0022-202X/84/8203-0235$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 82:235–238, 1984

Vol. 82, No. 3
Printed in U.S.A.

The Distribution of Melanocytes in the Leptomeninges of the Human Brain*

MARK H. GOLDGEIER, M.D.,† LAWRENCE E. KLEIN, M.D., SIGRID KLEIN-ANGERER, M.T.A., GISELA MOELLMANN, PH.D., AND JAMES J. NORDLUND, M.D.

Department of Dermatology, Veterans Administration Medical Center (LEK, JJN), West Haven, and Department of Dermatology, Yale University School of Medicine (SK-A, GM, JJN), New Haven, Connecticut, U.S.A.

The purpose of this study was to determine the qualitative and quantitative distribution of melanocytes in human leptomeninges by histochemical and ultrastructural techniques and to search for melanocytes in the mesothelial linings of the pleural and peritoneal cavities. Knowledge of the extracutaneous distribution of pigment cells will facilitate the interpretation of systemic symptoms in depigmentation disorders, such as vitiligo and the Vogt-Koyanagi-Harada syndrome.

In 15 brains examined, leptomeningeal pigment cells were found principally over the ventrolateral surfaces of the medulla oblongata. Only isolated pigment-containing cells were found in the meninges covering other parts of the brain. The mean number of pigment cells in the medullary meninges of 5 brains was 325/mm^2 ± 96. The presence of melanosomes as single, membrane-bound granules in all stages of melanization confirms that the melanin-containing dendritic cells of the leptomeninges are melanocytes and not macrophages.

No pigmented cells were observed in the pleural or peritoneal samples examined.

The human pigmentary system develops during the first 2 months of embryogenesis. Pigment cells, which arise in the spinal portion of the neural crest, migrate to peripheral sites such as the skin, the oral mucosa, and the uvea. The leptomeninges and its pigment-containing cells arise from the cephalic portion of the neural crest. It was noted decades ago that these pigmented cells were most numerous in the meninges investing the ventrolateral surfaces of the medulla oblongata and the upper cervical cord [1,2]. These observations were, however, not followed up with modern histochemical and ultrastructural techniques by which the pigmented cells could be identified as melanocytes.

Most recently, pigmented benign and malignant tumors from the leptomeninges were studied by electron microscopy and were found to contain melanin-synthesizing cells. These observations suggested that the tumors were of melanocytic origin [3–5]. It remained uncertain, however, whether pigment-containing cells that normally resided in the leptomeninges were, in fact, melanocytes from which such pigmented neoplasms could arise or whether all meningeal pigmented tumors were ectopic or metastatic [3–11].

Epidermal melanocytes are destroyed in patients with vitiligo. One-third of these patients also have characteristic destructive lesions of chorioretinitis observable by funduscopic examination [12]. This latter finding suggests that vitiligo may be a systemic disease afflicting the entire pigment system. Some patients with vitiligo and symptomatic chorioretinitis have an aseptic meningitis, i.e., they have the symptom complex known as the Vogt-Koyanagi-Harada syndrome. We have postulated that extracutaneous pigment cells in anatomic sites such as the leptomeninges [13] or possibly in the mesothelial linings of body cavities, may be involved in disorders of the pigmentary system such as vitiligo. To determine whether this concept is correct, it was necessary to know the normal distribution of pigment cells at these sites.

MATERIALS AND METHODS

Patients

Tissues were obtained from 15 sequentially available patients at autopsy, 18–36 h after death. Approval for these studies was obtained from the legal guardians of the deceased and from the Human Investigation Committee at the West Haven VA Medical Center. The skin was inspected by two of us (MHG and LEK) and the medical records checked to exclude patients with a history of disorders of the pigment system or the meninges, although none was found.

Tissues

The entire surface of the brain and the thoracic and abdominal cavities were examined for gross evidence of pigmentation. Leptomeningeal specimens, 1–2 cm^2, were removed from 9 different parts of the brain, including the ventrolateral aspect of the medulla oblongata, the cerebellum, the frontal and parietal lobes of the cerebral hemispheres, and the ventral surface of the pons. The pleura overlying the lingular lobe of the left lung and the peritoneum covering the posterior part of the fundus of the stomach were also removed for examination.

