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Morphology of Melanocytes in Hair Bulbs and Eyes of Vitiligo Mice

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The vitiligo mouse C57BL/6J Ler-*vit/vit* is a new, murine model for vitiligo in humans. It was studied with respect to morphology and fine structure of melanocytes in hair and eyes before and during depigmentation. The coat of vitiligo mice lightens progressively with age because of an increase in the ratio of white to pigmented hairs with each molt. The bulbs of white hairs are devoid of pigment, and they lack melanocytes. In other respects the epithelium is morphologically normal as determined by light and electron microscopy. The bulbs of pigmented hairs are histologically normal. By electron microscopy, however, some of the melanocytes are shown to have undergone degenerative changes. In addition, disruption of the basement membrane underlying the melanocytes and herniation of melanocytes into dermal papillae were observed at various stages of hair growth. Papillary melanophages are prominent in pigmented as well as in white hair bulbs. Newborn vitiligo mice have no uveal pigment. Pigment appears in the iris and ciliary body by Day 4 and in the choroid by Week 3. On Day 4, along with pigmentation, conspicuous spherical amelanotic cells appear over the anterior border of the iris. These cells become numerous in the ensuing weeks and gradually acquire large melanophagosomes. They occur also in the stroma of the iris and the ciliary body, associated with necrotic melanocytes. The spherical cells are identical to the clump cells of Koganei and are far more numerous in vitiligo mice than in controls. Macroscopically, no progressive decrease in iridial pigment is apparent for the life of the vitiligo mouse. In the choroid, an amelanotic patch surrounds the optic nerve. In the pigmented areas, melanocytes show compartmentalization of melanosomes and degeneration. The retinal pigment epithelium generally appeared continuous. In older animals some epithelial cells contained large fat bodies or were devoid of melanin. (Am J Pathol 1987, 127:380–388)

A MURINE MODEL for vitiligo, an acquired cutaneous depigmentary disorder,[1,2] has recently been established and given the provisional genetic designation C57BL/6J Ler-*vit/vit*.[3] Vitiligo in human patients is due to progressive patchy loss of cutaneous melanocytes, usually seen first in adolescence or early adulthood but occasionally also in children. In the vitiligo mouse, the acquired depigmentation in a progressive replacement of pigmented hairs by white hairs during successive molts or after hair plucking.[3] Dopa-reactive melanocytes disappear from both the hair follicles and ear epidermis by 6 months of age.[4] Reciprocal skin transplants between C57BL/6J and vitiligo mice and transplants onto nude mice suggest a programmed pigment cell death.[3] Also, necrosis of some keratinocytes and nonmelanocytic dendritic cells was observed in the epidermis of the ear.[4]

We report here histologic and ultrastructural studies on anagen hair bulbs during early stages of coat depigmentation. In addition, we have investigated the status of ocular pigment in young and adult vitiligo mice, because ocular pigment abnormalities may occur in human vitiligo patients[5] and always occur in the Smyth vitiligo chicken.[6] Our observations indicate that the vitiligo mouse mimics important pathologic events in human vitiligo patients[1,7] and the Smyth chicken.[8]

Materials and Methods

Animals

Vitiligo (C57BL/6J Ler-*vit/vit*) and control (C57BL/6J) mice were propagated and maintained in

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animal care facilities at the Yale University School of Medicine.

Skin

The procedures described in this section were performed on vitiligo and C57BL/6J control mice at the ages of 7 weeks (n = 7), 11 weeks (n = 2), and 18 weeks (n = 7). Hairs were plucked from a pigmented or depigmenting dorsal area, approximately a half inch in diameter. A central 2-sq mm patch of skin was removed surgically from lightly anesthetized mice 2 days, 4 days, and 2 weeks after plucking. The specimens were fixed and processed as described below.

At 3 weeks after plucking, regenerating hairs adjacent to the biopsy sites and now in the telogen stage, were plucked and taped to microscope slides. These hairs were counted and categorized as pigmented or amelanotic with the aid of a transmission photomicroscope at a magnification of 400×.

Eyes

Mice were euthanized with ether. Immediately both eyes were removed surgically, given an injection of fixative, and then immersed in fixative, described below. After washing, the eyes were cut circumferentially and separated into a posterior portion (eye cup), which included all parts behind the ciliary body, and an anterior portion. The tissue was processed further as described in the next paragraph. During the buffer wash, the eye cup was backlit and the area of the central amelanotic patch was evaluated subjectively or photographed. Eyes were obtained from mice at the ages of 1 day (n = 4), 4 days (n = 4), 7 days (n = 3), 3 weeks (n = 3), 4 weeks (n = 5), 8 weeks (n = 4), 12 weeks (n = 3), 16 weeks (n = 4), 30 weeks (n = 3), 6 months (n = 3), 9 months (n = 3), and 12 months (n = 3).

Fixation and Processing

Tissues were fixed in half-strength Karnovsky's mixed aldehyde fixative[9] for 4 hours at room temperature, and then immersed for 1 hour in 1% osmium tetroxide containing 1.5% potassium ferrocyanide.[10] They were dehydrated in ethanol and embedded in Epon 812.[11] For light microscopy, the tissue blocks were serially sectioned at 1 μ thickness and stained with a mixture of methylene blue and Azure II. For electron microscopy, ultrathin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and viewed with a Zeiss 9S-2 transmission electron microscope.

Results

Hair Bulbs

All abnormalities described below were found in mice of all ages examined, in anagen hair bulbs 2 days, 4 days, and 2 weeks after plucking. Serial, 1-μ-thick vertical sections of skin, providing longitudinal views of hair bulbs, were scrutinized by light microscopy for the presence of melanin and melanocytes in individual hair bulbs. The ratio of hair bulbs containing or lacking pigment roughly corresponded to the ratio of pigmented and white telogen hairs harvested later from sites immediately adjacent to the biopsied sites. Pigmented hair bulbs in vitiligo mice were histologically similar to those in C57BL/6J control mice (Figure 1a and b). However, the melanocytes of vitiligo mice had irregular, crooked dendrites (Figure 1b). The bulbs of white hairs were histologically normal but devoid of melanin and melanocytes (Figure 1c).

