Ultrastructural Evidence for Destruction in the Halo Nevus

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SUMMARY

Nine halo nevi in various stages of regression were examined by electron microscopy for fine structural evidence of an immunological mechanism of tumor cell destruction and halo formation. Early regressing lesions (Stage I) showed nevus cells associated with infiltrating lymphocytes, monocytes, and plasma cells, but without nevus cell destruction. In later lesions (Stages II and III), vacuolar cytolysis was commonly observed in nevus cells. In Stage III lesions, portions of nevus cells are found within macrophages. The electron microscopic findings of lymphocyte, monocyte, and plasma cell infiltration of the tumor followed by vacuolar cytolysis support the concept of an immune reaction in regressing halo nevi.

INTRODUCTION

Halo nevus is a spontaneously regressing mole which develops an enlarging area of depigmentation about the disappearing lesion. The presence of an accompanying inflammatory response in the lesion is taken by some investigators as morphological evidence for an immunological mechanism of tumor cell death and macroscopic halo formation (17). Recently, circulating antibodies against the cytoplasm of melanoma cells have been demonstrated in patients with regressing halo nevi (3, 18). The authors of these papers suggest that halo nevus represents the successful early rejection of melanoma. A cell-mediated response in melanoma patients with regressing halo nevi has also been reported (5).

The mechanism of halo formation is not known, but it may be secondary to the inflammatory response (15) or to a cytotoxic antibody against melanocytes (14). Halos, in addition to being present around benign moles (21), may be found around spontaneously regressing melanoma (6), about moles and metastases in patients treated surgically for melanoma (26), or about primary and secondary melanoma in patients who respond to either nonspecific immune stimulation (2) or adoptive immunization (26). The work of Lewis and Copeman (18) and of Copeman et al. (3) has provided information on which to base a hypothesis for an immune mechanism of tumor cell destruction in halo nevus syndrome. We have examined 9 halo nevi for fine structural evidence of such an immunological mechanism.

MATERIALS AND METHODS

Seventy-five halo nevi from 6 patients were examined clinically (including Wood's light scan). Nine of the 75 halo nevi in different stages of regression were removed from 4 patients, and a compound nevus was excised from each of 2 patients, neither of whom revealed evidence of past or present halo nevi or malignant melanoma.

Small portions of epidermis and dermis including the nevus, surrounding halo, and a narrow rim of normal skin were obtained by punch biopsy from midback, scapular, thigh, or deltoid regions. Biopsy was done under local anesthesia by a peripheral nerve-block technique. Excised specimens were immediately immersed in 2% glutaraldehyde in 0.1 M phosphate buffer. Fixed specimens were cut in half, and one half was placed in formalin and processed for routine histology. The other half was dissected into 3 portions: normal skin, halo, and mole. Extraneous fatty tissue was removed and the specimens were minced into 1-cm blocks. Specimens were rinsed several times in 0.1 M phosphate buffer followed by postfixation in 1% osmium tetroxide in 0.1 M phosphate buffer. The fixed specimens were dehydrated in a graded series of alcohols and embedded in Luft's 1:1 epon mixture (19). Thick and thin sections were cut on a Sorvall MT2 ultramicrotome. Thin sections were poststained with uranyl acetate and lead citrate and examined in either a Philips 300 electron microscope or a Zeiss EM 9A electron microscope.

RESULTS

Clinical Examination

Each of the 75 halo nevi was classified in 1 of the following 4 main stages of clinically apparent regression.

In Stage I were inflamed nevi that showed erythema and edema. In Stage II were erythematous and slightly raised nevi with faint halo formation. The halo was more pronounced under 3600-A light (Wood's lamp). In Stage III were flat and less pigmented nevi without erythema but with halo formation evident under natural light. In Stage IV were oval white depigmented areas of epidermis without clinical evidence of nevi.

Light Microscopy

Stage I. Nevus melanization appeared intact in the epidermis. The papillary dermis was edematous with some...
vascular dilation, but nevus cell nests were intact. In the reticular dermis there was an early infiltration of mononuclear cells at the margins of the tumor.

**Stage II.** Patchy demelanization was evident in the epidermal basal layer overlying the tumor with more marked loss of melanin in the epidermal basal layer peripheral to the tumor. There was minimal infiltration of the junctional nests of nevus cells by mononuclear cells in the papillary dermis. The reticular dermis showed marked infiltration of the nevus cell nests by these mononuclear cells associated with degeneration of the nevus cells. Occasional nevus cells had pyknotic nuclei and a finely vacuolated cytoplasm.

