Ultrastructural Studies on the Invasion of Melanomas in Initial Lymphatics of Human Skin

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The way in which melanoma cells invade the initial lymphatics of the skin was investigated in this study. Samples of sixty melanomas were examined by transmission electron microscopy. Tumor cells invading lymph vessels were demonstrated in 20 specimens. In most cases the melanomas penetrated the subendothelial space as single cells. These fused with the endothelial cytoplasmic membrane and subsequently destroyed the endothelial wall. J Invest Dermatol 98:64–67, 1992

RESULTS

Light Microscopic Findings. IL were present in all 60 samples examined. They were identified by means of the following criteria and could thus be distinguished from blood vessels [5–7]. The extremely thin-walled vessels are wider in the lumen than blood vessels and their outline is irregular [6]. They lack the well-developed basal lamina and pericytes of the dermal blood vessels.

In 20 of the 60 samples melanoma cells were seen in close proximity to IL, partly intraluminally. Four invasions of tumor cells in the lymph vessels were observed at level III, 14 at level IV, and two at level V. Initial invasion was recognized in three samples (level III).

Ultrastructural Results. Dilated lymphatics were easy to identify because of their extremely thinned endothelium, their interendothelial junctions, and a typical subendothelial network instead of the well-developed basal lamina of blood vessels [5–7]. Compared with the healthy skin, IL in melanomas showed some additional features that varied considerably from one sample to another: there were signs of intensified metabolic activity in the endothelium, marked by an increased number of mitochondria and thicker endothelial cells (Fig 1).

For investigations on the invasion of the lymphatics by melanoma cells, only samples in which melanoma cells (Fig 2) could clearly be identified were used. The criteria were as follows: a large nucleus, composed almost exclusively of heterochromatin, with deep invaginations of the nuclear membrane and a large nucleolus, foamy in structure with cytoplasmic and eosinophilic inclusions here and there. Melanosomes and pre-melanosomes in varying numbers were predominantly located at the periphery of the cell, surrounding an increased number of comparatively large mitochondria [8,9]. It was also necessary to differentiate them from melanophages (Fig 3).

Various phases in the relationship of melanoma cells to lymphatics were observed. In some tumor cells located close to IL, narrow cytoplasmic processes were discovered running in the direction of the vascular wall (Fig 4). In other samples these pseudopodium-like cytoplasmic protrusions drove the fibrous network apart (Fig 5). The cytoplasmic processes seemed to push themselves forward between the endothelial wall and the fibrous network, covering the former, so that parts of the tumor cells came into direct contact with the abluminal surface of the endothelium. Partial fusion of the cytoplasmic membranes of the tumor and the endothelial cell was evident in two samples; in these areas of fusion the cell membranes were no longer seen (Fig 6). In other samples melanoma cells pen-
trated the lumen of the lymphatics. Here fragments of destroyed endothelium were recognized in front of the invading tumor cell and, at the side, the edges of the broken endothelial wall (Fig 7).

DISCUSSION

Lymphatic fluid in peripheral collecting lymphatics always contains several cellular elements [10]. Normally the cells arrive at the lumen of the lymphatics from the tissue via the interendothelial openings [11]. Using mice ears as a model we discovered that inflammatory cells [i.e., neutrophil granulocytes and macrophages] located in the tissue drove the abluminal fibrous network of IL apart by cytoplasmic processes. Subsequently the cells traveled between the endothelium and the fibrous network and arrived at the lumen via an open junction. The present investigations also exhibited migration of inflammatory cells via interendothelial openings in cases in which there was a marked inflammatory infiltration existent (Fig 8). Inflammatory and melanoma cells both seem to use the initial path to

Figure 1. Activated lymphatic endothelial cells with numerous mitochondria, large smooth endoplasmic reticulum, and four electron-dense granules, perhaps engulfed melanosomes (*). SF, subendothelial fibrous network; N, nucleus; En, endothelial cell; C, collagen bundles. Bar, 1 μm.

Figure 2. Melanoma cell with numerous melanosomes at the periphery of the cell. N, nucleus; Nc, nucleolus; Mt, mitochondria. Bar, 1 μm.

Figure 3. Macrophage with organell-rich cytoplasm and numerous melanized phagosomes (P) varying in size. N, nucleus; Nc, nucleolus. Bar, 1 μm.

Figure 4. Cytoplasmic progress of a melanoma cell (→) in direction of the subendothelial fibrous network. MC, melanoma cell; SF subendothelial fibrous network; En, endothelial cell. Bar, 1 μm.
Figure 5. Cyttoplasmic protrusion of a melanoma cell (MC) driving apart the subendothelial fibrillar material (SF) of the network (●) around the lymphatic endothelial cell (En). Bar, 1 μm.

Figure 6. A broad cytoplasmic process in close association to the endothelium (En). There are partial fusions between melanoma cell (MC) and lymphatic endothelial cell (●). SF, subendothelial fibrinous network. Bar, 1 μm.

Figure 7. Melanoma cell (MC) penetrates the lumen of a lymphatic. Fragments of the destroyed endothelium (En) are seen (→). SF, subendothelial fibrinous network. C, collagen bundles. Bar, 1 μm.

Figure 8. Inflammatory cell (IC) migrates in the perilymphatic space to an interendothelial junction (★). SF, subendothelial fibrinous network; N, nucleus; En, endothelial cell. Bar, 1 μm.
the lymphatics; both form cytoplasmic processes that penetrate the abluminal fibrous network. Then their behavior is differentiable: the melanoma cell does not migrate any further in the perilymphatic space to an interendothelial opening. After fusion with the outer surface of the endothelial cell and following its destruction the melanoma cell arrives at the lumen of the lymphatics.

According to the results we have obtained so far, the tumor cells seem to penetrate the IL individually and not in formation.

It is conceivable that physiologic guide-ways exist leading to the lymphatics and that these are used by inflammatory cells as well as melanoma cells. We suspect that connective tissue fibers, which radiate through the fibrous network, function as guide-ways, as do elastic fibers [12].

Knowledge of the location of the so-called attachment molecules like fibronectin and laminin on the lymphatic wall is probably essential for an understanding of the invasion of IL by tumor cells [13,14]. Laminin and fibronectin were often evident in the wall of blood vessels of the skin [15,16]. In previous investigations (so far unpublished) we have demonstrated collagen type IV, laminin, and fibronectin along the abluminal endothelial surface. Closely packed tumor cells and melanophages located in the immediate vicinity of blood vessels but not penetrating the vascular wall were striking (Fig 9). The thick walls of the blood vessels seem to resist the infiltrating melanoma cells.

Our findings can be summarized as described in Fig 10. Tumor cells mostly invade IL in melanomas. Located close to these vessels melanoma cells develop pseudopodium-like cytoplasmic processes that penetrate the abluminal fibrous network. The resulting perilymphatic space, bordered by the endothelial wall on the one side and the fibrous network on the other, is occupied by parts of the melanoma cell, which then fuse partially with the endothelial cell wall and destroy it. Obviously the melanomas invade IL as single cells and not in formation.

REFERENCES