Differences in ultrastructure between the pigmented hair bulbs of vitiligo mice and those C57BL/6J control mice were observed with respect to melanocytes and epithelial integrity. In the controls, melanocytes resided on a continuous epithelial basement membrane, and numerous melanosomes were seen in neighboring keratinocytes (Figure 2a). In vitiligo mice, two types of pigmented hair bulbs were distinguished. One appeared to contain morphologically normal melanocytes exclusively, whereas the other contained a mixture of normal and abnormal melanocytes (Figure 2b and 2c). The ratio of normal to abnormal melanocytes in the latter varied drastically among the hair bulbs of a given sample and among the different ages of animals (7, 11, and 18 weeks). Criteria for abnormality included an exten-

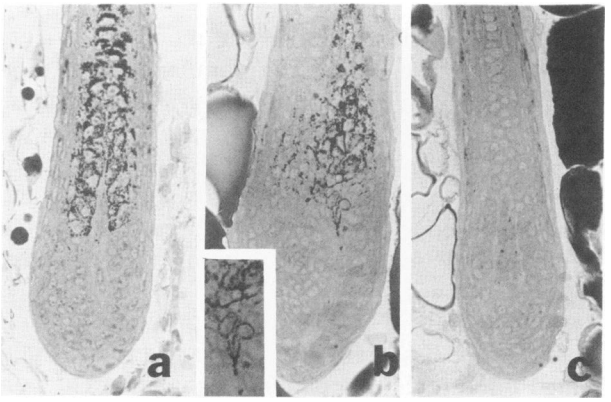

Figure 1—Histology of regenerating hairs from an 11-week-old C57BL/6J mouse showing normal pigmentation (**a**) and from an 18-week-old vitiligo mouse showing pigmentation (**b**) and amelanosis (**c**). **Inset**—Higher magnification of the melanocyte with a dendrite extending irregularly in a ventral direction. (Methylene blue and Azure II, ×118, **inset**, ×170)