**Stage III.** Complete demelanization of the peripheral epidermal layer was present; it was present to a lesser extent in the epidermal basal layer overlying the tumor. Fewer nevus cells, mononuclear cells, and melanophages were present in the papillary dermis. Similarly, in the reticular dermis fewer histiocytes, mononuclear cells, and nevus cells and an occasional melanophage were present. In Stages I to III, mononuclear cells were seen adjacent to some nevus cells (oil immersion), as well as clustered about and within nevus cell nests.

**Stage IV.** The remaining halo was histologically identical to vitiligo.

**Electron Microscopy**

**Stage I.** The inflammatory cell response was made up of both lymphocytes and monocytes. Both types of cells were found between the widened endothelial cell junctions of the reticular and papillary dermal vessels. These cells were present in the dermis adjacent to nevus cell nests. Occasionally, plasma cells were found in these extravascular locations (Fig. 1). When present they were found sometimes in contact with nevus cells. At plasma cell-nevus cell junctions, the cell membranes of both cells were thickened and electron-dense material was found between the 2 opposing membranes (Fig. 1).

Lymphocytes and monocytes were also within the nevus cell nests, surrounding the nevus cells, and separating them. When lymphocytes and nevus cells were in contact (Fig. 2), the involved cells had thickened cell membranes, and small ruptures were present along the points of contact. Electron-dense flocculent material was found between the 2 membranes (Fig. 3). Vacuoles were not present in the nevus cells in either Stage I or the control nevi.

**Stage II.** Small cytoplasmic vacuoles were found in some isolated nevus cells (Fig. 4). These vacuoles appeared to be derived from the endoplasmic reticulum (Fig. 4) and were filled with medium electron-dense granular material. Other nevus cells contained large, electron-lucid vacuoles. These cells had small amounts of melanin (Fig. 5) in their cytoplasm.

**Stage III.** Nevus cell destruction was extensive. Nuclei were somewhat separated from cytoplasm which contained elongated vacuoles (Fig. 6), and macrophages contained phagocytized portions of nevus cells. The phagocytized nevus cell cytoplasm contained electron-lucid vacuoles in addition to degenerated organelles (Fig. 7). In Stage II and III halo nevi, lymphocytes were found in the epidermis (Fig. 8). Nevus cells in junctional nests also contained vacuoles and appeared necrotic. Dendritic basal melanocytes were rounded with vacuolar change of the cytoplasm (Fig. 9). Langerhans cells were not affected by the degeneration process. Keratinocytes exhibited the same vacuolar change as nevus cells and melanocytes, but only around the membrane-bound complex melanin granules (Fig. 10). Keratinocytes with large vacuoles had a complete absence of melanin.

**Stage IV.** In the well-developed halo, keratinocytes were virtually free of vacuoles and there was a total absence of melanin (Fig. 11). In the dermis of the halo area there were disrupted mast cells, lymphocytes, pigment laden macrophages, and occasional nevus cells.

**DISCUSSION**

The nature of the inflammatory response and the tissue necrosis in halo nevus have been examined by many investigators (7, 8, 10, 13, 25, 29). The mononuclear infiltrate and the mechanism of depigmentation have been discussed but not resolved. Fine structural studies (1, 4, 27) have focused on the depigmented epidermis, the absence of melanocytes and melanin, and the replacement of melanocytes by Langerhans cells. Abnormal nevus cells with vacuolated mitochondria were reported by Epstein et al. (5), but these authors found little or no inflammatory response about the examined halo nevi. Stegmaier et al. (25) noted inflammatory cells surrounding nevus cells and suggested that vacuolated cells lacking melanin in the dermis might be degenerated nevus cells.

It is well known that malignant melanoma can arise in junctional or compound nevi (24). The recent evidence of Lewis and Copeman (18) and Copeman et al. (3) suggests that the halo nevus may represent the host rejection of an early malignant melanoma.

Evident from the present study is the structural delineation of a progressive involution of a melanocytic nevus (including melanocyte and nevus cell death) and the subsequent evolution of the depigmented halo.

From the morphology of the tissue obtained from halo nevi in different stages of regression, a scheme of events can be suggested that seems to correlate with available published immunological data. The earliest change in the lesion is a migration of lymphocytes and monocytes from local vessels into the tumor mass. Nevus cells that are tightly nested in both the dermis and epidermis are separated and surrounded by the inflammatory cells. Attachment of inflammatory cells to nevus cells causes focal nevus cell membrane destruction.

