Virchows Arch A (1987) 411:283–291

*Virchows Archiv A*


# Ultrastructure of tumour invasion and desmoplastic response of bronchogenic squamous cell carcinoma

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**Summary.** Using ultrastructural methods we studied the interaction of tumour cells and lung parenchyma in deep areas (i.e., more than about 3 mm from the tumour surface) of 50 bronchogenic squamous cell carcinomas. The tumour periphery, studied previously, had shown organized associations of tumour cells and lung epithelial cells and a surprising lack of invasion of non-epithelial tissue compartments. The deeper areas, where the tumour cells and the lung parenchyma had been in contact for longer periods, consisted of irregular groups of tumour cells and desmoplastic stroma which was very similar to granulation tissue. The deeper areas also contained many intact lung epithelial cells, arranged in compressed and distorted alveolar structures. Where non-neoplastic epithelial cells and tumour cells had direct contact, they formed common junctional complexes and basal laminae. In part of the tumours, the cells were largely devoid of a basal lamina. However, in most instances a continuous basal lamina surrounded every tumour cell group studied, even when these formed irregular strands or seemed to be completely isolated.

**Key words:** Tumor invasion – Ultrastructure – Desmoplasia – Squamous cell carcinoma – Lung parenchyma – Basal lamina

## Introduction

The capacity for invasion is one of the main properties distinguishing malignant from non-malignant cells. In order to invade surrounding tissues, tumour cells must necessarily alter or break down structures in their environment. However, in previous ultrastructural studies on various types of intravascularly administered tumour cells in mice, we have consistently found that the tumour cells inserted themselves into host tissues in a remarkably organized fashion, with hardly any morphological signs of tissue destruction (Dingemans 1973; 1974; Roos and Dingemans 1981; Dingemans and Roos 1982). From these results the question arose whether such orderly behavior constitutes a general attribute of invasive tumour cells, or is related to the artificial model systems used. It seemed therefore worthwhile to compare the experimental tumours with human material. To this end, we collected a large series of human bronchogenic squamous cell carcinomas and studied the interaction of tumour cells and pre-existing lung parenchyma.

The tumour periphery, where the cells have only recently come into contact with the lung parenchyma, has been described previously (Dingemans and Mooi 1986). It appeared that, also in this human material, tumour cells and pre-existing tissue integrated in a highly organized way with full preservation of the alveolar tissue pattern. Furthermore, virtually all the tumour cells were located in the epithelial tissue compartment, without migrating through the basal lamina (BL) which separates the epithelium from the interstitium. This means that, unexpectedly, the peripheral "invasion front" of a bronchogenic squamous cell carcinoma behaves as a carcinoma in situ despite the light microscopic appearance of a complex mixture of tumour cells and non-neoplastic lung cells.

The subject of the present paper is the deeper areas of bronchogenic squamous cell carcinomas, where the contact between tumour cells and lung parenchyma has existed for a longer period. In these deeper areas, a prominent desmoplastic response has developed, resulting in an irregular

