Ultrastructural Observations on the Hair Bulb Melanocytes and Melanosomes in Acute Alopecia Areata

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It is well recognized that alopecia areata (Aa) may preferentially affect pigmented hair and may spare white hair, and that regrowing hair in the disease is often initially white. In addition, there is an association with vitiligo and ocular depigmentation. To date, the pathomechanisms of the melanocyte effects are unclear. We have studied 10 patients with untreated acute alopecia areata, and three normal patients without hair loss.

Morphologic changes, studied by conventional light and electron microscopy, in the cytoplasm of affected melanocytes often predated nuclear hyperchromatism. Increased numbers of bizarre melanosomes were found in affected melanocytes compared with normal ones; such melanosomes had incomplete or "aborted" melanization, resulting in poor pigment deposition, and were disrupted, enlarged and rounded, with loss of normal ellipsoidal shape. An unusual outer root sheath (ORS) distribution of hair bulb melanocytes was seen.

Other atypical melanosome effects included marked pigment displacement into peribulbar and DP melanophages. In the DP clumped melanin granules formed giant spherical complexes without discernible limiting membranes, which were sometimes associated with lymphocytes.

These morphologic changes indicate an active involvement of hair bulb melanocytes in alopecia areata. J Invest Dermatol 94:803–807, 1990

Alopecia areata (Aa) is believed to be characterized by the sudden precipitation of anagen stage III hair follicles, at the site of disease activity, into early catagen. Though light microscopic (LM) studies of this condition are numerous [1–4], there is still a relative paucity of ultrastructural reports. These studies [5–7] outline the major histopathologic changes in Aa, which include lymphocytic infiltration to the matrix and ORS of the hair bulb, edema of the DP, rupture of the BL separating the matrix and the DP, catagen-like DP, and autophagic vacuolization of the hair bulb cells.

The importance of each of these events in the overall pathomechanism of the condition is unclear due to the difficulties in standardizing disease activity. Working with regrowing white hair from Aa patients, Messenger and Bleehen were [7] of the opinion that the primary target cells in Aa were the upper bulb pre-cortical keratinocytes. The failure of such cells to keratinize properly resulted in the production of a defective fiber. The striking keratinocytic degeneration observed by Messenger is impressive, though normal anagen-catagen transformation may account for some of the changes.

Melanocyte degeneration in Aa has, to our knowledge, not been reported previously. This may be because it was overlooked or because patient or lesion selection had not been precise enough.

MATERIALS AND METHODS

Biopsy specimens were excised with a 4-mm punch biopsy instrument after lignocaine hydrochloride (2% w/v) anaesthesia, with informed consent, from selected untreated areas of alopecia in the scalps of 10 adults. Control biopsies were taken from three normal adult scalps. The age range of patients and normals was 15–32 years.

Though duration of hair loss was variable, biopsies were taken only during periods of acute and active hair loss and from sites of maximal disease activity. Biopsied material included skin from within, on, and over the advancing border of active lesions and contained some exclamation mark club hairs. Sites of long-established or regrowing lesions were avoided.

A minimum of seven follicles from each biopsy was fixed immediately after excision for 4 h in Karnovsky's fixative, a mixture of 2% formaldehyde and 2.5% glutaraldehyde [8] buffered to pH 7.4 with 0.1 M sodium cacodylate buffer, and kept overnight at 4°C in 70% ethanol.

The biopsies were post-fixed in cacodylate-buffered 1% osmium tetroxide for 1 h, dehydrated in graded ethanol, and embedded in TAAB 812 resin.

One-micrometer and ultrathin sections were cut with a LKB Ultratome. Semithin sections were stained with methylene blue/azure II and examined by LM. Ultrathin sections were stained with uranyl acetate and lead citrate and then examined in a Hitachi H300 electron microscope.

RESULTS

Gross Morphology of Affected Melanocytes Melanocytes of the controls had the characteristic features of normal follicular melanocytes with melanosomes present in all four stages of synchronized pigment deposition, as outlined by Toda and Fitzpatrick [9].
The most striking change in severely affected hair bulb melanocytes was the greatly increased electron density of both the nucleus and cytoplasm. Some cells were seen in late stages of cell death, while others were in early stages (Fig 1).

Affected pigment cells were generally dendritic, and located at or close to the DP basal lamina in the mid-bulb region of the follicle. Groups of three or more affected cells were common here, in direct contact or more usually scattered individually.

In three patients, ORS melanocytes were present at or about the critical level of the bulb, i.e., at or about the level of the DP (Fig 2). These melanocytes appeared unaffected, showing no cellular hyperchromatism or melanosomal abnormality; however, they were small and non-dendritic, with fewer melanosomes.

