Virchows Archiv A Pathol Anat (1990) 417:395–404

Virchows Archiv A
Pathological Anatomy and Histopathology

The ultrastructure of signet-ring cell non-Hodgkin's lymphoma

B.P. Eyden, P.A. Cross, and M. Harris

Department of Histopathology, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, UK

Received February 27, 1990 / Accepted May 31, 1990

Summary. New ultrastructural findings are reported from two lymphomas of vacuolar signet-ring cell morphology (SR^+), one of B cell and one of T cell lineage. When these lymphomas were compared ultrastructurally a difference in the relationship of the endoplasmic reticulum (ER) to the vacuole was noted, although the fine structure of the vacuoles themselves was similar and they were interpreted as giant multivesicular bodies (mvbs). Smooth ER was found near the vacuoles in both cases. Dark mvbs with a complex, reticulate form are emphasised as readily identified but hitherto unreported cell components in these tumours. A further B cell lymphoma of centroblastic/centrocytic type which was SR^- was found to be rich in mvbs and may be a transitional form between SR^- and SR^+ lymphomas. In addition, the occurrence of mvbs has been studied quantitatively in a number of other lymphomas and in B and T lymphocytes in reactive nodes. Although increased numbers of mvbs were found in neoplastic compared with reactive lymphocytes, and in T compared with B cell lymphomas, these differences were not statistically significant. The possible roles of endoplasmic reticulum and mvbs in the generation of SR^+ change are discussed.

Key words: Ultrastructure – Non-Hodgkin's lymphoma – Signet-ring cell – Endoplasmic reticulum – Multivesicular body

Introduction

The signet-ring cell (SR^+) appearance is a distinctive light microscopical phenomenon most often seen in adenocarcinomas where it is usually due to mucus granule accumulation (Ming 1971; Shousha 1982; Wong et al. 1986; Remmele et al. 1988), and sometimes to the presence of large intracytoplasmic lumina (McCarty and Paull 1983; Ro et al. 1988). The appearance has also been observed in rare examples of other tumours (Rubinstein 1972; Ramzy 1976; Cramer and Heggeness 1985; McCaughey et al. 1985; Sheibani and Battifora 1988), and it has been occasionally recorded in non-Hodgkin's lymphomas.

In SR^+ lymphomas, several variants have been described. In the Russell body type – so far seen only in B cell lymphomas – the appearance is due to the accumulation of immunoglobulin-containing cisternae of rough endoplasmic reticulum (rER) (Kim et al. 1978; van den Tweel et al. 1978). In the vacuolar type, which is seen in both B and T cell lymphomas (Kim et al. 1978; Grogan et al. 1985; Weiss et al. 1985; Cross et al. 1989), the cytoplasm is dominated by a large, membrane-limited vacuole. A third type is marked by the presence of granular/fibrillar material lacking an enclosing membrane (Navas-Palacios et al. 1983).

There are over 20 cases of vacuolar SR^+ lymphoma recorded in the literature, all but 4 of them of B cell type (Kim et al. 1978; van den Tweel et al. 1978; Vernon et al. 1979; Iossifides et al. 1980; Moir 1980; Harris et al. 1981; Pileri et al. 1981; Vernon 1981; Spagnolo et al. 1982; Navas-Palacios et al. 1983; Silberman et al. 1984; Stramignoni et al. 1984; Grogan et al. 1985; Weiss et al. 1985; Hanna et al. 1986; Tungekar 1986; Allevato et al. 1985; Cross et al. 1989). We have had the opportunity of studying a B and T cell example (Harris et al. 1981; Cross et al. 1989). In this paper we present a more detailed ultrastructural analysis of these two cases with new observations. By analysing both these and SR^- lymphomas, an attempt is made to clarify the roles of certain membranous organelles in the generation of the signet-ring cell phenotype.

Materials and methods

Twenty-two lymphomas were studied by conventional light microscopy, immunohistochemistry and electron microscopy as already described (Harris et al. 1981; Cross et al. 1989). These included two vacuolar SR^+ lymphomas and an mvb-rich SR^- lymphoma

Offprint requests to: B.P. Eyden

Table 1. Clinical details

Age (years)	*Sex*	*Site*	*Diagnosis*
Case 1			
58	Male	Left groin node	ML[a]; B cell; SR^+ (vacuolar type); cb/cc[b]
Case 2			
75	Male	Skin nodule, chin	Peripheral T cell lymphoma; SR^+ (vacuolar type)
Case 3			
43	Male	Retroperitoneal mass	ML; B cell; SR^- ("mvb rich"); cb/cc

[a] Malignant lymphoma
[b] Centroblastic/centrocytic

(Table 1). Two non-specifically reactive lymph nodes were also studied. The number of mvbs per cell was estimated by examination of 80–100 cells in each case.

Results

Vacuolar SR^+ lymphomas: cases 1 and 2

The B cell lymphoma (case 1) was classified as malignant lymphoma, follicular and diffuse, centroblastic/centrocytic using the Kiel system; centrocytes greatly outnumbered centroblasts. Case 2, after morphological and immunohistochemical studies, was classified as a low-grade cutaneous T cell lymphoma of signet-ring cell type.