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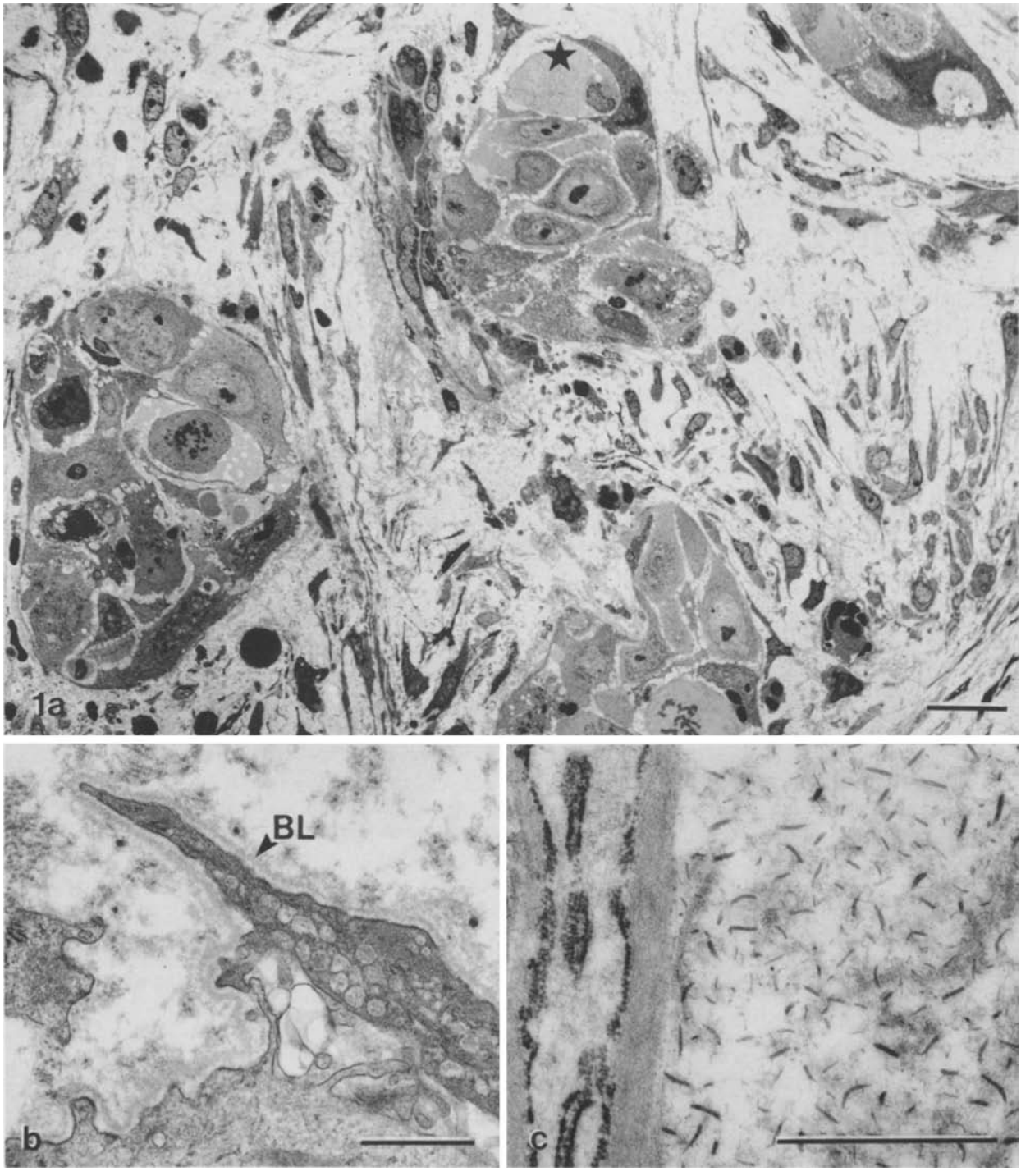


**Fig. 1a.** Survey of deep tumour area, showing 4 tumour cell groups within electron-lucent stroma. Predominant stromal cells are elongated myofibroblasts. Detail indicated by asterisk is enlarged in **b**. **b** Detail showing edge of tumour cell group. BL covers tumour cells even in areas where surface is highly irregular. **c** Detail of electron-lucent matrix in sample treated with cuprolinic blue. Proteoglycan is visible as system of short stripes, filling extracellular space. Although cuprolinic blue treatment is not optimal for preservation of cellular details, cell at left is recognizable as myofibroblast by presence of peripheral sheaths of microfilaments and somewhat dilated endoplasmic reticulum, lined by ribosomes. **a** ×750 (*bar represents 10 microns*); **b** ×18000 (*bar represents 1 micron*); **c** ×35000 (*bar represents 1 micron*)

histological picture very different from that in the tumour periphery. The questions to be answered were, first, does the prolonged contact between tumour cells and lung parenchyma lead to degeneration of the parenchymal cells and, if so, are there morphologic indications of the mechanism of this degeneration? Second, do the tumour cells in the deeper tumour areas leave the confinement of the epithelial BL? and, third, what is the nature of the newly formed stroma?

## Materials and methods

The collection and preparation of specimens for electron microscopy from an essentially non-selective series of 43 resected bronchogenic squamous cell carcinomas have been described in detail in a previous paper (Dingemans and Mooi 1986). Briefly, two or three large pieces of tumour tissue, containing the tumour lung interface at one end, were taken from each case and fixed in Karnofsky's fixative. From these pieces, both superficial and deeper samples were taken and embedded for electron microscopy; in selecting the samples, large blood vessels and airways were avoided as far as possible. The deeper samples were situated at a distance of about 3–8 mm from the tumour-lung interface; the actual centers of the tumours (average diameter 4 cm) were often necrotic and therefore unsuitable for ultrastructural study. To obtain samples from peribronchial tumour tissue, 7 bronchial biopsies were also collected, and processed in toto. Apart from the presence of bronchial tissue, the histology of these biopsies corresponded to that of the deeper areas of the resected tumours. Electron microscopic study of the bronchial biopsies was restricted to areas in which the tumour tissue was in contact with lung parenchyma rather than with peribronchial connective tissue.