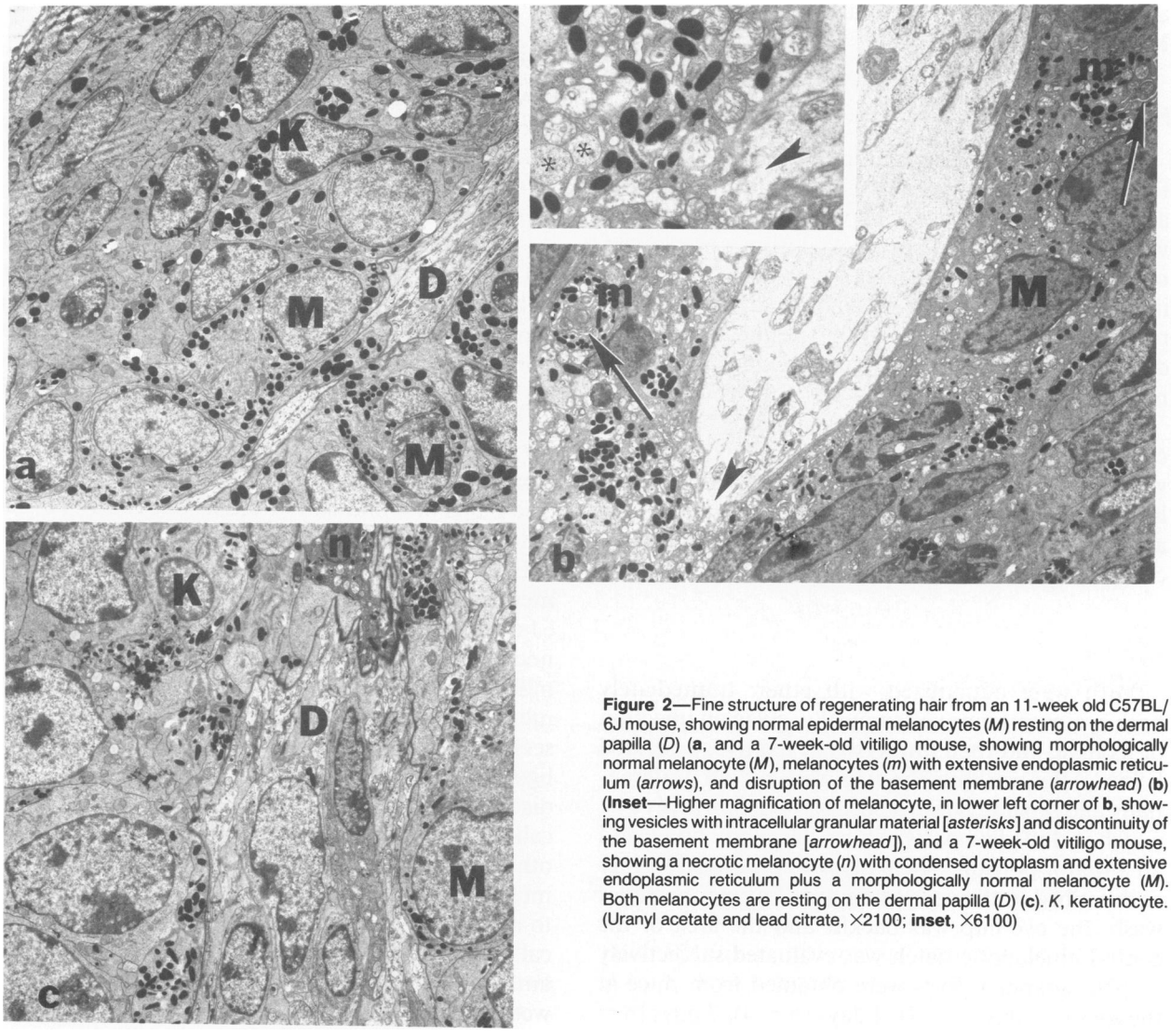

Figure 2—Fine structure of regenerating hair from an 11-week old C57BL/6J mouse, showing normal epidermal melanocytes (*M*) resting on the dermal papilla (*D*) (**a**, and a 7-week-old vitiligo mouse, showing morphologically normal melanocyte (*M*), melanocytes (*m*) with extensive endoplasmic reticulum (*arrows*), and disruption of the basement membrane (*arrowhead*) (**b**) (**Inset**—Higher magnification of melanocyte, in lower left corner of **b**, showing vesicles with intracellular granular material [*asterisks*] and discontinuity of the basement membrane [*arrowhead*]), and a 7-week-old vitiligo mouse, showing a necrotic melanocyte (*n*) with condensed cytoplasm and extensive endoplasmic reticulum plus a morphologically normal melanocyte (*M*). Both melanocytes are resting on the dermal papilla (*D*) (**c**). *K*, keratinocyte. (Uranyl acetate and lead citrate, ×2100; **inset**, ×6100)

sive, often whorled endoplasmic reticulum (Figure 2b) and/or vesicles containing granular material (Figure 2b, inset). In some cases the granular material appeared also extracellularly and, rarely, in vacuoles within keratinocytes. Necrotic cells with condensed cytoplasm were observed frequently (Figure 2c) and were presumed to be melanocytes because they were found on the basement membrane and contained free premelanosomes.

On occasion, a melanocyte appeared to have disrupted the basement membrane and to have herniated into the dermal papilla (Figure 3a). The portion of the melanocyte that extended into the papilla did not necessarily have an identifiable plasma membrane (Figure 3a). These cells contained small premelanosomes and vesicles filled with granular material. Evidence suggestive of the dropping of melanocytes into the dermal papillae was the restriction of basement membrane disruption to areas beneath melanocytes (Figure 3b) and the presence of papillary melanophages. Disruptions of basement membrane were observed beneath morphologically normal and abnormal melanocytes.

The membrane-bounded compartments in papillary melanophages (Figure 3a, inset) contained as few as 2 and as many as 22 melanin granules per ultrathin cross-sectional view. Papillary melanophages occurred in pigmented as well as in amelanotic hair bulbs of vitiligo mice. In control mice, such cells were rare, and the compartments were never seen to hold more than 5 granules per cross-sectional view.

The bulbs of white hairs lacked melanocytes. The

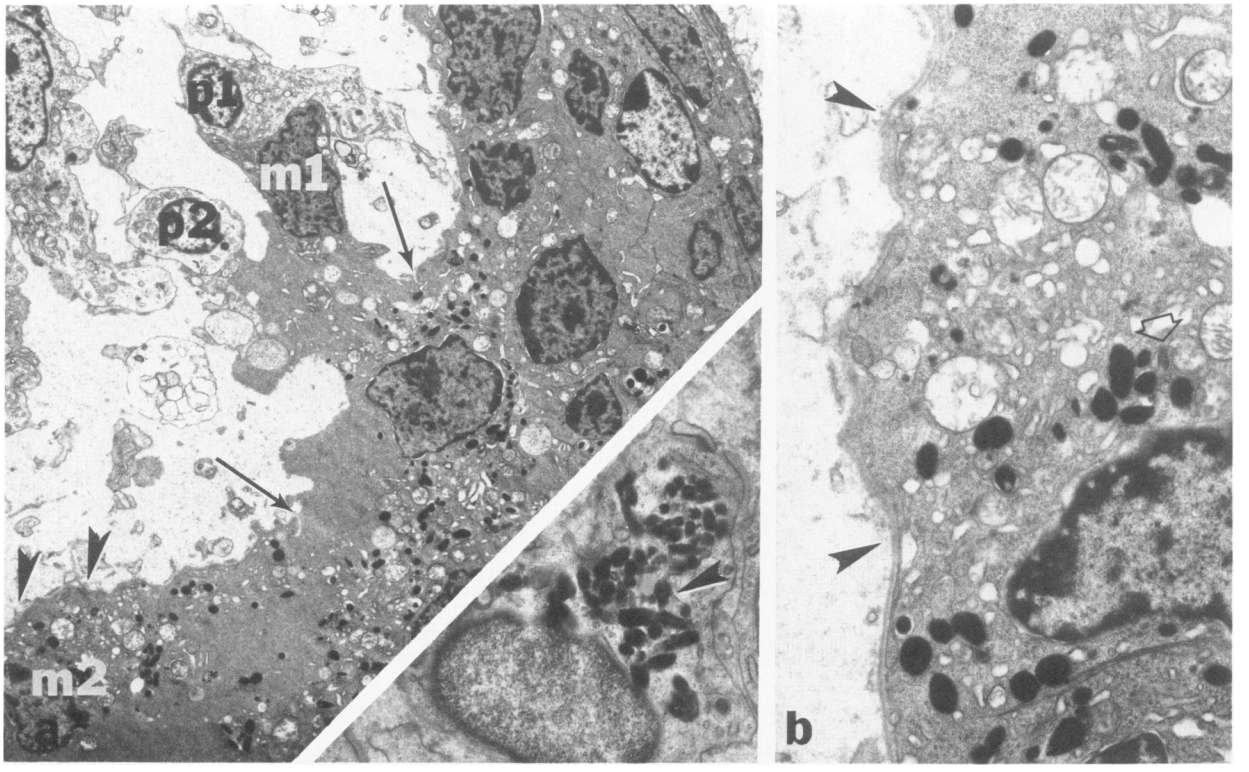

Figure 3—Abnormalities in anagen hair from vitiligo mice. **a**—Regenerating hair from a 7-week-old vitiligo mouse showing a melanocyte (*m 1*) herniating into the dermal papilla and being attacked by presumptive mononuclear phagocytes *p 1* and *p 2*. The basement membrane abruptly ends (*arrows*) on both sides of the herniating melanocyte. There is a melanocyte in the lower left corner (*m 2*) whose basement membrane is fragmented (*between arrowheads*). **b**—Higher magnification of melanocyte m 2 from Figure 3a and its basement membrane abnormality (*arrowheads*). *Open arrow* points to a premelanosome. **Inset**—Cell in the dermal papilla of an 18-week-old vitiligo mouse showing compartmentalization of melanosomes (*arrowhead*). (Uranyl acetate and lead citrate; **a**, ×2400; **inset**, ×14,000; **b**, ×9700)

