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ELECTRON MICROSCOPIC STUDY OF KERATOACANTHOMA

Y. Takaki, M. Masutani and A. Kawada

From the Department of Dermatology, Juntendo University School of Medicine, Tokyo, Japan

Abstract. A typical lesion of keratoacanthoma was studied at the ultrastructural level. The surface of proliferating cells revealed very irregular undulation showing numerous microvilli in association with the increase of desmosomes. Mitochondria, rough-surfaced endoplasmic reticulum, RNA particles and vacuoles were also increased. Various stages of abnormal keratinization were observed. Interestingly, desmosome-like structures were found within the cytoplasm of the abnormally keratinized cells.

Keratoacanthoma, though bearing a striking resemblance to squamous cell carcinoma, is differentiated from true malignancy because of its tendency to show spontaneous regression and benign clinical course. There have been several reports concerning the ultrastructure of this tumor. Most of them have focused on the inclusion bodies resembling viral particles in the nuclei (2, 4–6, 8, 11, 12) and there is much less information on the ultrastructural changes of the proliferating cells themselves (1, 2, 8). This paper is concerned with electron microscopic study of this tumor with particular references to the following findings: abnormal keratinization in the tumor cells and the presence of desmosome-like structures within abnormally keratinized cells.

MATERIAL AND METHODS

Biopsy specimen was obtained from a typical lesion of 4 weeks duration on the right infraorbital region of a 34-year-old woman. This lesion regressed completely 4 months after the biopsy without any treatment.

The biopsy specimen was divided into two portions. One portion was processed for light microscopy by routine paraffin-embedding and staining with H-E and PAS stain. The other one was fixed in buffered 1% osmium tetroxide for 2 hours, dehydrated in graded ethanols and embedded in Araldite. Thin sections were stained with uranium acetate and lead hydroxide, and were examined in Hitachi HU-11A electron microscope.

RESULTS

Light microscopy

The tumor consisted of irregular epithelial proliferation (Fig. 1). In general, the basal layer was intact, though in some areas where the basal layer became disorganized, the cell masses appeared poorly demarcated from the surrounding stroma. The surface of this tumor showed irregular grooves and hyperkeratosis intermingled with areas of parakeratosis. The proliferating cells were predominantly spinous cells showing well-developed prickles and a slight atypicality. Mitotic figures were scanty. Horn pearls were present in small number and some showed partial keratinization of their centres. There was a rather marked inflammatory reaction such as infiltration predominantly of lymphocytes and vascular dilatation in the surrounding stroma. Many lymphocytes and

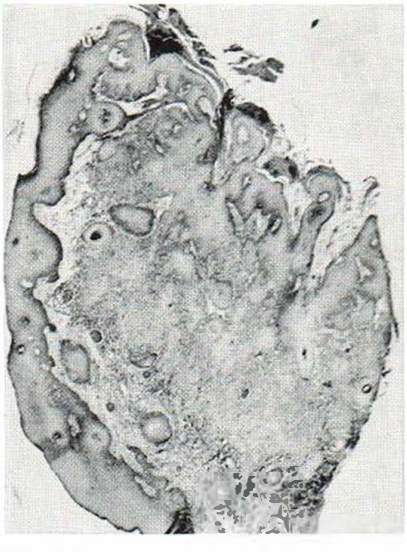

Fig. 1. Low-power view of histologic section. H-E stain.