As indicated in our preliminary data (Harris et al. 1981; Cross et al. 1989), SR^+ cells in both lymphomas had a comparable appearance which by light microscopy (Fig. 1, inset) consisted of a crescentic nucleus and a large, predominantly clear vacuole. By electron microscopy, this was limited by a single membrane (Fig. 1)

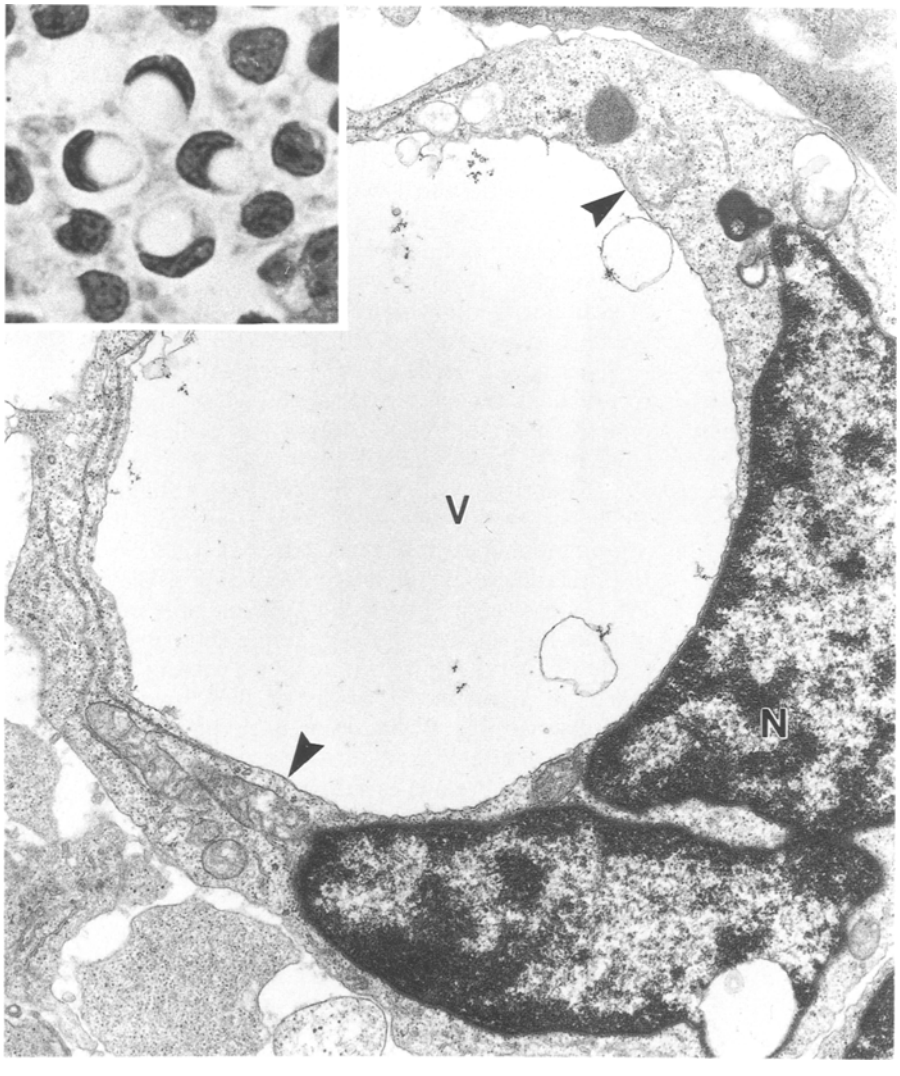


Fig. 1. Correlated light and electron microscopy of B cell vacuolar SR^+ lymphoma. *Inset*: Haematoxylin and eosin touch preparation (×1100). Electron micrograph: *N*, nucleus; *V*, vacuole limited by single membrane (*arrowheads*). ×13000