Histochemistry

To test for dopa oxidase (tyrosinase) activity, unfixed specimens were incubated in a solution of L-dopa (1 mg/ml) in sodium phosphate buffer, pH 6.6, overnight at 37°C. They were subsequently fixed in formalin for 2 h, dehydrated in ethanol, and cleared with xylene. The tissues were mounted on glass slides and examined by light microscopy.

Samples were stained with hematoxylin and eosin, or with Prussian blue (for iron) or Fontana-Masson silver (for melanin).

Electron Microscopy

Pigmented (gray) samples of meninges from the ventrolateral medulla of brains from Caucasian subjects were fixed for 2–4 h in a cold, sodium cacodylate buffered mixture of formaldehyde and glutaraldehyde [14]. The tissues were refixed in a buffered mixture of osmium tetroxide and ferrocyanide [15]. Following gradual dehydration in ethanol, the tissues were embedded in Spurr's epoxy mixture [16]. Semithin sections of the embedded material were examined by light microscopy, and tissue containing pigmented dendritic cells was thin sectioned, stained with uranyl acetate and lead citrate, and viewed in a Zeiss 9S2 transmission electron microscope.

RESULTS

Gross Examination of the Meninges, Pleura, and Peritoneum

The ventrolateral aspect of each medulla oblongata had a visible brown-gray, finely reticulated pigmentation (Fig 1). The pigment was localized within the leptomeninges and was removed by dissection of the leptomeninges from the underlying

Manuscript received March 28, 1983; accepted for publication October 21, 1983.

* Dermatology Unit Publication #21.

This work was supported by the Veterans Administration and by NIH Grant AM 25252.

† Currently with the Department of Medicine, Dermatology Unit, University of Rochester Medical Center, Rochester, New York 14642.

Reprint requests to: James J. Nordlund, M.D., Department of Dermatology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

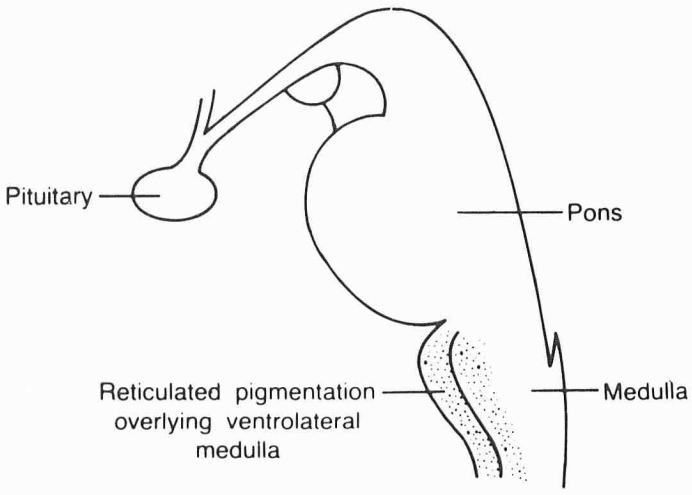

FIG 1. Schematic drawing showing typical distribution of pigmentation in the leptomeninges of the human brain.