pigment-free keratinocytes were morphologically normal, and the basement membranes were uninterrupted.

Eyes

The choroids of newborn vitiligo mice were amelanotic, in contrast to the pigmented choroids of newborn C57BL/6J mice. Pigment became apparent histologically by 3 weeks, but its distribution was not uniform. A central amelanotic patch, irregular in shape and surrounding the optic nerve, was present in all animals examined (n = 36) (Figure 4a). Eyes from mice ranging in age from 3 weeks to 1 year were examined with backlighting, and the amelanotic patches were compared. The size of the patches varied by no more than 10% regardless of the age of the mouse or the extent of hair depigmentation. Histologically, the patches were generally devoid of melanin.

As in the pigmented pelage,[3] melanization of the pigmented choroidal areas in both young and adult vitiligo mice was markedly reduced in comparison with that of similar areas in C57BL/6J mice (Figure 4b and c). Occasional pigmented clumps (Figure 4c) were indicative of adendritic melanocytes. There was ultrastructural evidence of compartmentalization of melanosomes (Figure 4d) and degeneration of melanocytes (Figure 4e). In some choroidal melanocytes the selectively compartmentalized melanosomes appeared partially digested, indicative of lysosomal activity (Figure 4d, arrowheads). The compartments resembled the acid phosphatase-positive melanophagosomes (or compound melanosomes) characteristic of the preamelanotic stage in the Smyth chicken model for vitiligo.[12]

Degeneration of choroidal melanocytes in the vitiligo mouse was characterized by disruptions of the plasma membrane and rarefaction of the cytoplasm (Figure 4e). In extreme cases, individual melanosomes, compartmentalized melanosomes, and mitochondria appeared in the extracellular matrix (Figure 4e).

Lymphocytic infiltrates were never observed, neither in the pigmented nor in the amelanotic areas.

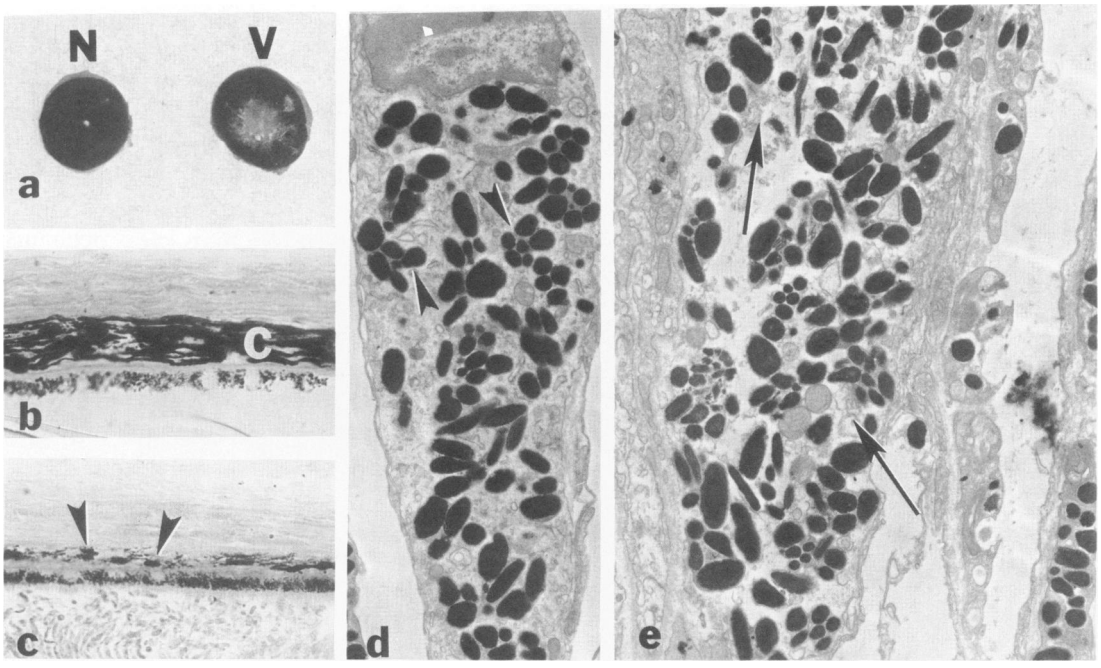

Figure 4—Initial choroidal abnormalities in the vitiligo mouse. **a**—Excised eye cups from a 6-month-old C57BL/6J mouse with normal pigmentation (*N*) and a 6-month-old vitiligo mouse with central amelanotic patch (*V*). **b**—Choroid (*C*) from a 16-week-old C57BL/6J mouse showing normal pigmentation. **c**—Pigmented area of choroid from a 16-week-old vitiligo mouse showing reduced pigmentation and clumping of melanocytes (*arrowheads*). (**b** and **c**, methylene blue and Azure II, ×160) **d** and **e**—Choroidal melanocytes from a 16-week-old vitiligo mouse, showing compartmentalization of melanosomes into membrane-bounded vesicles (*arrowheads*) (**d**) and melanocytic degeneration (*arrows*) (**e**). (**d** and **e**, uranyl acetate and lead citrate, ×8000)

Large spherical pigmented cells, presumed to be macrophages and described in more detail below, appeared between the retinal pigment epithelium and the photoreceptor cells by 30 weeks of age. These cells occurred predominantly near the optic nerve but were also found in the more peripheral areas of the posterior eye cup. The retinal pigment epithelium generally appeared continuous in all animals examined. However, in older vitiligo mice (ie, 9–12 months) retinal pigment epithelial cells occasionally contained large fat bodies, or they were devoid of melanin granules.