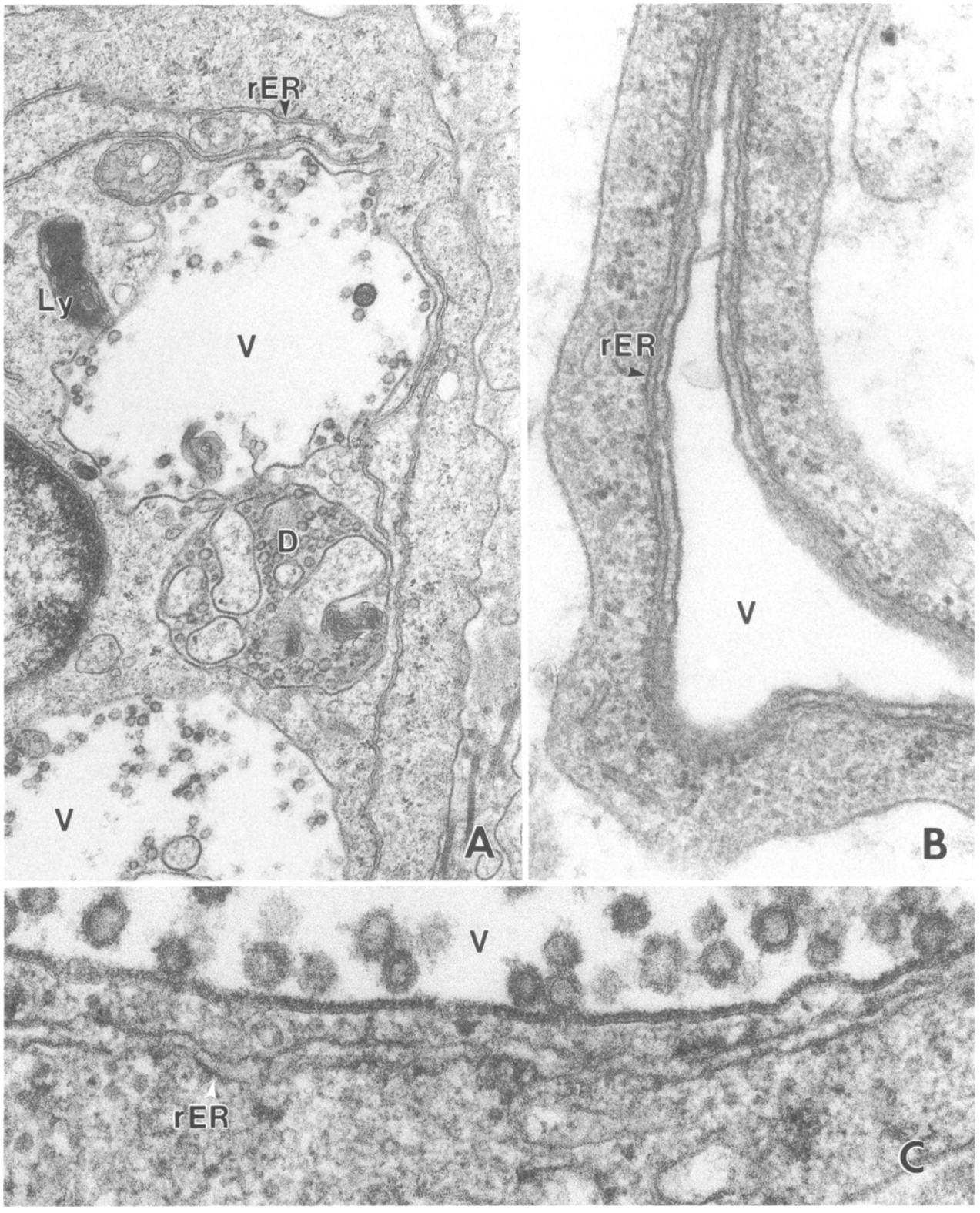


Fig. 2A–C. Rough endoplasmic reticulum (rER) in B and T cell vacuolar SR^+ lymphomas. **A, C** rER in T cell lymphoma. *Ly*, Dense lysosomal body; *V*, vacuole (light mvb); *D*, dark multivesicular body (mvb). ×40000 and ×133000 respectively. **B** Close rER-vacuole relationship in B cell lymphoma. *V*, Vacuole. ×70000

and enclosed varying numbers of microvesicles; these were few and peripheral in large vacuoles, and more numerous and evenly distributed in smaller examples. In case 1, large vacuoles were found only in centrocytes.

In both lymphomas, elements of rER were present (Figs. 2A, 5A). In the B cell tumour narrow cisternae of rER (possessing only a few ribosomes, however) exhibited intimate apposition to the surface of many of

the vacuoles (Fig. 2B). This rER-vacuole association was not seen in the T cell lymphoma, although an occasional rER cisterna was noted near the vacuole (Figs. 2A, 2C). Smooth endoplasmic reticulum (sER) in both cases was represented by aggregates containing reticulate profiles (Fig. 3A, B) and by more loosely organised collections of individual cisternae (Fig. 3C).

Mvbs, containing microvesicles and occasionally other membranous or unstructured material, were prominent in both cases. *Light* and *dark* forms were distinguishable, principally by different degrees of microvesicle packing. Light mvbs tended to have few or loosely arranged microvesicles (Figs. 2A, 5A). They varied from small, oval or rounded structures, about 200 nm across (Fig. 5C) to the large vacuole responsible for the SR^+ phenotype, the latter being interpreted as a light mvb of exceptional size and containing few microvesicles. Dark mvbs showed greater close-packing of microvesicles (Figs. 2A, 4A, B) than light forms and a similar size range, except for a slightly lower maximum. Striking reticulate dark mvbs were noted, in small numbers in the B cell tumour, but more prominently in the T cell lymphoma (Fig. 4A, B).

A number of membranous elements suggestive of early vacuole formation were observed. In the B cell lymphoma, assemblies of light mvbs near to small or moderately sized vacuoles (Fig. 5A), or groups of closely juxtaposed small vacuoles (Figs. 5B, 5C) were encountered in centrocytes, centroblasts and cells of intermediate morphology. In both B and T lymphomas, occasional groups of simple, rounded vesicles were identified, some of them near the Golgi (Fig. 5D).