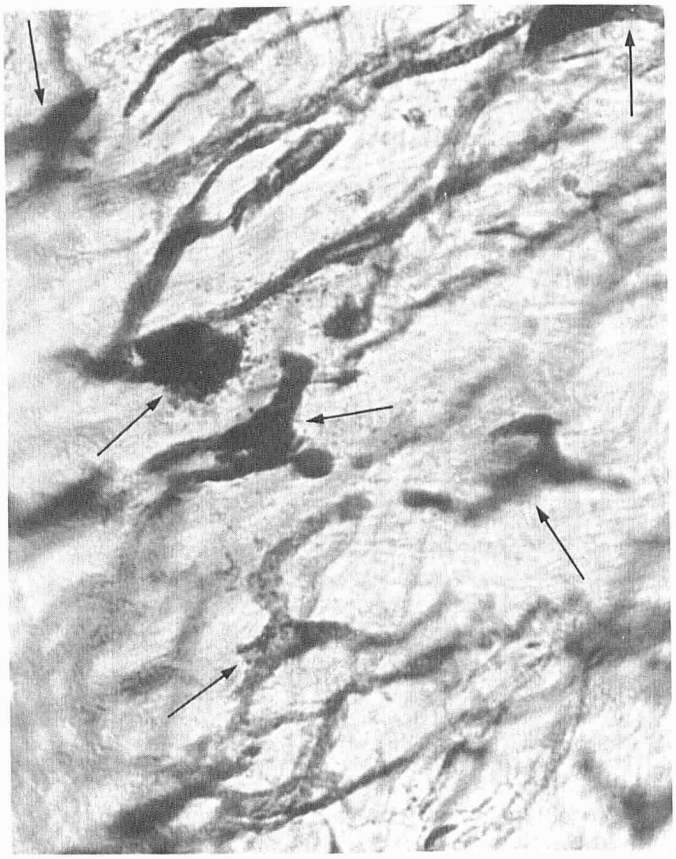

FIG 2. Photomicrograph (470×) from ventrolateral portion of medullary leptomeninges stained with dopa. The cell bodies of several melanocytes are visible (*arrows*) as well as a network of dendritic processes within and beyond the plane of focus.

nervous tissue. The amount of pigmentation in the meninges did not correlate with the depth of skin color. No surface pigmentation was visible over any other region of the brains examined. There was no pigmentation visible in the lining tissues of the pleural and peritoneal cavities.

Light Microscopy

Routine hematoxylin and eosin sections of meninges showed pigment-containing cells only within tissues obtained from the ventrolateral surface of the medulla (Figs 2, 3). These cells had long dendritic extensions that were heavily laden with pigmented granules. These extensions were intertwined with those

FIG 3. High-magnification photomicrograph (1880×) of leptomeningeal melanocytes showing cell bodies (*arrows*) and dendritic processes (*arrowheads*), filled with melanin granules.

TABLE I. *Numerical density of melanocytes in the leptomeninges overlying the ventrolateral medulla oblongata*

Case #	Age	Skin color	Sex	Mean number of melanocytes/mm^2 ±SE[a]
1	63	B	M	360 ± 60
2	68	W	M	246 ± 25
3	82	W	M	483 ± 70
4	74	W	F	250 ± 50
5	66	W	F	343 ± 47

[a] Five different areas, each 1 mm^2, were evaluated in each case.

from nearby cells and formed a network. No pigmented cells were seen in sections of pleura or peritoneum stained with hematoxylin and eosin.

No dopa-positive cells were found in the pleura, peritoneum, or in most parts of the leptomeninges. The dendritic cells of the ventrolateral area of the medulla were so deeply pigmented that staining due to dopa was difficult to assess. The mean number of dendritic, pigmented cells in the medullary meninges of 5 brains was 325/mm^2 ± 96 (Table I).

Prussian-blue staining for the presence of iron, indicative of macrophages, was consistently negative in all tissues examined. On the other hand, the Fontana-Masson silver method, used to detect melanin, deeply stained cytoplasmic granules of the dendritic cells of the ventrolateral medullary areas. Rarely, a stained dendritic cell was observed in other areas of the meninges. No cells in the pleural or peritoneal samples examined were stained by the Fontana-Masson technique.

Electron Microscopy

Fine structural examinations of ventrolateral medullary meninges revealed that the pigmented cells and their dendrites were filled with melanosomes (Fig 4). Most of the melanosomes were fully melanized (stage IV) but melanosomes in stages II and III of melanization were also identified (Fig 4*C*,*D*).