The morphologic changes associated with cell death were distinct from both apoptosis or coagulative necrosis/pyknosis. Nuclear pyknosis was not the dense clumping and margination of chromatin seen in apoptosis; instead, the increased electron density was homogeneous throughout. The nuclear and cytoplasmic condensation was not associated with fragmentation of the cell into a number of membrane-bound, ultrastructurally well-preserved entities. While apoptotic bodies of keratinocytic origin were present within keratinocytes, few of such bodies were of melanosomal origin.

Organelles in affected cells were discernible in the early stages of degeneration, though these were often hypertrophied.

Melanophages, heavily laden with pigment, were common, but engulfed fragments of melanocytes were not seen. Marked pigment displacement occurred in apparently "amelanotic" hair bulbs within numerous DP melanophages and peribulbar macrophages. Macrophages were more common than normal in the alopecic hair bulb matrix, apparently entering via the DP (Figs 3 and 4). The direction of their movement was inferred by the manner in which the BL ruptured. No inflammatory response was associated with the cell degeneration seen.

In hair bulbs, where neither melanocytes nor pigment could be identified, dendritic cells, including Langerhans cells (LC), were present (Fig 5). LC and closely associated plasma cells were seen in some dermal papillae, often in close proximity to melanin complexes. Such cells of the immune system are not usually found in these regions of the normal hair follicle.

**Ultrastructure of Affected Melanocytes**

Affected cells were packed with melanosomes presenting a variety of bizarre appearances (Figs 6 and 7). Rounded rather than ellipsoidal, the internal structure of these melanosomes was variable in amount and organization of deposited pigment; some were quite empty. The well-defined melanization sequence of normal melanocytes as described by Toda and Fitzpatrick [9] was lacking in many of these cells. The only other cell organelle prominent in all the affected melanocytes was the Golgi system, which was often hypertrophied, with distended, disrupted, and irregular cisternae (Fig 6).

Giant spherical melanin complexes occurred in the hair bulb of some follicles, composed of many individual, fully melanized melanosomes, differing from the "giant melanosomes" of other conditions such as nevi. Up to 100 individual melanosomes were present in these non-delimited complexes (Fig 8), which were located in the matrix close to the DP or within DP cells. Giant melanin complexes were also seen in the DP or abutting the basal lamina. Rupture of the BL occurred close to the complexes. Lymphocytes were seen in some cases close to such pigment complexes.
DISCUSSION

While melanocyte involvement is believed to be a component in the pathogenesis of Aa, it is unclear whether this is a primary or secondary feature of the disease. Galbraith et al. [10] suggested from Western blot analysis of serum antibody reactivity with melanoma cell antigens in Aa that autoreactivity to pigmented cells occurs in certain patients with Aa.

Some authors have selected long-established lesions as their choice of biopsy site, while others have chosen regions of regrowth, where follicular activity is near normal; both have their inherent drawbacks. Of particular importance to us is the examination of biopsies containing exclamation mark club hair follicles, a reliable indicator of disease activity.

An unexpected finding in the hair bulbs of Aa patients was the unusual occurrence of melanocytes in the ORS. Melanocytes are normally found here only to the level of the sebaceous gland. Whether this location in Aa is a consequence of any disease process is unclear. Such cells were invariably smaller, non-dendritic, and weakly melanized. On comparison with normal melanocytes (Fig. 1), differences in their overall morphology are obvious. Interpretation of this is difficult due in part to the shrinkage artefact in Fig. 2. However, a reduced number of melanosomes and lack of dendrites in the ORS melanocytes suggest a lowered melanocytic activity in these cells.

The main features of the affected melanocytes, both at the DP/matrix junction (in groups of 2 to 4) and in the inner matrix (scattered singly), were nuclear and cytoplasmic condensation, indicative of impending cell death, and dystrophy of the melanosomes and associated Golgi systems. Condensation was homogeneous throughout, without the nuclear chromatin margination seen in early stages of apoptosis [11].

The intact plasma membranes and their close adherence to normal neighboring matrix cells distinguish this type of cell degeneration from that of apoptosis, while the retention of post-pyknotic cytoplasmic organelle integrity likens it [12]. A third type of cell death, originally described by Johannsson [13] and called dark cell phenomenon, more closely relates to the morphologic features found in many of the affected melanocytes. Though the chromatin is often seen to be focally clumped in this type of cell death, there is no overall segregation.

Apoptotic bodies, which characteristically increase in number during anagen-catagen transformation [14] and keratinocyte degeneration in severe alopecia areata [7], were also observed at an increased level.

Whether the degenerative changes reported here are correlated with stages in the hair-growth cycle, or are truly the result of a pathologic process, must be considered. As only occasional melanocytes are affected, the ultrastructural changes do not appear to be closely related to any growth-cycle change. Increased cell death among follicular melanocytes may be expected at catagen and telogen, during follicular involution, and the paucity of identifiable melanocytes in these follicles may reflect either increased cell death or failure to identify them due to their amelanotic status in this growth stage.