Stromal pigment was absent also from the iris and ciliary body of newborn vitiligo mice, while present in C57BL/6J controls. Pigment became apparent microscopically by the fourth day. Once melanization of the anterior uveal tract was established, it was retained for at least up to 1 year of age, as judged by macroscopic observation of enucleated eyes. Histologically, the intensity of melanization of the anterior border layer of cells and stroma of the iris, but not of the posterior pigmented iridial epithelium, was markedly reduced in comparison with the same areas in C57BL/6J controls.

Accompanying the delayed onset of pigmentation in the anterior uveal tract at 4 days of age was the appearance of amelanotic spherical cells on the surface of the anterior border layer of the iris (Figure 5a). These cells looked metabolically active because of an extensive Golgi apparatus and numerous surface folds indicative of ruffling (Figure 5b). Occasionally they contained melanosomes of all stages of maturation within lysosome-like compartments (Figure 5c). By 4 weeks of age, such spherical cells were heavily pigmented and present not only along the anterior border layer but also in the stroma of the iris, in close association with the pigmented epithelium, and at the anterior-chamber angle (Figure 6a).

During the time of depigmentation of the pelage, the pigmented spherical cells of the eyes became more numerous in the iris (Figure 6a) and were prominent also in the stroma of the ciliary body. Occasionally they were closely associated with the ciliary epithelium (Figure 6b). By electron microscopy, the pigmented spherical cells were seen to contain densely packed melanin granules, some of which were compartmentalized into membrane-bounded vesicles containing up to a dozen granules per ultrathin cross-section (Figure 6c). The cells had extensive surface folds. In the iris, such melanophages were seen adjacent to stromal melanocytes, which could have normal morphologic features or could contain small compartments of melanosomes and appear to be degenerating (Figure 6d).

The pigmented epithelial cells of the posterior face of the iris were ultrastructurally normal.

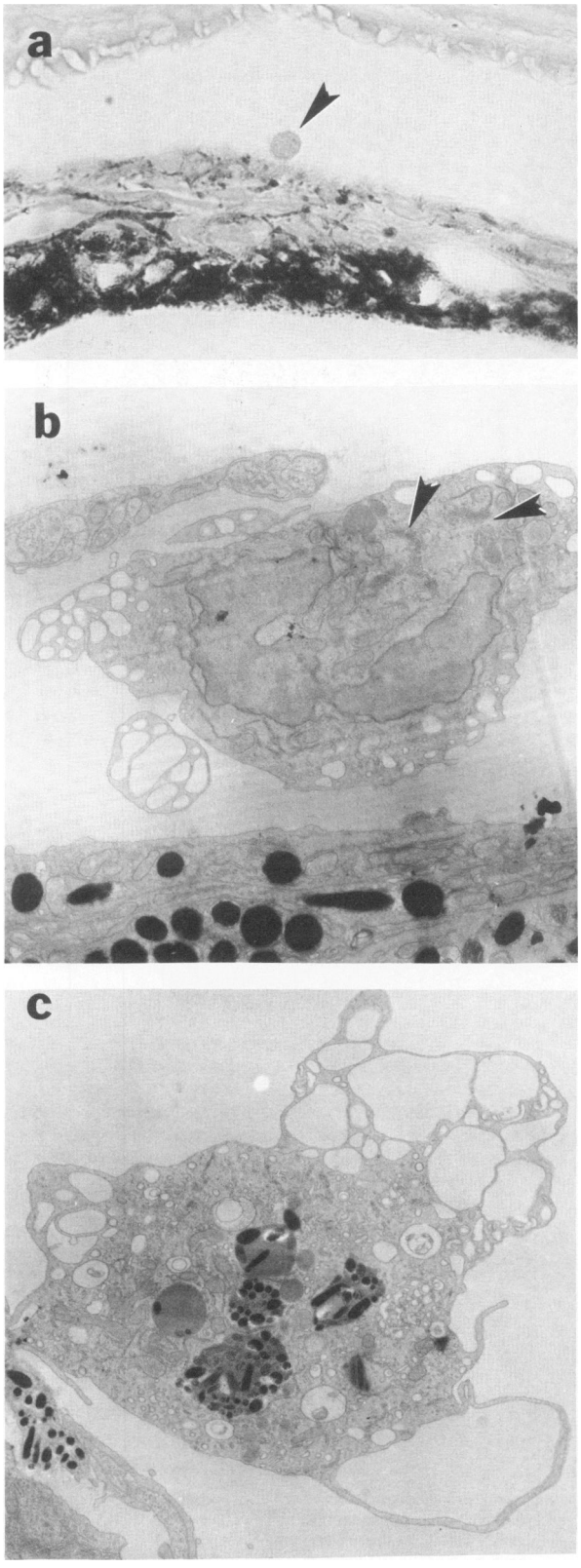

Figure 5—Initial abnormalities in the anterior uveal tract of the vitiligo mouse. Amelanotic spherical cells at the anterior border layer of the iris from a 1-week-old vitiligo mouse, seen with light microscopy (*arrowhead*) (**a**) and with electron microscopy (**b** and **c**) showing a pleomorphic nucleus, an extensive Golgi apparatus (*arrowheads*), cell surface folds (**b** and **c**), and lysosomelike compartments containing early- and late-stage melanosomes (**c**). (**a**, methylene blue and Azure II, ×200; **b** and **c**, uranyl acetate and lead citrate, ×6400)

Discussion

Various mechanisms have been postulated as possible causes for the loss of pigment cells in vitiligo.[1] Circumstantial evidence can be cited for an autoimmune, chemical, autotoxic, or neurologic basis. In the majority of patients, however, the loss of pigment cells is not associated with lymphocytic infiltrates or evidence for a chemical or neurologic mechanism. A relatively recent development is the finding of melanocyte-binding immunoglobulins in the sera of patients and animals with vitiligo.[13–15] The hypothesis of autodestruction of melanocytes is based on the known cytotoxicity of melanin precursors.[16–19] Dermal melanophages are a common finding in vitiligo. They represent the end point of melanocyte destruction.