Mvb-rich SR^- lymphoma: case 3

This was a follicular and diffuse centroblastic/centrocytic lymphoma. Tumour cells were divided into more or less cohesive masses (Fig. 6A) by prominent hyaline sclerosis (Fig. 6B). Centrocytes had cleaved nuclei, prominent dissected heterochromatin and nuclear pockets (Fig. 6C). Centroblasts, in far fewer numbers, had almost no heterochromatin. Cytoplasmic features were similar in both cell types. A third of centrocytes contained mvbs, which were numerous in some cells. They were of variable shape, about 200–600 nm across, and contained microvesicles 40–60 nm in diameter. Dark and light forms were present (Fig. 6D). Some lysosomal organelles containing microvesicles and lamellar profiles, and some dense residual bodies, were present.

Table 2. Numerical analysis of multivesicular bodies (mvbs) in lymphomas and reactive lymphocytes

	% of cells studied with mvbs (range)	Range of no. of mvbs/cell	Average no. mvbs/cell for cells containing mvbs (range)
B cell lymphomas ($n=15$)	8.44 (0.9–34.0)	1–5	0.15 (0.01–0.79)
T cell lymphomas ($n=7$)	23.7 (3.3–56.7)	1–36	0.69 (0.03–2.1)
Reactive B cells ($n=2$)	3.17 (1.18–4.0)	1–2	0.03 (0.01–0.06)
Reactive T cells ($n=2$)	0	0	0

Differences in the three parameters were not statistically different amongst the four populations

Mvb numbers in lymphomas and reactive lymph node tissue

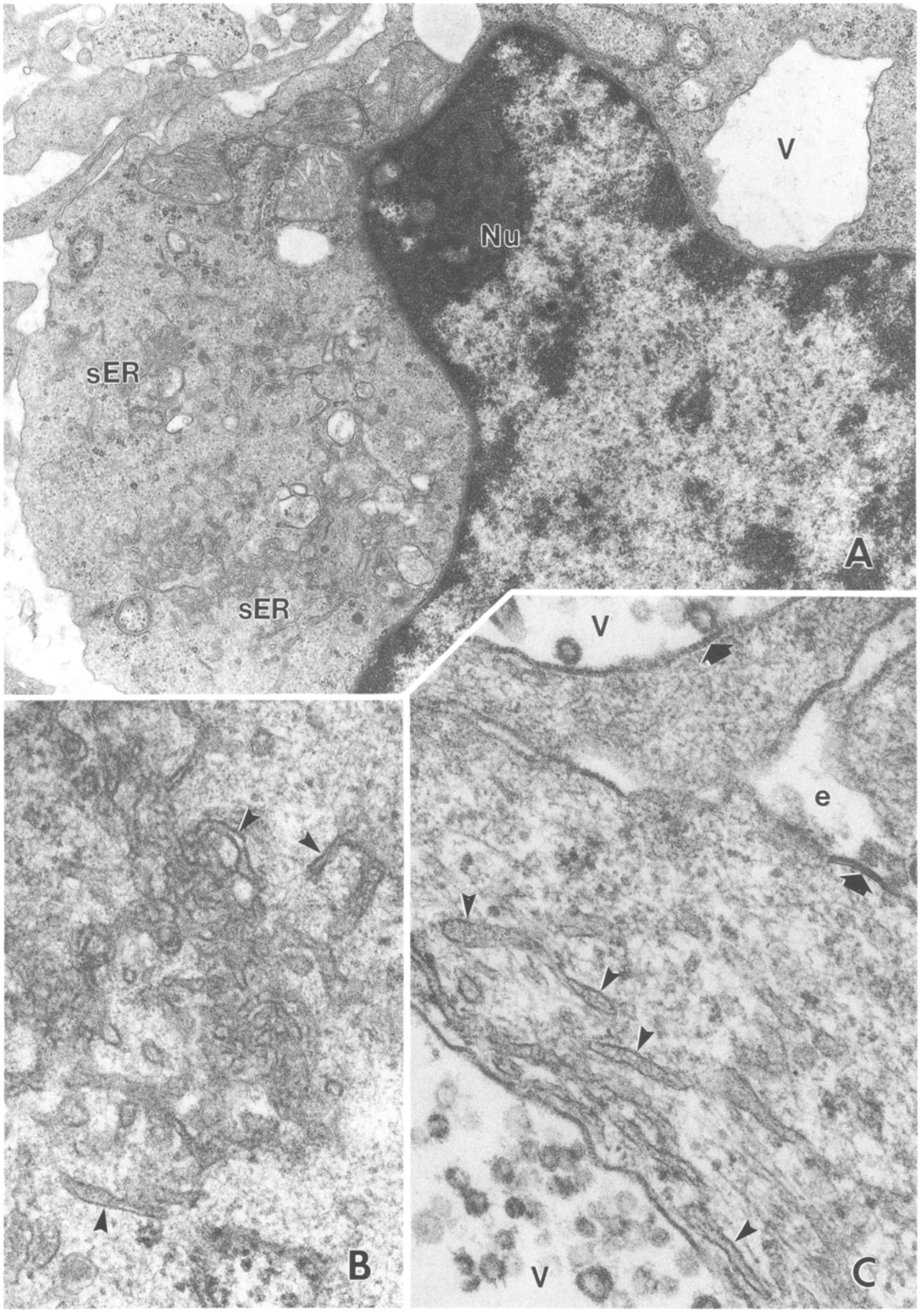
The occurrence of mvbs in this material is shown in Table 2. No distinction was made between light and dark forms.