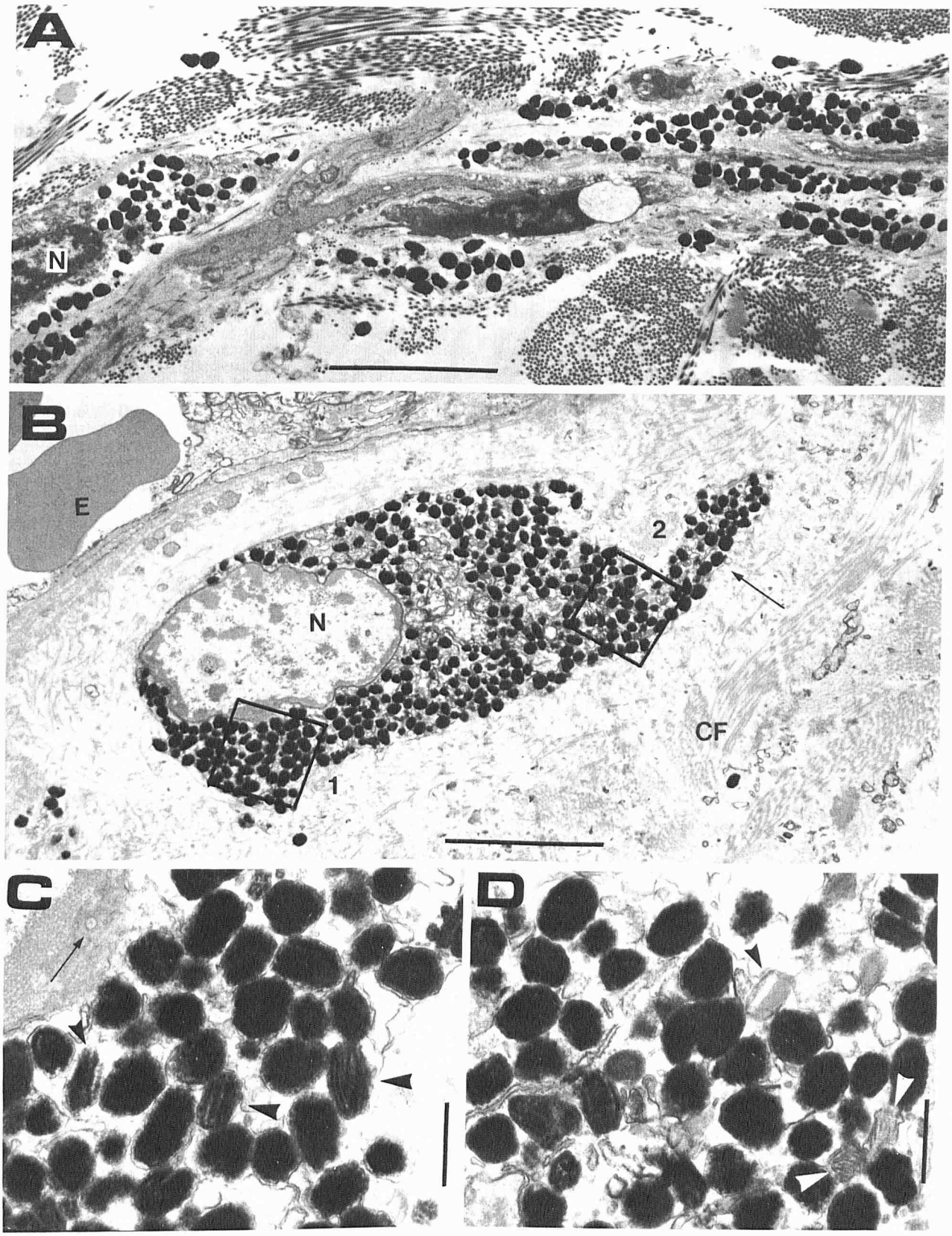

FIG 4. Electron micrographs of pigment in human leptomeninges. *A*, Dendritic network of melanocytic processes. *N* = nucleus of a melanocyte. *Scale bar*: 5 μm. *B*, Section through cell body of a melanocyte and proximal portion of a dendrite (*arrow*). *N* = nucleus of melanocyte. *Boxes 1 and 2*: contents shown at higher magnification in *C* and *D*. *E* = erythrocyte in lumen of a meningeal venule. *CF* = collagen fibers. *Scale bar*: 5 μm. *C*, High-power view of *box 1*, in *B*, illustrating fully melanized melanosomes, stage III (*arrowheads*). *Arrow* indicates a nuclear pore. *Scale bar*: 0.5 μm. *D*, High-power view of *box 2* in *B*, illustrating substructure of premelanosomes, stage II (*arrowheads*), in different planes of sectioning. *Scale bar*: 0.5 μm.

DISCUSSION

Our ultimate goal is to determine whether, in addition to ocular melanocytes, pigment cells at other extracutaneous sites become involved in disorders of pigmentation, especially vitiligo. Examination of three such possible sites, the cranial leptomeninges, the pleura, and the peritoneum from persons without vitiligo, revealed pigment cells only within the leptomeninges. There the pigment was confined to sites overlying the ventrolateral aspect of the medulla oblongata. Histochemical stains suggested that the pigment was melanin.