An extensively studied, avian model for vitiligo is the Smyth chicken.[8] It has provided evidence of an inherent melanocyte dysfunction suggestive of autodestruction of pigment cells and subsequent involvement of the immune system. Feather melanocytes and neural-crest–derived cultured melanocytes produce morphologically abnormal melanosomes and have an increase in activity and intracellular distribution of tyrosinase prior to autophagocytosis of melanosomes.[12] Histologically, the malfunctioning melanocytes are first hypertrophic and later clumped.[6] We have concluded that an increase in toxic melanin precursors, resulting from hypermelanization, induces autophagocytosis of melanosomes and melanocyte death.[12] Melanocytes cultured from neural tubes of Smyth chicks,[12] or from the skin of patients with vitiligo,[20] do not proliferate as rapidly in culture as those from the respective controls.

In our study of the vitiligo mouse, we have examined melanocytes in the epithelium of regenerating hair bulbs and have demonstrated necrosis and herniation through the basement membrane into the dermal papilla. Associated with this aberration was extracellular granular material, which had previously been demonstrated in the epidermis of human patients,[7] in Smyth chicken feathers,[6] and ear epidermis of the vitiligo mouse.[4] The morphologic evidence of melanocytic dysfunction in hair bulbs complements recent skin transplantation experiments that were suggestive of a programmed melanocyte death in the vitiligo mouse. Reciprocal skin transplants between C57BL/6J and vitiligo mice, and transplants onto nude mice, in all cases retained or developed the donor's pigmentation.[3]

It is important to consider that the onset and progression of amelanosis in the vitiligo mouse occur between hair cycles. The switch from a pigmented hair with some abnormal melanocytes to an amelanotic hair without any melanocytes is apparent immediately at the outset of the hair's regeneration and