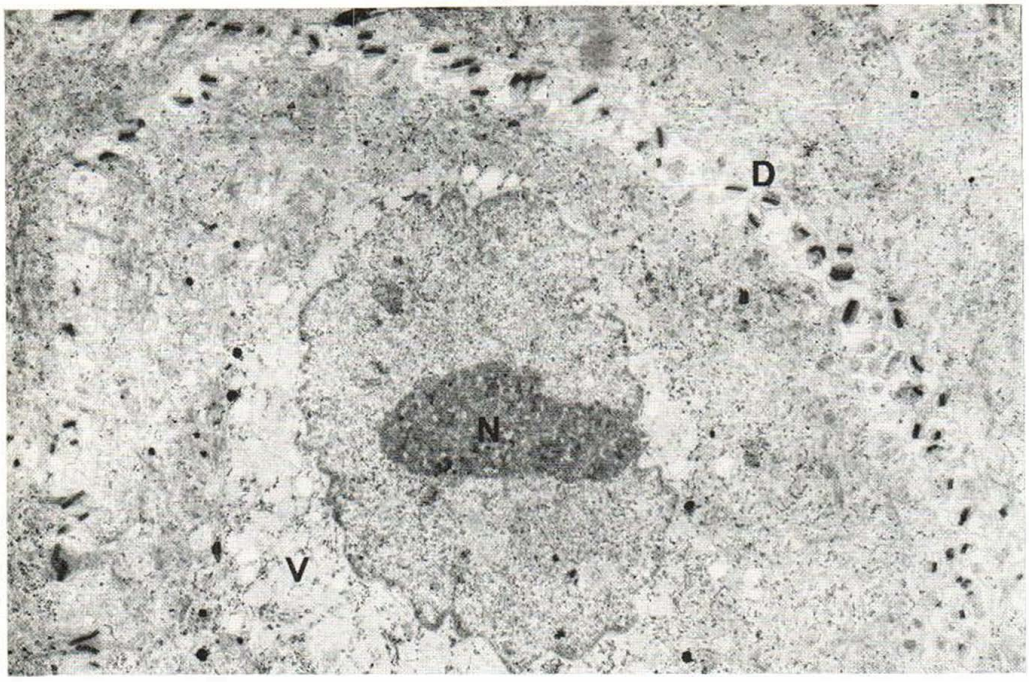

Fig. 2. Electron micrograph of a proliferating cell of keratoacanthoma. The cytoplasm near the nucleus is occupied with numerous mitochondria or vacuoles. The cell surface shows numerous microvilli and desmosomes are increased in number. *D*, desmosome; *N*, nucleus; *V*, vacuole. × 7 000.

neutrophils were also noted in the epidermis proliferated.

Electron microscopy

The stratum corneum usually showed parakeratosis. The granular and basal layers appeared almost normal. The epithelial proliferation consisted mostly of spinous cells which revealed a marked increase in rough-surfaced endoplasmic reticulum, free RNA particles and vesicles or vacuoles of various sizes (Fig. 2). In addition, there were numerous enlarged mitochondria which were occasionally degenerate. In general, tonofilaments were decreased in quantity and had a tendency to aggregate forming disorganized short thick fascicles, whereas those anchored to the attachment plaques of desmosomes were well developed (Fig. 3). The cell surface frequently showed very irregular undulation forming numerous microvilli and the intercellular spaces were enlarged. The intact desmosomes were seemingly increased in number (Fig. 2). Among the proliferating cells was found an unusual keratinization of various stages. The partially keratinized cells revealed aggregation of tonofilaments in disorganized fascicles, thickening of the cell membrane in association with the decrease or lack of desmosomes and enlargement of the intercellular spaces between the adjacent cells. In more keratinized cells their cytoplasms were filled with keratinous mass composed of irregularly aggregated tonofilaments which were frequently mixed with ribosomes and many vesicles or vacuoles (Figs. 4, 5 *a*). In addition, there were more compact keratinous masses in which the filaments had a higher opacity than in the normal keratin pattern (Fig. 6). In these, there were a few vacuoles of various sizes but no membrane-coating granules.

In some of these keratinized cells, desmosome-like structures were occasionally detected. These structures were definitely located within their cytoplasms, some were noted in the vicinity of the cell membrane and attachment plaques (Fig. 7 *a, b*). The relationship between these structures and tonofilaments was not clear. In some instances, desmosome-like structures were found to be engulfed between the apposed cytoplasmic membrane showing invagination (Fig. 5 *b*) and also