Electron microscopic examination of the pigmented cells revealed the presence of oval, membrane-limited cytoplasmic granules identical to premelanosomes, melanosomes, and fully melanized melanin granules. Because of their shape and because of the singlet distribution of the melanosomes, these cells have the appearance of bona fide melanocytes and not of melanin-containing macrophages. The presence of pre- and partially melanized melanosomes suggests that the cells were capable of synthesizing melanin.

The mean number of pigment cells per unit area (325/mm^2) in the medullary meninges represents a numerical density of about one-third of that found in the epidermis. Occasionally isolated dendritic cells with Fontana-Masson-positive granules were seen in the leptomeninges overlying the cerebellum and the cerebral hemispheres, but no cells were observed to be stained with dopa in these regions. This discrepancy may be due to sampling error because of the paucity of such cells or it may reflect the ability of the Fontana-Masson silver stain to react with argentaffin cells other than melanocytes.

Vitiligo is a depigmenting disorder in which not only cutaneous but also ocular pigment cells are destroyed. In patients with the Vogt-Koyanagi syndrome, cutaneous depigmentation is associated with acute anterior uveitis, alopecia, poliosis, and dysacousia. In the Harada syndrome, cutaneous pigmentation is associated with posterior uveitis and aseptic meningitis. Thus, each of the organs affected by the Vogt-Koyanagi and Harada syndromes, i.e., the epidermis, hair bulbs, eye, ear, and meninges, are known to contain normal melanocytes. In this study, we have confirmed that melanocytes are normally present in the leptomeninges. These leptomeningeal melanocytes might also become involved in vitiligo. We hypothesize that the aseptic meningitis observed in the Harada syndrome is due to the destruction of leptomeningeal melanocytes. We predict that aseptic meningitis is common in patients with vitiligo although it may be subclinical as is the vitiligo-associated chorioretinitis observed with the funduscope.

Other studies are also consistent with the in situ origin of at least some meningeal melanocytic tumors.

REFERENCES

1. Baader O: Uber die Pialmelanose. Z Mikrosk Anat Forsch 22:735, 1935
2. Symmers W: Pigmentation of the pia mater with special reference to the brain of modern Egyptians. J Anat 40:25–27, 1905
3. Limas C, Tio FO: Meningeal melanocytoma ("melanotic meningioma"). Its melanocytic origin as revealed by electron microscopy. Cancer 30:1286–1294, 1972
4. Savitz MH, Anderson PJ: Primary melanoma of the leptomeninges: a review. Mt Sinai J Med 41:774–791, 1974
5. Silbert SW, Smith KR, Horenstein S: Primary leptomeningeal melanoma. An ultrastructural study. Cancer 41:519–527, 1978
6. Bouton J: Primary melanoma of the leptomeninges. J Clin Pathol 11:122–127, 1958
7. Gibson JB, Burrows D, Weis WP: Primary melanoma of the meninges. J Pathol Bacteriol 74:419–438, 1957
8. Pappenheim E, Bhattacharji SK: Primary melanoma of the central nervous system. Arch Neurol 7:101–113, 1962
9. Reed WB, Becker SW Sr, Becker SW Jr, Nickel WR: Giant pigmented nevi, melanoma and leptomeningeal melanocytosis. Arch Dermatol 91:100–119, 1965
10. Russell DS, Rubinstein LJ: Neurocutaneous melanosis and melanoma of the leptomeninges, Pathology of Tumors of the Nervous System. Baltimore, Williams & Wilkins, 1977, pp 55–57
11. Tolnai G, Campbell JS, Hill DP, Peterson EW, Hudson AJ, Luney FW: Primary malignant melanocytosis of leptomeninges. Arch Neurol 15:404–409, 1966
12. Albert DM, Nordlund JJ, Lerner AB: Ocular changes in vitiligo, Pigment Cell, vol 5. Edited by SN Klaus. Karger, Basel, 1979, pp 62–72
13. Nordlund JJ, Albert DM, Forget BM, Lerner AB: Halo nevi and the Vogt-Koyanagi-Harada syndrome—manifestations of vitiligo. Arch Dermatol 116:690–692, 1980
14. Karnovsky MJ: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 27:137A–138A, 1965
15. Karnovsky MJ: Use of ferroxyanide-reduced osmium tetroxide in electron microscopy. J Cell Biol 51:146a, 1971
16. Spurr AR: A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26:31–43, 1969