In a few cases, samples from resected carcinomas were treated with cuprolinic blue for the demonstration of proteoglycans (Scott 1980; Van Kuppeveld et al. 1984).

## Results

On superficial examination, the deeper parts of the tumours contained only a few organized structures. The tissue consisted of an extensive, irregular stroma in which equally irregular groups and strands of closely packed cells were growing (Fig. 1a). In these characteristics it differed strongly from the peripheral tumour tissue described previously.

### *Tumour cells and tumour-stroma interface*

The groups of tumour cells dispersed in the stroma ranged from large sheets and nodules to thin, irregular strands and small aggregates, apparently isolated from the larger masses. The tumour cells were interconnected by desmosomes which seemed to be no less abundant in the small strands than in the larger nodules. There was no consistent morphological difference between the tumour cells in the deeper tumour areas and those in the periphery described previously.

In over half of the tumours an almost continuous BL was present around all tumour cell groups studied, even when they were small and seemed to grow freely in the stroma. In contrast with the situation in the periphery, the BL usually had a well demarcated lamina lucida and lamina densa (Fig. 1b). The tumour cells were connected to the BL by numerous hemidesmosomes. Except for some areas of multilayering and small tumor cell protrusions which occasionally breached it, the BL had few irregularities. In the remaining tumours, parts of the tumour cell groups studied were directly exposed to the stroma without an intervening BL. The proportion of the tumor cell surface devoid of BL varied from case to case, but in almost none was it completely absent. When the BL had discontinuities, these were by no means restricted to the smallest and most irregular tumour cell strands but were also observed in large nodules (Fig. 2). When a BL was discontinuous, it was mainly present in the form of short stretches lining recessions of the tumour cell surface and connected to it by hemidesmosomes. The free tumour cell surface, however, was usually irregular with many protrusions (Fig. 2b). A detailed analysis of the prevalence of the BL in these tumours, based on immunohistochemistry and quantitive electron microscopy, will be published separately (Havenith, Dingemans, Cleutjens, Wagenaar and Bosman, in preparation).

Since most tumour cell groups were embedded in an almost structureless matrix (see below), it was generally impossible to assess whether connective tissue destruction had occurred in the immediate vicinity of the tumour cell surface (McNutt 1976; Hashimoto et al. 1972; Tarin 1969). Only when myofibroblasts were closely adjacent to the tumour cells was it possible occasionally to detect signs of local degeneration in the form of swelling of cell extensions or the formation of small myelin-like figures. Characteristically, such changes were not found in myofibroblasts that were located slightly farther from the tumour-stroma interface (Fig. 2b).

### *Non-neoplastic epithelium*

Next to the tumour cell groups, most tumours contained groups of pulmonary epithelial cells (Fig. 3). In most cases, these appeared viable and intact (Fig. 3b). They were always interconnected by junctional complexes and lined an intercellular lu-