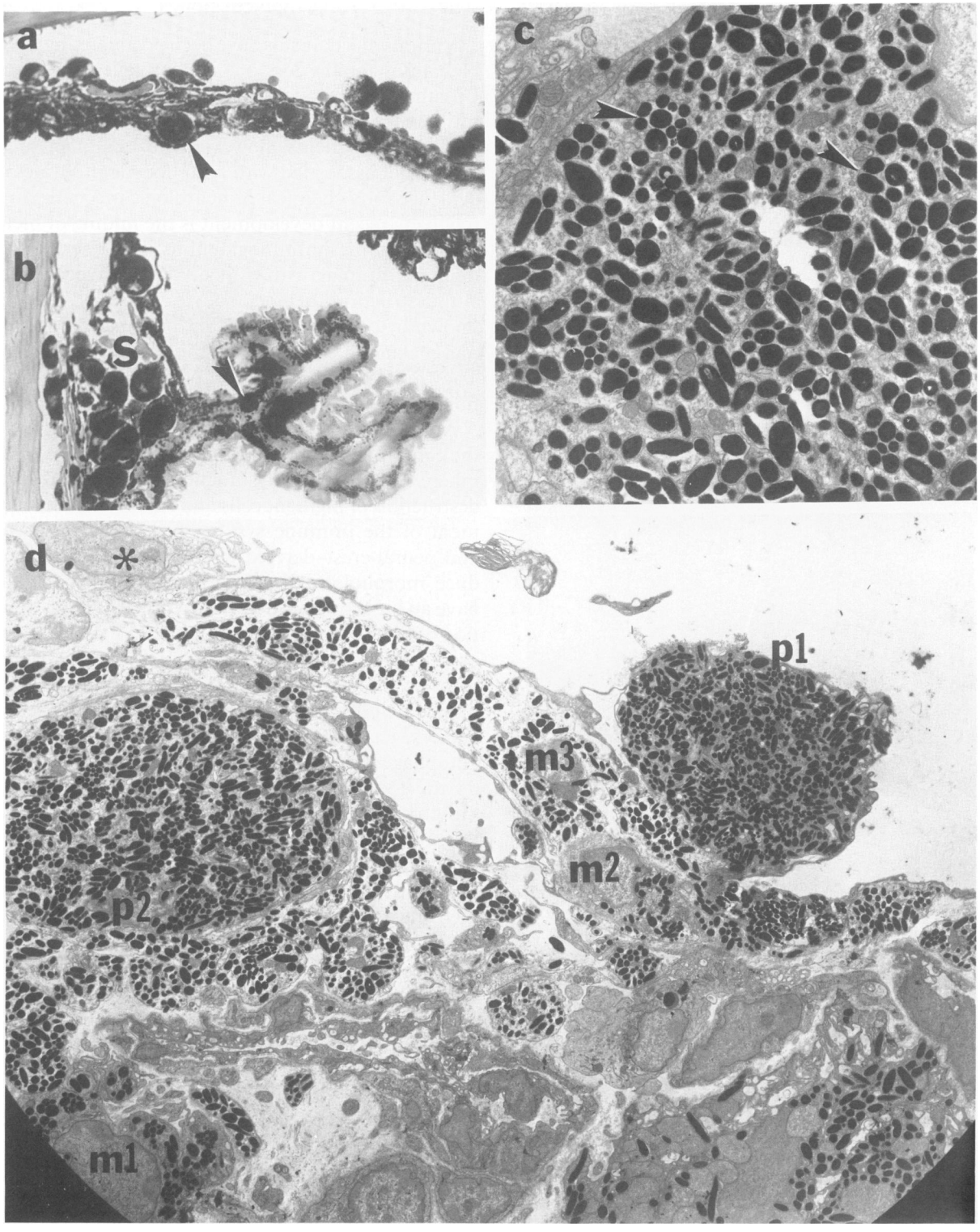

Figure 6—Later abnormalities in the anterior uveal tract of the vitiligo mouse. **a**—Iris from a 30-week-old vitiligo mouse showing large, spherical pigmented cells along the anterior border layer and within the stroma, and associated with the pigmented epithelium (*arrowhead*). **b**—Ciliary body from a 30-week-old mouse showing pigmented spherical cells concentrated within the stroma (*S*) and occasionally near the ciliary epithelium (*arrowhead*). **c**—Melanophage within the stroma of a ciliary body from a 16-week-old mouse showing numerous small membrane-bounded compartments of melanosomes (*arrowheads*). **d**—Iris from a 16-year-old mouse showing two melanophages, one at the anterior border layer of the iris (*p 1*) and one in the stroma (*p 2*); two stromal melanocytes with compartmentalized melanosomes (*m 1*, *m 2*); and one degenerative stromal melanocyte (*m 3*). An amelanotic spherical cell, containing a single melanosome, is also present (*asterisk*). (**a** and **b**, methylene blue and Azure II, ×160; **c** and **d**, uranyl acetate and lead citrate, **c**, ×91,000, **d**, ×3900)

not while hair growth is in progress. Therefore, terminal degeneration and/or elimination of melanocytes must occur during telogen or catagen, or immediately before the next anagen. Sugiyama and Kukita[21,22] have postulated that the melanocyte population responsible for hair color in mice is cyclic. They suggested that melanocytes dedifferentiate during the telogen and catagen stages, and thereafter proliferate, redifferentiate, and repopulate the bulb during early anagen.[21] Interruption of such a remodeling process would dramatically abolish pigmentation of the new hair. The abnormal and necrotic melanocytes that we observed during both the early (Day 2) and late (Week 2) anagen stages may represent a preterminal manifestation of an intrinsic melanocytic defect that spreads to all melanocytes of a given hair bulb, but not necessarily to neighboring hair bulbs, before the next hair cycle begins.

A mixture of morphologically normal and abnormal melanocytes was also found in the pigmented areas of the choroid. However, some choroidal melanocytes, unlike those in the hair bulbs, showed compartmentalization of melanosomes. In addition, the vitiligo mouse always had a central amelanotic patch surrounding the optic nerve similar to that characteristic for Smyth chickens[23] and occasionally present in patients.[5] However, contrary to the Smyth chicken, which has been selected genetically for extensive feather amelanosis coupled with blindness, the size of the amelanotic patch in the vitiligo mouse did not increase with age or extent of hair depigmentation. Like human patients with vitiligo, the vitiligo mice do not lose their eyesight. We speculate that the central amelanotic choroidal patch of vitiligo mice may be an expression of the congenital piebaldism[3] in these animals.

Finally, there is suggestive evidence, discussed in the literature,[24] for an immune connection in vitiligo. The incidence of vitiligo is increased in persons with disorders such as autoimmune thyroiditis, pernicious anemia, uveitis, alopecia areata, and some forms of adrenal insufficiency.[1] In addition, individuals with active vitiligo,[13,14] as well as animals with acquired progressive loss of pigment,[15] have serum antibodies that bind surface antigens derived from cultures of pigmented melanocytes. Whether these antibodies are a cause or a consequence of melanocyte destruction remains to be determined. An immune response provoked by melanocyte dysfunction or death has been implicated in the Smyth chicken[12] but not in the vitiligo mouse. Abnormal melanocytes develop in feather follicles and the choroid prior to inflammation of these tissues.[6] Bursectomy delays[25] and cyclosporin A blocks[26] the loss of plumage pigmentation. The aberrant melanocytes persist in bursectomized birds that retain their pigmentation.[27]

Like human patients with vitiligo, vitiligo mice develop a loss of reactivity to contact allergens,[3,28] as assessed by a diminution of cutaneous delayed-type hypersensitivity responses. Vitiligo mice also have a lower resistance to injected B16 melanoma cells than do C57BL/6J controls.[3] Neither we nor Bell et al[4] found histologic evidence of lymphocyte involvement associated with the melanocyte pathology. Mononuclear leukocytes are represented by macrophages (melanophages) in the hair papillae and in the uveal tract, where they are identical to Type 1 clump cells of Koganei.[29] In most mammals the clump cells of Koganei are generally located in the vicinity of the iris sphincter muscle and in the ciliary body near the root of the iris.[29] These are precisely the sites where amelanotic spherical cells occur in young vitiligo mice. The clump cells of Koganei become more frequent and more congested with melanin with increasing age,[30] in pathologic conditions, and after experimentally induced photocoagulation.[29] Therefore, the ocular amelanotic spherical cells in young vitiligo mice are in all likelihood precursors of the melanin-laden macrophages seen in older vitiligo mice.

In conclusion, we confirm on the basis of the above morphologic analyses and comparisons that the vitiligo mouse, as indicated earlier,[3] can be considered a new and convenient experimental animal model for vitiligo in human beings. In both organisms, melanocytes are eliminated selectively without a significant correlative lymphocytic infiltrate. Progressive loss of melanocytes in a patchy fashion is the hallmark of vitiligo. In the vitiligo mouse such losses occur in the pelage, the patches being represented by individual hair bulbs, in the uveal tract, as well as in those parts of the epidermis that harbor pigment cells.[4] The eyes were examined because in 39% of vitiligo patients discrete depigmentation involving the choroid and retinal pigment epithelium had been observed.[5] That lack of pigment is generally restricted to an area surrounding the optic nerve, an area that is congenitally amelanotic in the vitiligo mouse.