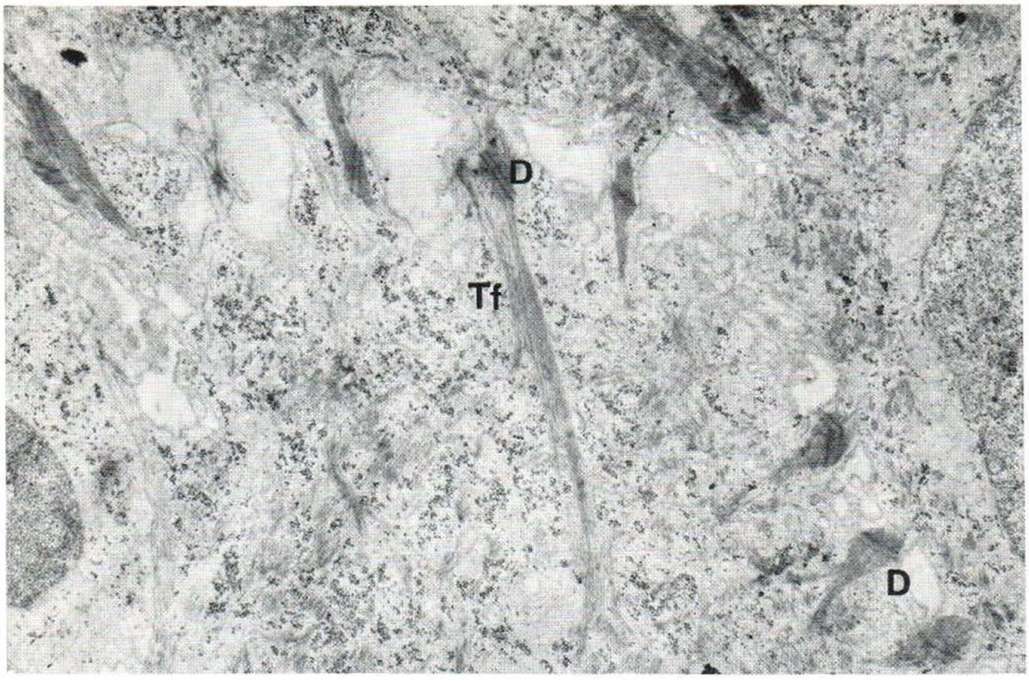

Fig. 3. Tonofilaments are decreased and tend to form short thick fascicles while these anchored to attachment plaques of desmosomes are well developed. *D*, desmosome; *Tf*, tonofilament. × 17 000.

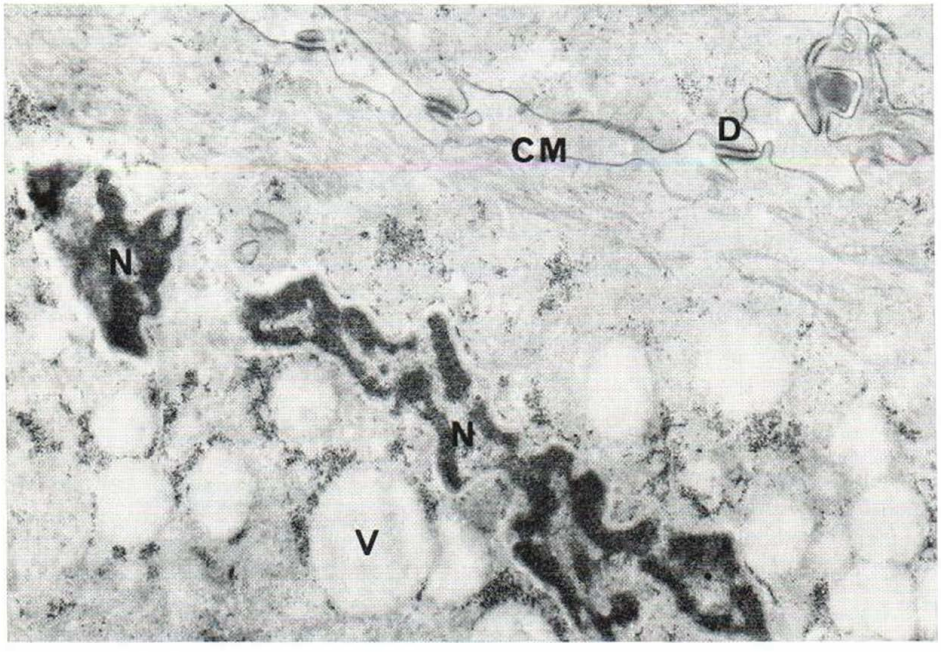

Fig. 4. Partially keratinized cell containing the indented nucleus and mitochondria or vacuoles of various sizes. The cell membrane is thickened and the intercellular space is enlarged. *CM*, cell membrane; *D*, desmosome; *N*, nucleus; *V*, vacuole. × 17 000.