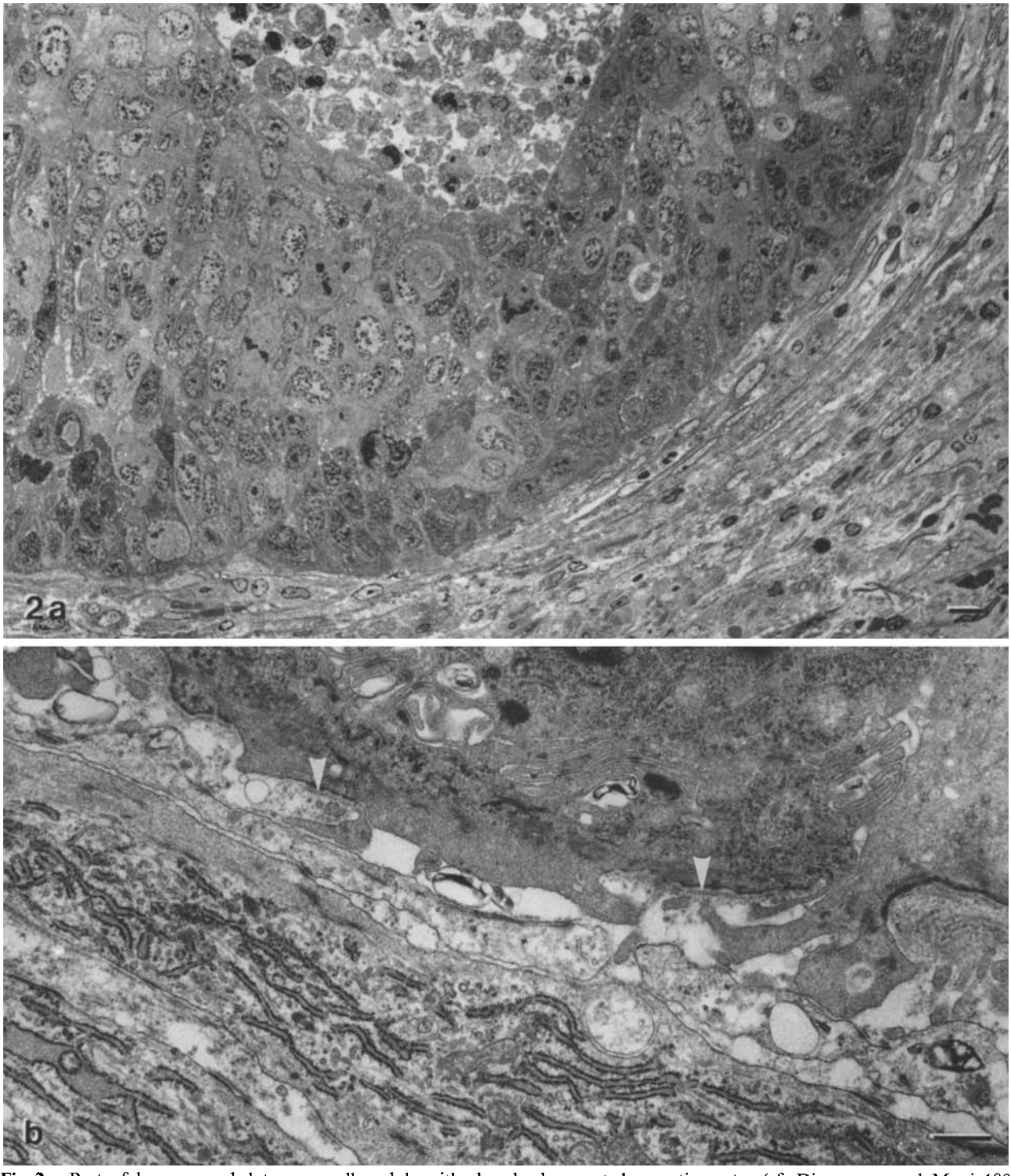


**Fig. 2a.** Part of large, rounded tumour cell nodule with sharply demarcated necrotic center (cf. Dingemans and Mooi 1984; 1986). **b** Detail of edge of nodule shown in **a**. The tumour cell surface is irregular, with numerous small protrusions. Patches of BL, most of them associated with hemidesmosomes, are mainly present on deeper surface areas (*arrow heads*). Stromal myofibroblasts have peripheral myofilaments, fusiform densities and extensive rough endoplasmic reticulum. Slight local degeneration is suggested by presence of myelin-like structures and small vesicles as well as swelling of myofibroblast extensions. Note that signs of degeneration are restricted to narrow zone at tumour-stroma interface. **a** ×500 (*bar represents 10 microns*); **b** ×10000 (*bar represents 1 micron*)