Discussion

This paper records several novel observations on signet-ring lymphoma which add to the existing descriptive information available on these uncommon tumours.

It is clear that the organelle responsible for the Russell body SR^+ appearance is rER (Kim et al. 1978; Dardick et al. 1983; Navas-Palacios et al. 1983). In the vacuolar lymphomas, however, ultrastructural studies suggest that rER is not finally responsible and that the vacuoles are giant mvbs: nonetheless, elements of rER or sER may be functionally related to vacuole formation. One such element is the vacuole-associated rER cisterna seen in the B cell tumour. Although we have designated this as rER, it is unusual in having few attached ribosomes and therefore in being smooth to a large extent. On the other hand, it is solitary and substantial and in these respects it is unlike typical sER which, as seen in lipid metabolising cells and neurons for example (Fawcett 1986; Richard et al. 1989), is reticulate or more commonly dispersed as multiple discrete cisternae. This cisterna has not been documented in other cases of SR^+ lymphoma, although Navas-Palacios et al. (1983) have illustrated lamellar profiles in the vicinity of the vacuole which may also be ER. The involvement of the cisternae with small and angular (see our Fig. 2B) as well as *large* vacuoles discounts the idea that it is a passive accretion of growing vacuoles. Its restric-

Fig. 3. Smooth endoplasmic reticulum (sER) in B (**A, B**) and T (**C**) cell lymphoma. **A** A cell intermediate between a centrocyte and a centroblast (note some peripheral heterochromatin and prominent nucleolus, *Nu*) with sER aggregates and a small vacuole (*V*). ×20000. **B** Detail of sER from **A** showing smooth nature of cisternae and reticulate profiles (*arrowheads*). ×70000. **C** Individual smooth cisternae (*arrowheads*) near a vacuole (*V*). Note the similarity of vacuole membrane and plasmalemma (*fat arrowheads*). *E*, Extracellular space. ×70000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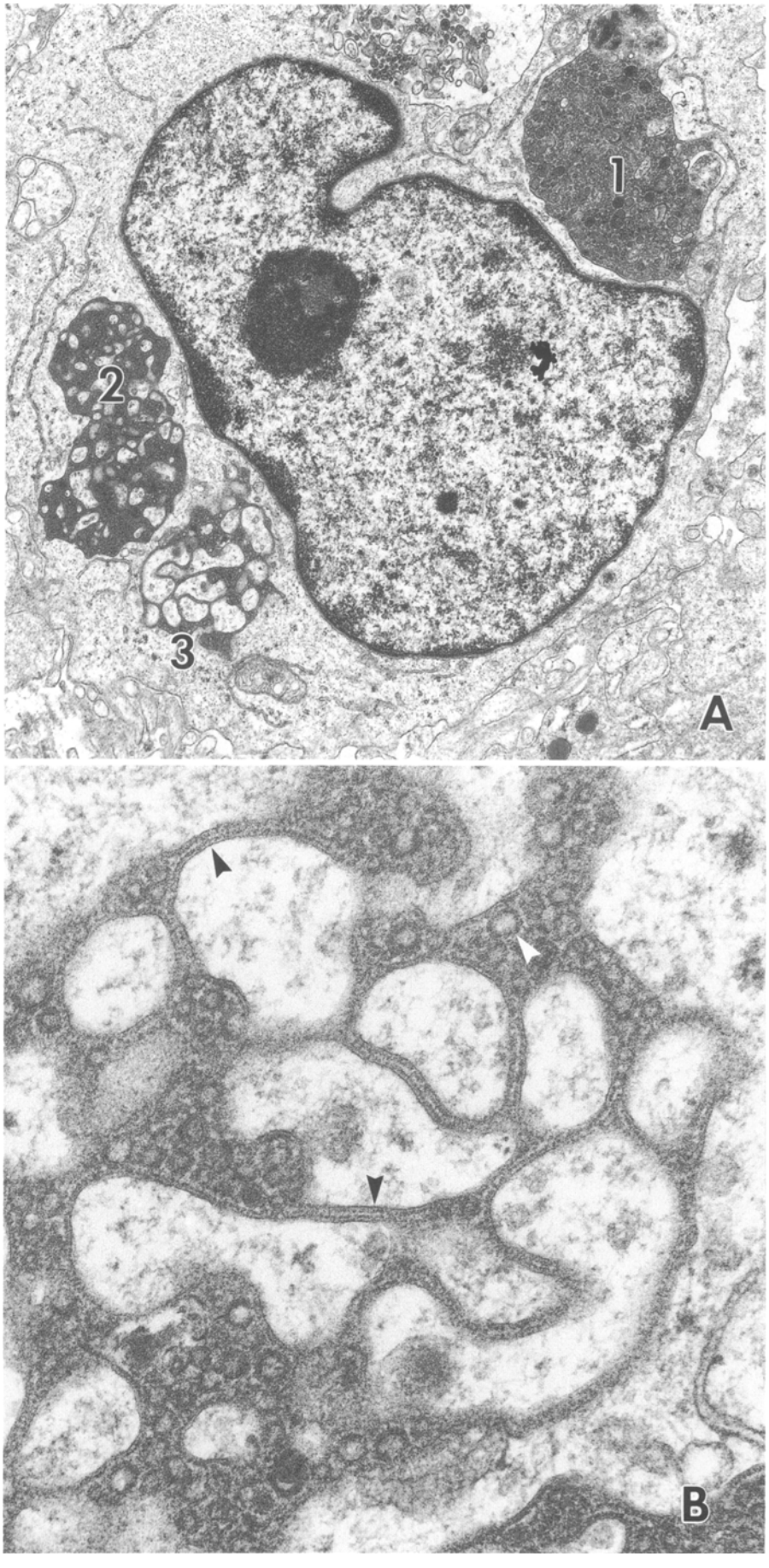