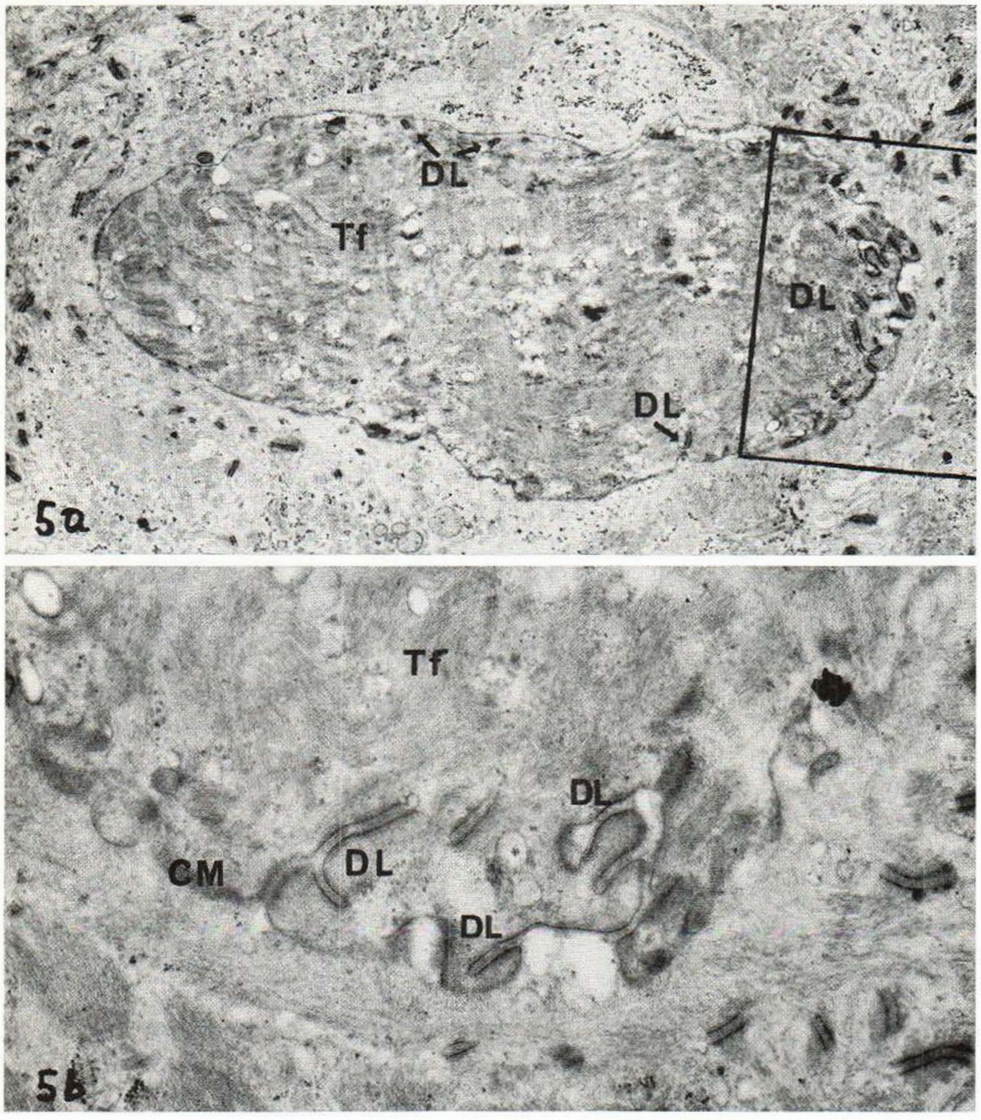

Fig. 5. (*a*) Keratinized cell revealing aggregation of tonofilaments in disorganized fascicles which are mixed with many vacuoles and ribosomes. × 9 000. (*b*) High magnification of part of Fig. 5 *a*. Desmosome-like structures are noted between the apposed cytoplasmic membrane showing invagination. × 38 500. *CM*, cell membrane; *DL*, desmosome-like structure; *TJ*, tonofilament.