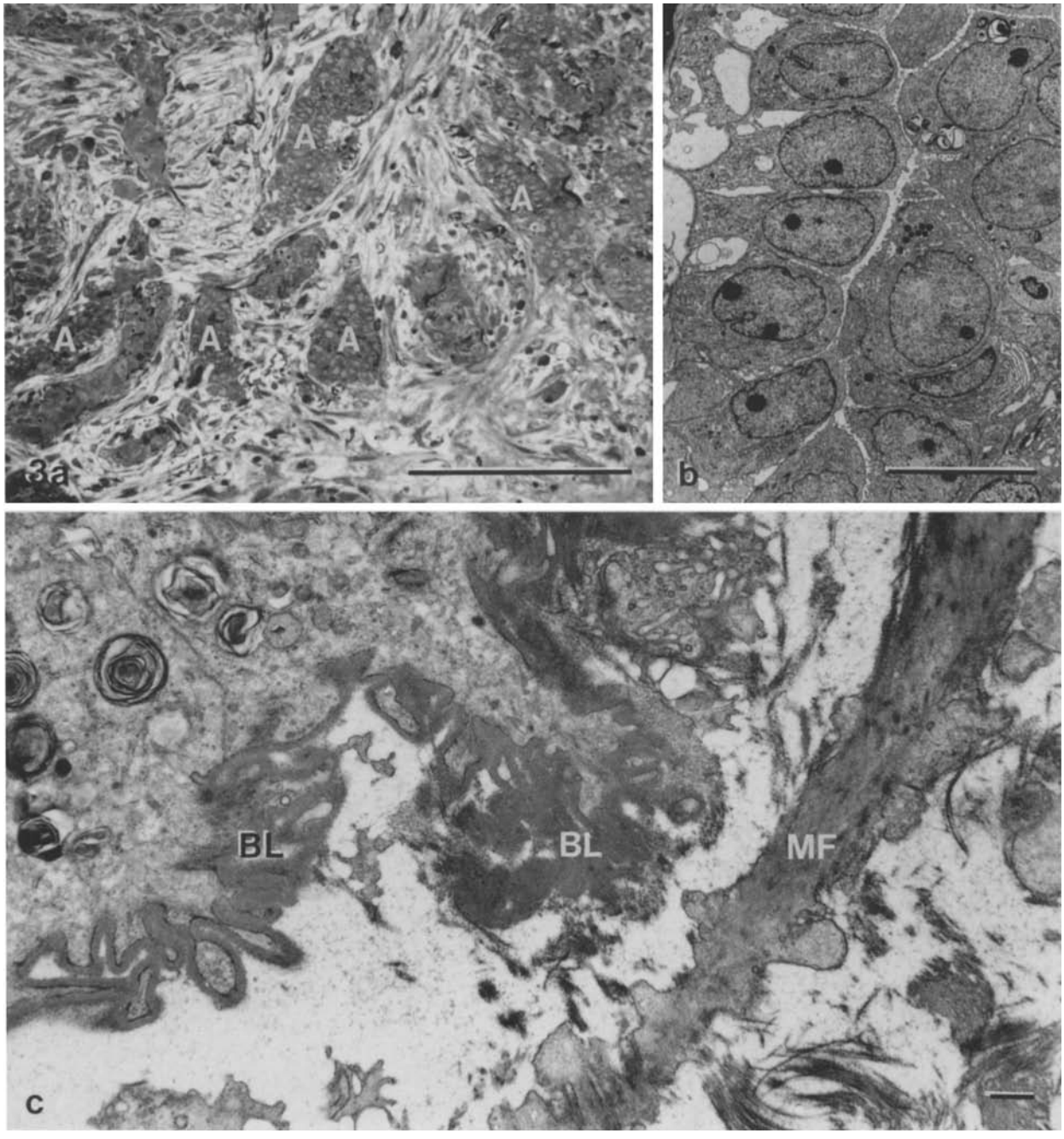


**Fig. 3a.** Light microscopic survey of trapped alveoli (*A*) and tumour cell strands, lying within extensive stroma. **b** Detail of **a**, showing part of alveolus with slit-like lumen. **c** Detail of trapped alveolus, characterized by osmiophilic surfactant bodies (*upper left*). Pleated, redundant alveolar epithelial BL is situated near myofibroblast (*MF*) which shows signs of vigorous contraction in the form of electron-lucent surface blebs (cf. Dingemans and Wagenvoort 1976). **a** ×350 (*bar represents 100 microns*); **b** ×2300 (*bar represents 10 microns*); **c** ×7300 (*bar represents 1 micron*)

men (which was often reduced to a narrow slit, undetectable by light microscopy). The luminal cell surface had many short, regular microvilli and the lumen often contained surfactant-like material. The cell groups therefore represented deformed and collapsed but otherwise intact non-neoplastic pulmonary alveoli trapped by the tumour. The BL surrounding such collapsed alveoli often seemed to be too wide and had accumulated to form irregularly pleated aggregates (Fig. 3c).