Fig. 4A, B. Dark mvbs in the T cell SR^+ lymphoma. **A** Tumour cell with three large dark mvbs (*1, 2, 3*) showing progressive depletion of internal material resulting in reticulate form 3. ×14000. **B** Detail of reticulate mvb 3 from **A** showing comparatively sparse microvesiculate interior (*white arrowhead*) and abundant limiting membrane (*black arrowhead*). ×78000

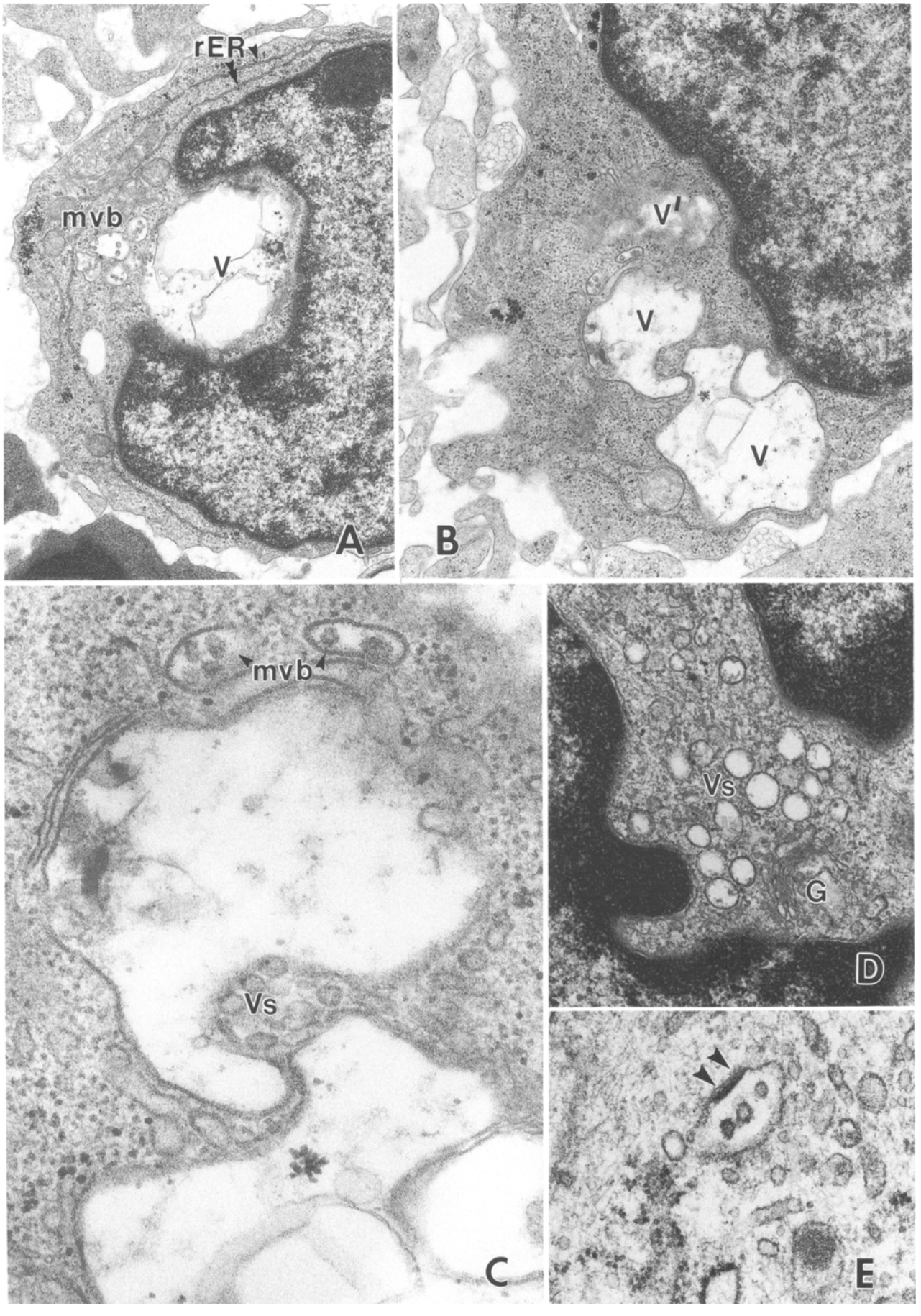

Fig. 5. Structures suggestive of early vacuole-formation in B (**A–C**) and T (**D**) cell SR^+ lymphoma. **A** Group of mvbs close to the surface of a moderately sized vacuole. ×14000. **B** Three associated, slightly irregular vacuoles (*V*; V′ is tangentially sectioned). ×21000. **C** Detail of **B** showing surrounding vesicles (*Vs*) and mvbs. ×71000. **D** Focus of small, round