a few half-desmosome-like structures were seen on the cell membrane of some keratinized cells.

The intranuclear inclusion bodies resembling viral particles were not detected.

DISCUSSION

Our study demonstrated that tonofilaments were decreased in quantity and desmosomes were increased in number although both structures were reported to be almost normal by Zelickson (11). These results are almost in agreement with those of Arao & Kuwabara (1). Though it is not altogether certain whether the increase of desmosomes, as seen in our case, is an invariable feature of keratoacanthoma or not, this finding is in contrast to the decrease of desmosomes as was usually recognized in squamous cell carcinoma (3, 10).

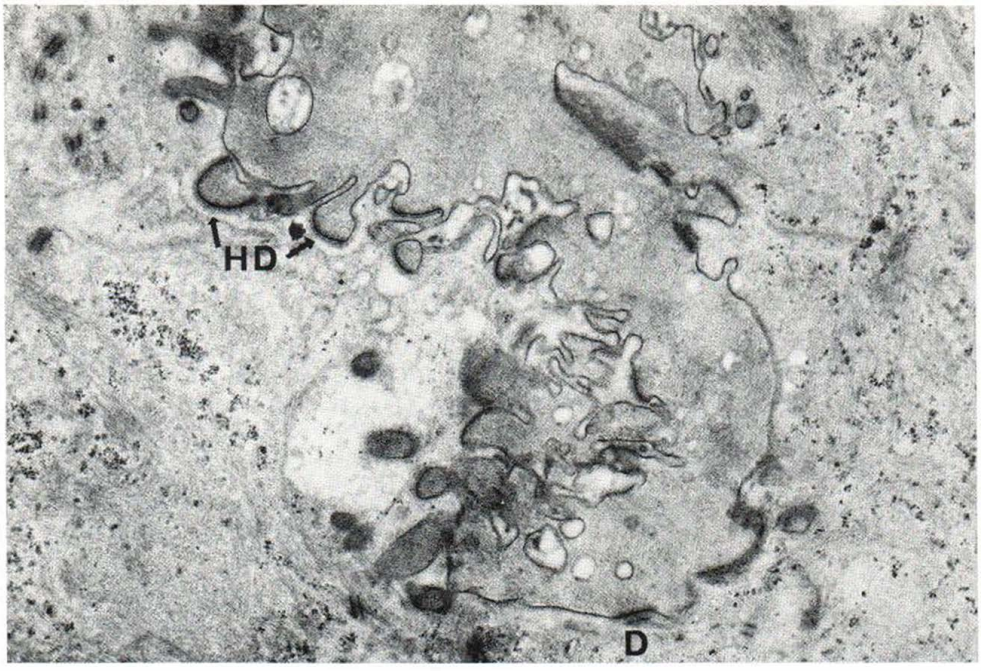

Fig. 6. Compact keratinous mass in which the filaments show a higher opacity than in normal keratin pattern. Half-desmosome-like structures are seen on the cell membrane. *D*, desmosome; *HD*, half-desmosome-like structure. × 25 000.