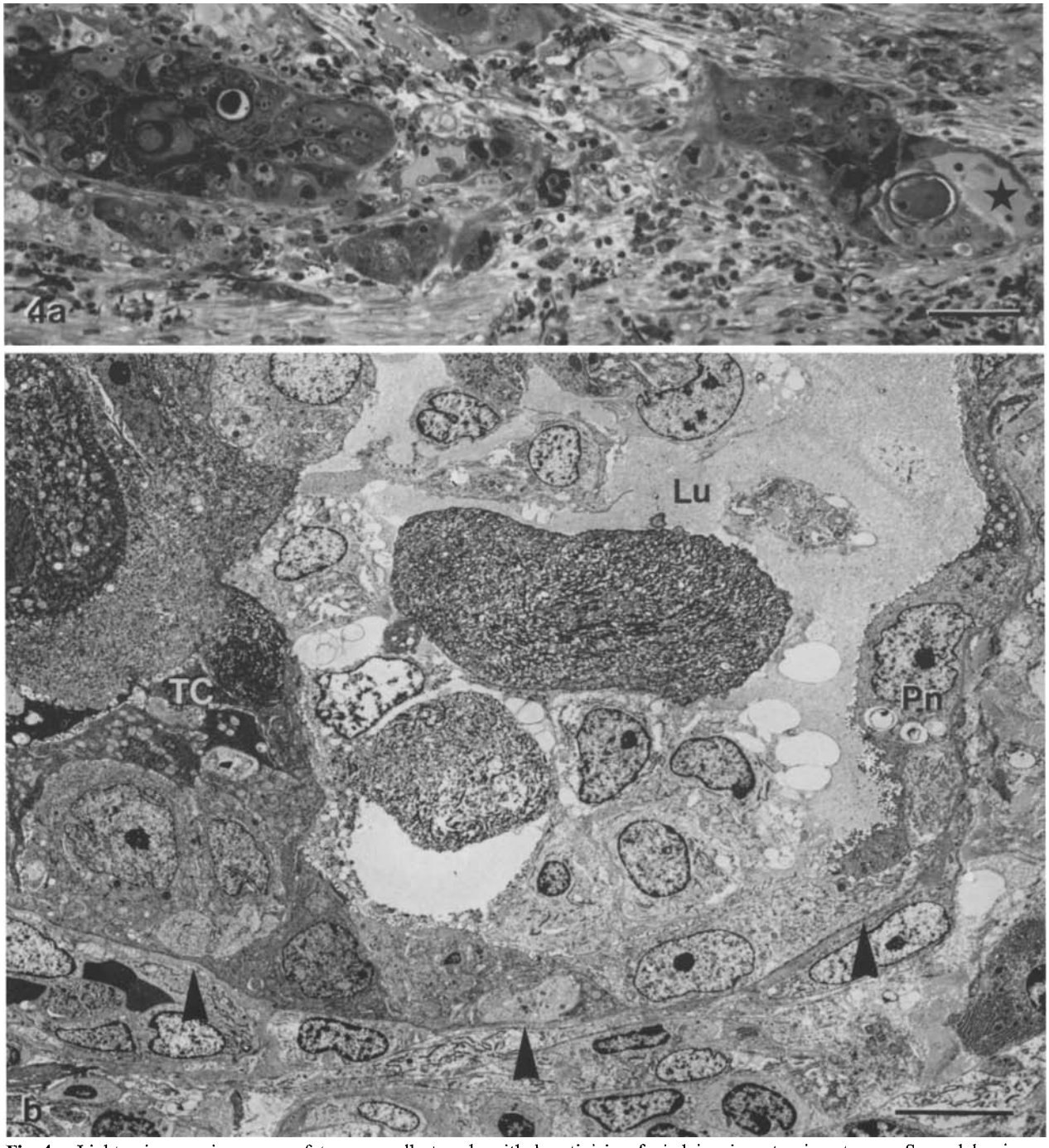


**Fig. 4a.** Light microscopic survey of tumour cell strands with keratinizing foci, lying in extensive stroma. Several lumina are present; largest of these, indicated by asterisk, is shown in detail in **b**. **b** The lumen (*Lu*) is lined partially by tumour cells (*TC*) and partially by pneumocytes (*Pn*) which share common BL (*arrow heads*). It contains desquamated, keratinized tumour cells. **a** × 150 (*bar represents 100 microns*); **b** × 1900 (*bar represents 10 microns*)