vesicles near the Golgi. ×32000. **E** Mvb from mycosis fungoides showing "straight" area of limiting membrane and surface plaque (*arrowhead*). ×53000

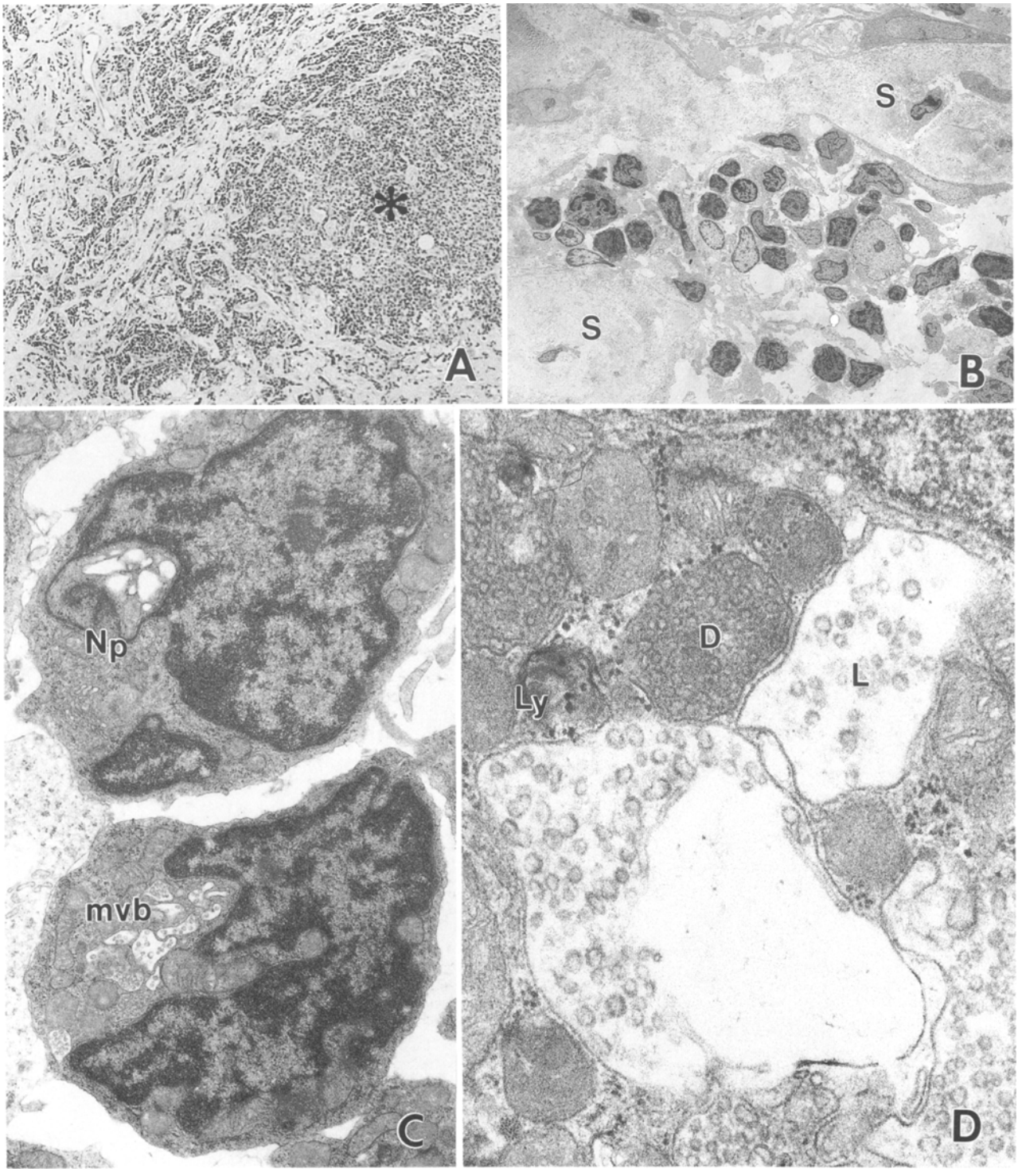

Fig. 6. Mvb-rich SR^- lymphoma. **A** Haematoxylin and eosin appearance showing loosely organised nodule of tumour cells (*). ×250. **B** Small group of tumour cells surrounded by collagenous stroma (*S*). ×1100. **C** Small centrocytes, one showing a nuclear pocket (*Np*), the other a cluster of mvbs. ×14000. **D** Cluster of light (*L*) and dark (*D*) mvbs. *Ly*, Lysosomal dense body. ×53000

tion to the B cell tumour hints at the possibility that among the SR^+ vacuolar lymphomas, there may be subtle, fine structural differences, possibly related to divergent vacuole functions or modes of formation.