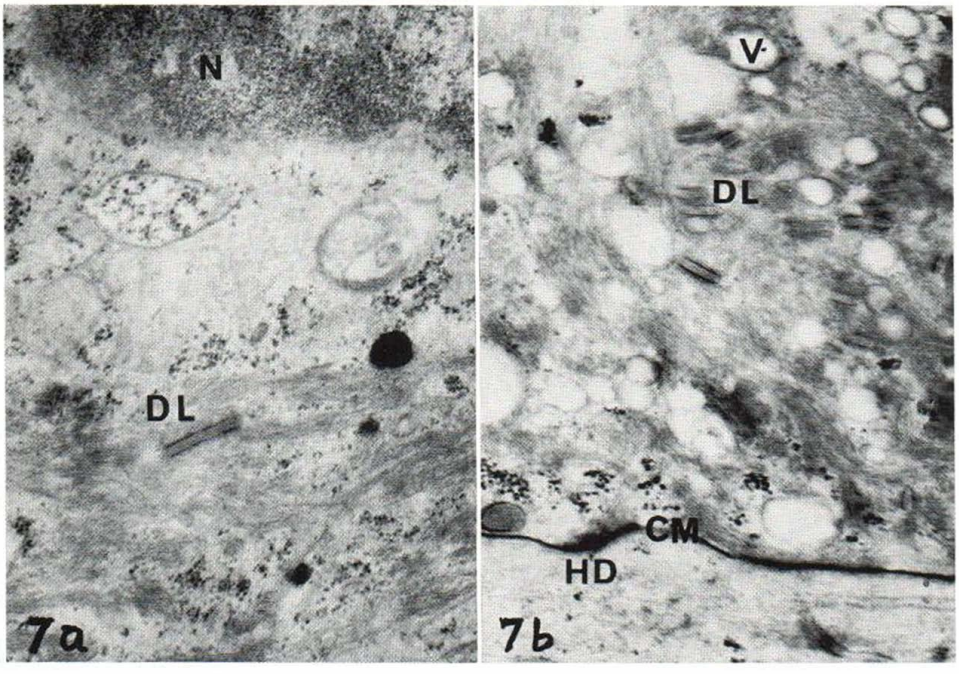

Fig. 7. Desmosome-like structures are seen within the cytoplasm of keratinized cell. Most of them reveal characteristic internal structures of desmosome. *DL*, desmosome-like structure; *HD*, half-desmosome-like structure; *N*, nucleus; *V*, vacuole. (*a*) × 35 000; (*b*) × 30 000.

Prutkin (7) has reported the ultrastructure of the epithelial tumor induced by dimethylbenzanthracene in ear auricles of the rabbits. This tumor is said to have similar clinicopathologic features to keratoacanthoma in man. According to his follow-up study, subcellular changes in the progressing stage increase the nucleus–cytoplasm ratio, accelerate formation of tonofibrils, increase the number of mitochondria, rough-surfaced endoplasmic reticulum and RNA particles, and also enlarge the intercellular spaces; in the regressing stage, degeneration of mitochondria and development of electron-dense, homogeneous bodies, and in later stages, autolysis of the various cell organelles are found. In addition, so-called intranuclear inclusion bodies are detected first in the late regressing stage but not in the progressing or mature stage. The findings, in our case, are similar to those in the mature or early regressing stage of above-mentioned experimental keratoacanthoma except for the decrease of tonofilaments and increase of desmosomes.

It is of interest that desmosome-like structures were occasionally found to be located within the cytoplasm of abnormally keratinized cells. In addition, in some instances, desmosome-like structures were noted between the apposed cytoplasmic membrane showing invagination. Recently Seiji & Mizuno (9) recognized the presence of these structures in the cytoplasm of the dyskeratotic cells undergoing mitosis in a case of Bowen's disease. They suggested that the dyskeratotic cell may lose its contact with surrounding epidermal cell during cell division and incorporate the desmosomes including part of the adjacent cell cytoplasm. It is of further interest that this phenomenon was observed in hyperplastic lesion such as keratoacanthoma as well as Bowen's disease. It may have a certain relation to abnormal keratinization though the exact mechanism remains unknown. On the basis of our findings, it is conceivable that under abnormal keratinization the cell loses its contact with neighbouring epidermal cells and then incorporates desmosomes into its own cytoplasm.

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Y. Takaki, M.D.
Department of Dermatology
Juntendo University
School of Medicine
Hongo, Bukyo-ku
Tokyo
Japan