References

1. Lerner AB: On the etiology of vitiligo and gray hair. Am J Med 1971, 51:141–147
2. Nordlund JJ, Lerner AB: Vitiligo—It is important. Arch Dermatol 1982, 118:5–8
3. Lerner AB, Shiohara T, Boissy RE, Jacobson KA, Lamoreux ML, Moellmann GE: A mouse model for vitiligo. J Invest Dermatol 1986, 87:299–304
4. Bell M, Rheins LA, Nordlund JJ: Cytologic alterations in the epidermis of the depigmenting C57BL/vi·vi mouse (Abstr). J Cell Biol 1984, 99:386a

5. Albert AM, Nordlund JJ, Lerner AB: Ocular abnormalities occurring with vitiligo. Ophthalmology 1979, 86:1145–1158
6. Boissy RE, Smyth JR Jr, Fite KV: Progressive cytological changes during the development of delayed feather amelanosis and associated choroidal defects in the DAM chicken line—a vitiligo model. Am J Pathol 1983, 111:197–202
7. Moellmann G, Klein-Angerer S, Scollay DA, Nordlund JJ, Lerner AB: Extracellular granular material and degeneration of keratinocytes in the normally pigmented epidermis of patients with vitiligo. J Invest Dermatol 1982, 79:321–330
8. Smyth JR Jr, Boissy RE, Fite KV: The DAM chicken: A model for spontaneous postnatal cutaneous and ocular amelanosis. J Hered 1981, 72:151–156
9. Karnovsky MJ: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy (Abstr). J Cell Biol 1965, 27:137A
10. Karnovsky MJ: Use of ferrocyanide-reduced osmium tetroxide in electron microscopy. Proceedings of the 11th Annual Meeting American Society Cell Biology, 1971, p 146
11. Coulter HD: Rapid and improved methods for embedding biological tissue in Epon 812 and Araldite 502. J Ultrastruct Res 1967, 20:346–355
12. Boissy RE, Moellmann G, Trainer AT, Smyth JR Jr, Lerner AB: Delayed-amelanotic (DAM or Smyth) chicken: Melanocyte dysfunction in vivo and in vitro. J Invest Dermatol 1986, 86:149–156
13. Naughton GK, Eisinger M, Bystryn J-C: Antibodies to normal human melanocytes in vitiligo. J Exp Med 1983, 158:246–251
14. Moellmann GE, Krass P, Halaban R, Kuklinska E, Lerner AB: On the subject of serum antibodies to melanocytes in vitiligo (Abstr). J Invest Dermatol 1985, 84:333–334
15. Naughton GK, Mahaffey M, Bystryn J-C: Antibodies to surface antigens of pigmented cells in animals with vitiligo. Proc Soc Exp Biol Med 1986, 181:422–426
16. Hochstein P, Cohen G: The cytotoxicity of melanin precursors. Ann NY Acad Sci 1963, 100:876–886
17. Halaban R, Lerner AB: Tyrosinase and inhibition of proliferation of melanoma cells and fibroblasts. Exp Cell Res 1977, 108:119–125
18. Pawelek J, Körner A, Bergstrom A, Bolognia J: New regulations of melanin biosynthesis and the autodestruction of melanoma cells. Nature 1980, 286:617–619
19. Wick MM, Byers L, Frei E III: Selective toxicity of L-dopa for melanoma cells. Science 1977, 197:468–469
20. Puri V, Ramaiah A, Mojamdar M: In vitro growth characteristics of melanocytes from normal and vitiligo subjects (Abstr). J Invest Dermatol 87:423
21. Sugiyama S, Kukita A: Melanocyte reservoir in the hair follicles during the hair growth cycle: An electron microscopic study, Biology and Diseases of the Hair. Edited by T Kobari, W Montagna. Tokyo, University of Tokyo Press, 1976, pp 181–200
22. Sugiyama S: Mode of redifferentiation and melanogenesis of melanocytes in mouse hair follicles: An ultrastructural and cytochemical study. J Ultrastruct Res 1979, 67:40–57
23. Smyth JR, Boissy RE, Fite KV, Albert DM: Retinal dystrophy associated with a postnatal amelanosis in the chicken. Invest Ophthal Vis Sci 1981, 29:799–803
24. Lerner AB, Moellmann GE: Vitiligo—The immune connection, Dermatologic Immunology and Allergy. Edited by J Stone. C.V. Mosby Company, 1985, pp 641–643
25. Lamont SJ, Smyth JR Jr: Effect of bursectomy on development of a spontaneous postnatal amelanosis. Clin Immunol Immunopathol 1981, 21:407–411
26. Pardue SL, Fite KV, Bengston L, Lamont SJ, Boyle ML III, Smyth JR Jr: Enhanced integumental and ocular amelanosis following the termination of Cyclosporin administration. J Invest Dermatol (In press)
27. Boissy RE, Lamont SJ, Smyth JR Jr: Persistence of abnormal melanocytes in immunosuppressed chickens of the autoimmune "DAM" line. Cell Tissue Res 1984, 235:663–668
28. Rheins LA, Palkowski MR, Nordlund JJ: Alterations in cutaneous immune reactivity to dinitrofluorobenzene in graying C57BL/vi·vi mice. J Invest Dermatol 1986, 86:539–542
29. Wobmann PR, Fine BS: The clump cells of Koganei: A light and electron microscopic study. Am J Ophthal 1972, 73:90–101
30. Hogan MJ, Zimmerman LE: Ophthalmic Pathology. 2nd edition. Philadelphia, W.B. Saunders, 1962

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