Sugiyama et al. [15] suggest that bulb melanocytes do survive the follicular cycle, undergo dedifferentiation, and thereafter differentiate and repopulate the next anagen follicle.

Superficially the aberrant melanosomes resemble those of choroidal melanocytes studied by Hu [16]. Hu observed qualitative and
quantitative changes in melanosomes of the rhesus macaque and the correlated them with increasing age. Similar progressive ultrastructural changes were also seen in lentigines and nevi of human skin where they were described as an aging population of skin melanocytes. Marked melanosomal vacuolization was the principal feature of Hu’s study.

Boissy et al [17] observed degenerative melanoctytic changes in the hair bulbs and eyes of the vitiligo mouse C57BL/6J Ler-vit/vit. Necrotic cells were found at the BL, which was often ruptured due to herniation of melanocytes into the DP. Breathnach [18] also noted similar vacuolization in dispersed tissue cultures of normal melanocytes. It can only be concluded that, whatever the cause, melanosomes in Aa have incomplete or abnormal assembly and melanization of internal components. This “abortion” of melanogenesis is indicative of a grave abnormality of the normal synthetic processes of the cell, and could give rise to toxic-by-products leading to cell death.

The marked pigment displacement present in some anagen bulbs of Aa patients (Fig 3) result from phagocytosis by macrophages and DP melanophages. In some cases the anagen matrix seemed amelanocytic though anagen is normally characterized by an increase in melanocyte number and activity.

The pigment free matrix contrasted sharply with pigment-laden connective tissue components of the hair bulb. Such pigment may be picked up, either from the DP or as the result of infiltration of the matrix by histiocyes, and subsequent removal of the granules via the DP vasculature.

In addition pigment-free histiocyes were seen to infiltrate the matrix. The direction of histiocytic movement was inferred by the way in which the BL was ruptured, i.e., BL material was found around the advancing end of the cell. The sheer volume of displaced pigment indicates abnormal melanocyte function in these apparently “amelanocytic” bulbs, as pigment amount is consistent with a normal melanocytic population in the matrix.

Pigment granules, 1 μm or less in length, which are transferred to keratinocytes and dermal papilla fibrocytes during normal hair growth, form small membrane-bound complexes. Consequently, the presence of giant apparently non-delimited melanosome complexes, up to 8 μm in diameter and containing up to 100 individual granules is probably the result of abnormal melanocyte/melanosome function. Similar spherical complexes are also seen in dermal melanoma cells [16]. All cells types in the region are similarly affected. The fully melanized complexes appear to be removed from the matrix by movement into the dermal papilla through the BL which ruptures in areas with these complexes. Although reduced melanocyte activity in Aa is widely appreciated, little ultrastructural evidence of the associated melanocyte degeneration exists. It is, however, unclear from the above results what part, if any, such abnormal morphologic changes play in the etiology of the disease, or whether they are secondary to some other process. The current belief that the disease has an immune component does not conflict with our findings, as a lymphocytic association was observed with some morphologic changes. The increased occurrence of LC and macrophages, both within the epidermal and dermal follicular components and plasma cells in some dermal papillae studied (unpublished observation), reflect a raised immune response in these lesions.

As a purely ultrastructural study of the hair bulb melanocyte in Aa, direct functional interpretations cannot be made. However, the significance of an active attack on melanocytes in the etiology of Aa or the possible mechanism of their induction may possibly be ascribed in part to a change in the integrity of the “epidermal-melanin unit” within the pilosebaceous system.

Melanocytes can be damaged by a build-up of toxic byproducts and free radicals generated during melanogenesis without a parallel increase in the production of the scavenger melanin [19]. The majority of the affected melanocytes in this study had a greatly increased number of melanosomes compared with unaffected cells, but with a distinct paucity of melanin. In addition, toxic products
released from affected cells are also likely to damage neighboring keratinocytes and cause a decrease or loss of melanocyte support by keratinocytes within the functional pigmentary unit. Keratinocytes are known to support the proliferation and viability of melanocytes by the production of basic fibroblast growth factor (bFGF) [20]. Defective keratinocyte function may ultimately lead to deficient fiber formation. A secondary autoimmune response to altered cell-surface proteins on damaged melanocytes and possibly keratinocytes, in addition to intra-cellular antigens sharing homology with the cell surface, like melanosomes [19], may finally lead to a considerable or irreversible loss of melanocytes.

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REFERENCES


Figure 7. Packing of bizarre melanosomes in affected melanocyte in Aa. Negligible pigment deposition in swollen melanosomes (SM) in the hair bulb matrix of a 32-year-old female with Aa. Bar, 1 μm.

Figure 8. Giant spherical melanosome complexes in Aa. Melanin complexes (MC) at ruptured basal lamina (RL), large apoptotic bodies (AB) and vacuolated melanocytes (VM) in the hair bulb of a 25-year-old male with alopecia areata. Bar, 5 μm.