*Association of tumour cells and non-neoplastic epithelium*

Apart from the tumour cell groups and the trapped non-neoplastic alveoli described above, most tumours contained nodules in which tumour cells and pulmonary epithelial cells were combined. They frequently lined a common lumen, in which situation they were connected by junctional complexes and shared the same BL; such lumina often contained desquamated, keratinized neoplastic cells (Fig. 4). In other cases, collapsed alveoli were

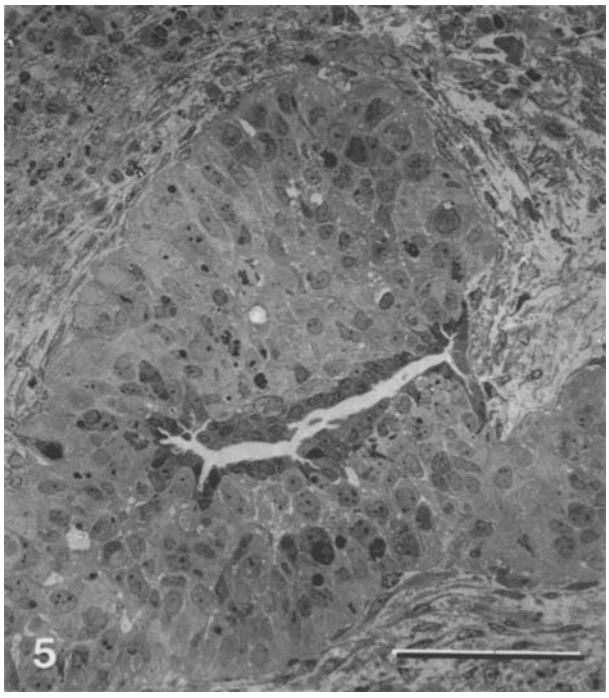


**Fig. 5.** Tumour cell nodule containing collapsed alveolus lined by pneumocytes that are more electron-dense than surrounding tumour cells. Pleated aggregates of BL, as illustrated in Fig. 4c, were invariably associated with such collapsed alveoli largely surrounded by tumour cells. ×250 (*bar represents 100 microns*)

deeply embedded in compact tumour cell groups, in which situation the pulmonary epithelial cells very often exhibited an increased electron density (Fig. 5). The BL surrounding tumour nodules which contained collapsed alveoli often showed the same pleating as the BL around the isolated collapsed alveoli mentioned above.

*Stroma*

Myofibroblasts were the predominant cell in the stroma. They were characterized by peripheral sheaths of myofilaments with dense bodies. In addition, they had a well developed rough endoplasmic reticulum and Golgi apparatus (Figs. 1c and 2b). Normal fibroblasts were not very numerous and when present had the same "active" appearance as the myofibroblasts. Generally there were also many plasma cells and capillaries, and variable numbers of mast cells, granulocytes, lymphocytes, and macrophages.

The following extracellular elements could be distinguished in the stroma: An electron-lucent ground substance which invested all formed structures and which constituted the main stromal element in most tumors (Fig. 1a, b). A proteoglycan-like meshwork was generally discernible within this ground substance, the nature of which was confirmed by the application of cuprolinic blue which specifically stains proteoglycans (Fig. 1c) (Scott 1980; Van Kuppeveld et al. 1984). There were also haphazardly arranged strands of fibrin, characterized by a periodicity of about 23 nm (Dvorak et al. 1983); irregular collagen fibers, most of them thin and with a poorly recognizable cross-striation; and strands and patches of elastin, recognizable by the presence of microfibrils around a homogeneous center, and finally aggregates of flocculent material of moderate electron density. In different tumours, different elements predominated. Dense collagen, consisting of bundles of parallel thick fibers with a prominent cross-striated pattern, was usually restricted to the vicinity of large airways and vessels.

## Discussion

Like the tumour periphery previously described, the deeper tumour areas contained many non-neoplastic pulmonary epithelial cells which were usually intact, even when in close contact with the tumour cells. In the deeper areas this contact often resulted in the formation of junctional complexes, the sharing of common BL and lumina, and so on. Our results therefore demonstrate that also in human material, malignant invasion is not necessarily associated with destruction of pre-existing surrounding cells. However, it was obvious that the number of the non-neoplastic epithelial cells present in the deeper tumour areas had decreased considerably. The mechanism of this decrease may be sought in the preferential localization of the neoplastic cells between the pre-existing septal capillaries and the non-neoplastic cells, which may lead to the degeneration of the latter. Such a phenomenon is suggested by pictures like Fig. 5, where a collapsed alveolar structure is embedded in a massive tumour cell nodule; the lining pulmonary epithelial cells are electron-dense, and therefore possibly degenerating.