While no physical continuity between this rER cisterna and the vacuole membrane has been established, the position of the cisterna is suggestive of a functional relationship. A similar intimate proximity is shown by sER and fat droplets in jejunal epithelium and steroidogenic cells (Rhodin 1975; Reger et al. 1989). Here juxtaposition facilitates utilisation of lipidic components withdrawn from the fat droplets as a means of fat processing

and for the synthesis of steroid hormones. sER in other systems, however, is known to synthesise membrane-directed lipids such as cholesterol, which is a universal membrane constituent (Houslay and Stanley 1982). The vacuole-associated cisternae, therefore, like the unequivocal sER also present in these vacuolar lymphomas, may synthesise membrane components. This may be a compensatory process to replace membrane being taken out of circulation and being irreversibly translocated to the vacuole membrane.

Mvbs have a wide distribution amongst mammalian cells (Ghadially 1988) and are probably universal in actively proliferating or metabolising cells. The widespread occurrence of mvbs, as reported here, in both SR^+ and SR^- lymphomas, as well as the reduced number in reactive lymphocytes, is therefore not surprising, although no study specifically aimed at documenting mvbs in lymphomas to our knowledge has been carried out. Our initial and unpublished findings of conspicuous numbers of mvbs in some T cell lymphomas prompted investigation of mvb number/cell as a discriminant between B and T cell subgroups. In spite of confirming a trend to increased numbers of mvbs in T cell compared to B cell lymphomas (and in neoplastic compared with reactive lymphocytes) the fact that the differences were not statistically significent has failed to substantiate this idea.

The present study emphasises mvb dimorphism into light and dark categories. In vacuolar SR^+ lymphomas, dark mvbs have been referred to and illustrated by few authors (Grogan et al. 1985; Cross et al. 1989) while the large, reticulate forms have not previously attracted attention even though they can represent a sizeable cellular component. They are similar ultrastructurally and probably related functionally to the "pleomorphic granules" described in cells of histiocytic tumours by Maier et al. (1985) and Kanitakis et al. (1988) who have considered them to be lysosomal. The reticulate dark mvbs may be aberrant. The high surface-to-volume ratio suggests loss of materials, solubilised by digestion, from the mvb interior to the cytosol, but at the same time an inability to bud off membranous vesicles for membrane recycling (de Duve 1984). Anteunis (1974) found that dark mvbs never contained the exogenous tracer that could be seen in light forms, and were therefore viewed as autophagic and developing towards residual bodies. For the dark mvbs in these lymphomas, a digestive function remains to be established.

The present study is also one of the few to pay attention to the early stages of vacuole formation. Harris et al. (1981) and Cross et al. (1989) have argued that the vacuole can be considered as a giant mvb and (Harris et al. 1981) that mvbs may fuse with the vacuole as a mechanism of vacuole growth. Iossifides et al. (1980) described assemblies of small mvb-like elements which were believed to form a larger solitary vacuole. Our data suggest an initial development of collections of small mvbs, vacuoles or simple vesicles, which we would postulate as undergoing subsequent fusion. In this context the centroblastic/centrocytic mvb-rich lymphoma is of interest. It is tempting to speculate that this case represents a transitional form between SR^- and SR^+ lymphomas in which there may be a block in the fusion mechanism of mvbs, preventing the development of a large vacuole and of the SR^+ appearance.

On the question of whether membranous elements such as mvbs and the vacuole are of exogenous or endogenous origin transmission electron microscopy is limited by the fact that the technique in this context cannot unambiguously define directionality of membrane movements. Several observations suggest that the light mvbs are endocytotic. The dense plaque of the kind seen on the mvb of our Fig. 5E, for example, is considered by Willingham and Pastan (1984) to indicate endocytotic origin; Grogan et al. (1985) have inferred endocytosis as a contributing process to vacuole formation from their electron micrographs of a T cell SR^+ lymphoma, and Anteunis (1974) found exogenous tracer in light mvbs in blood lymphocytes. However, given Friend's (1969) data of mvbs deriving from the Golgi, an internal origin cannot be entirely discounted, nor can a composite origin involving both. Without more sensitive techniques, such as the use of exogenous tracers on living cells to follow endocytotic and subsequent pathways, combined with immunoelectron-microscopical localisation of organelle-specific markers such as thiamine pyrophosphatase for Golgi elements, the supramolecular events leading to vacuole formation must remain speculative.

Acknowledgements. It is a pleasure to thank Naushad Survay, Julie Cook and Daxa Chauhan for technical assistance.

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