At this point a diffusible cytotoxic substance (lymphotoxin) may be released by the lymphocytes, causing necrosis of basal melanocytes and nevus cells through osmoregulatory disruption (cytoplasmic vacuolarization). It has been shown by Russell et al. (23) that lymphotoxin, when applied as a purified compound to cultured target cells, does...
produce a vacuolar degeneration. This type of cytolysis is identical to that observed between target cells and sensitized lymphocytes (22). Stegmaier et al. (25) have suggested the presence of a diffusible cytotoxic substance in developing halo nevi, because the halo develops while melanocytes are 3-(3,4-dihydroxyphenyl)-L-alanine-positive and only a few lymphocytes have invaded the epidermis.

Langhof et al. (16) reported the presence of an anti-melanin antibody in patients with vitiligo. A critical review of this work by Wasserman and Van Der Walt (28) concludes that melanin is not antigenic and that the melanin preparation of Langhof’s group contained melanocyte cellular debris. We also believe that melanin is not antigenic because the cytoplasmic vacuoles in nevus cells and melanocytes appear to originate from the endoplasmic reticulum, not premelanosomes or melanosomes. This belief is further strengthened because keratinocytes in Stage III halo nevi undergo cytolysis only around the membrane-bound complex melanin granules which contain small bits of phagocytized melanocyte cytoplasm (9, 20). The vacuoles in keratinocytes from Stage III halo nevi disappear by Stage IV, and dead and dying keratinocytes are not found in the epidermis. Keratinocytes also are not sloughed, because hyperkeratosis and scaling are absent in the depigmented halo and the epidermis overlying the regressing nevus.

Dr. William Terry and his colleagues (personal communication) have demonstrated that cultured lymphocytes from halo nevus patients quickly attach to several different lines of cultured human melanoma cells. The tumor cells swell, become vacuolated, and die. If sheep erythrocytes are added to the culture mixture and the supernatant is removed, the lymphocytes attach to the sheep erythrocytes and few lymphocytes remain attached to melanoma cells. This evidence points to a possible T-cell-mediated response, in addition to the humoral response (3, 18). Melanoma patients with regressing halo nevi have intact or increased delayed hypersensitivity reactions (5). A strongly positive delayed hypersensitivity reaction has been noted in one of our halo nevus patients (11). Induced delayed hypersensitivity reactions to synthetic haptenes and microbial antigens have been shown to result in regression of a number of human skin tumors when reactions are induced at tumor sites (12). Klein and his associates have observed that cutaneous metastases of melanoma convert to “benign halo nevi” with dense mononuclear infiltrates following nonspecific immunotherapy with dinitrochlorobenzene. Biopsies from these lesions prior to the immune challenge showed an absence of cellular infiltrate.

Although the data to date are insufficient to answer the question pertaining to the possibility that halo nevus represents an early host-rejected melanoma, further investigation of this lesion should be of value as a human model for studying the mechanism of host destruction of a neoplastic population of melanocytes.

ACKNOWLEDGMENTS
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REFERENCES


Figs. 1 to 11. Stain used: uranyl acetate + lead citrate.

Fig. 1. Extravascular plasma cell (P) attached to nevus cell (N). Note electron-dense material between cells (arrows). × 14,000.

Fig. 2. Lymphocyte (L) attached to nevus cells (N). Note the increase of electron density of both cells along contacting cell membranes (arrow). × 10,000.

Fig. 3. High-power view of Fig. 2, showing increased electron density of cytoplasm of both cells and electron-dense material between contacting cell membranes (arrows). × 18,000.

Fig. 4. A single nevus cell undergoing early vacuolar changes. The vacuoles are filled with medium electron-dense material (arrows). × 5,000.

Fig. 5. High-power view of a nevus cell showing early vacuolar cytolysis from endoplasmic reticulum (Arrow 1) and later vacuole change showing fission of vacuoles (Arrow 2). × 15,000.

Fig. 6. A late stage of nevus cell degeneration. The cytoplasmic vacuoles have coalesced to form channels in the cytoplasm separating cellular components (arrows). A lymphocyte (L) is attached to the degenerating nevus cell. × 7,600.

Fig. 7. A macrophage with 2 phagocytized bits of nevus cells (Arrow 1). Each phagocytized portion of nevus cell contains a single melanosome (Arrow 2). × 8,000.

Fig. 8. A lymphocyte (L) within the epidermis is adjacent to a necrotic keratinocyte and the epidermal basement membrane (BM). × 12,000.

Fig. 9. A condensed melanocyte (M) undergoing vacuolar cytolysis (V). Note the vacuoles in and about the complex melanin granules of the keratinocytes (arrows). × 8,000.

Fig. 10. In keratinocytes, vacuoles are present in or about the complex melanin granules (arrows). × 7,400.

Fig. 11. Keratinocytes in the well-developed halo are devoid of melanin. Note the desmosome (arrow). × 7,000.
Cell Destruction in Halo Nevus
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