In the majority of the tumours, all malignant cell groups studied were found to have a complete BL. In the large rounded tumour cell nodules, this seemed to correspond to the original BL of the alveolar epithelium which surrounded all tumour cell nodules in the periphery which had filled the preexisting alveoli. In the case of the more irregular tumour cell strands with a complete BL, it seemed at least in part to be newly formed. This notion is supported by our frequent finding of a complete BL around tumour cell groups that had already invaded the walls of pulmonary blood vessels (to be published; cf. Pitelka et al. 1980). Also the re-

cent immunohistochemical findings of others (Gusterson et al. 1984; Carter et al. 1985) demonstrate that invasiveness and the presence of a continuous BL are by no means mutually exclusive.

Many authors have argued that a BL is not a static but a highly dynamic structure, the presence or absence of which in a given situation may be of limited significance (Luibel et al. 1960; Erlandson and Carstens 1972; Pitelka et al. 1980; Parssons et al. 1982; Halter and Glick 1983; Gusterson et al. 1984). This is supported by our observations: in some tumours even the thinnest tumour cell strands, which seemed to move freely through the stroma, had a continuous BL, whereas others consisted of larger, rounded, seemingly non-invasive cell nodules with only scantly BL remnants. From the foregoing it is clear that there are two answers to the question formulated in the introduction, namely, whether the tumour cells in the deeper areas leave the confinement of the epithelial BL. Firstly, in many tumours, the cells had direct contact with the stroma without an intervening BL and can therefore be regarded as lying outside the epithelial tissue compartment. Secondly, however, it should be emphasized that this "escape" from the confinement of the BL appears to be a gradual process and it is often impossible to determine which tumour cells are still within the epithelial tissue compartment and which are not.

Normal lung parenchyma has little connective tissue. Thus, the extensive stroma present in parenchymatous areas invaded by the tumours must be newly formed. It represents an example of desmoplasia, which is especially prominent in mammary, colonic, and prostatic carcinomas, but which may occur to some extent in almost any organ (Liotta, 1982; Madri and Carter 1984; Haemmerli et al. 1985). It consisted mainly of an electron-lucent matrix in which a proteoglycan meshwork could usually be discerned. In addition, fibrin was often present in massive amounts. Both proteoglycans and fibrin are common constituents of tumour stroma (Iozzo 1985; Dvorak et al. 1983). Myofibroblasts were by far the most frequent cells in the stroma. Since the original descriptions of myofibroblasts in tumour stroma (Tremblay 1979; Dvorak et al. 1979; Seemayer et al. 1979), they have been found in a great variety of neoplasms. Their occurence is reported to be closely linked to invasion (Seemayer et al. 1979; Ohtani and Sasano 1980; 1983), although we found them in tumours with a complete BL around every cell group no less than in those with a discontinuous BL. Various contraction phenomena in human carcinomas are attributed to the activity of myofibroblasts (Tremblay 1979; Seemayer et al. 1979; 1980). In our material, they seemed to be responsible for at least part of the collapse of trapped alveoli. This was suggested by the presence of signs of contraction (Dingemans and Wagenvoort 1976) in myofibroblasts located in the immediate vicinity of collapsed alveoli with a pleated BL (Fig. 3).

The combination of proteoglycans, fibrin, myofibroblasts, and ingrowing capillaries occurs not only in tumour desmoplasia, but also in granulation tissue which develops whenever tissue remodelling, fast cell proliferation and cell migration are required, such as during wound healing and regeneration (Vasiliev 1958; Takeuchi 1976; Dvorak et al. 1979; 1981; Seemayer et al. 1980; 1981; Schürch et al. 1981; Thompson et al. 1985). The fact that invasive tumour cells grow in a newly created environment which shares many features with granulation tissue, may have important functional implications for the process of invasion and warrants extensive further study (Dvorak 1986).

*Acknowledgements.* Our thanks are due to Sj. Sc. Wagenaar and Ella Vaarkamp (Sint Antonius Ziekenhuis, Nieuwegein, The Netherlands), who carefully collected most of the specimens and provided the diagnoses. We also thank the technical staff of the Department of Electron Microscopy, especially Mr. M.A. Van den Bergh Weerman, for the processing of hundreds of tissue blocks.

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Accepted April 6, 1987