite picture shows the coexistence of Nrer and Birbeck granule rods (arrow). Some Nrers are almost smooth-surfaced (ser) and attached to the bulb (B), while others resemble worm-like bodies (w) and comma bodies (c).

viruses ultrastructurally, such as the measles virus [7, 8], however, this is not supported by our own studies [9]. A comprehensive study of RNA paramyxoviridae performed for all major connective tissue diseases failed to detect any significant signals [9]. In addition, histiocytic proliferation is remote from the pathophysiology associated with connective tissue diseases. Despite the above, it is still possible that WLB represent some unknown paramyxovirus (LE virus) that has so far escaped detection.

One should not forget that the Langerhans cell was once considered as an effete melanocyte [10, 11]. Today, the Langerhans cell is at the centre of cutaneous immunology. Our present study has shown that WLB are consistent markers of mild-to-moderate proliferation of histiocytes, and as such, are expected to exert some function(s).

In conclusion, hybrid cells containing BG and WLB (or comma bodies) exist but are very rare. In histiocytic tumours, BG are usually markers of malignancy, whereas WLB are specific to mild-to-moderate histiocytic proliferation. For the detection of BG, one should consider several different presentations, ranging from the common tennis-racket configuration to a grid-like presentation. WLB also present different profiles, such as a cotton sugar-like appearance, puffy swelling, and octopus bodies. Narrow rough-surface endoplasmic reticulum (Nrer) could be related to WLB or even to the rods of BG. Morphologically, WLB and comma bodies resemble LEV, although the latter can be tubular. Nrer is the most frequent organelle in histiocytic proliferative disorders, and therefore often coexists with other organelle markers, such as BG rods and WLB; consequently, hybrid cells, containing various combinations of rod, WLB, comma body, and Nrer, are theoretically possible and are in fact observed. ■

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