Ultrastructural findings in oral hyperpigmentation of HIV-infected patients

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Oral hyperpigmentation has been observed in six HIV-infected patients, in two of whom systemic medication (ketokonazole, clofazimine) was supposed to be etiologically involved. Histologically, pigment was found in epithelial basal cells and particularly in subepithelial connective tissue. Ultrastructurally, the presence of premature melanosomes in subepithelial keratinocytes was of interest. Stimulation of melanocytes during HIV infection may occur in association with immunopathologic changes in the oral mucosa.

Key words: electron microscopy; HIV infection; mouth; oral pigmentation.

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Recently, abnormal hyperpigmentation of skin (1), of finger- (2) and toe nails (3) in patients with HIV-infection has been described. Clinical and histologic findings in oral hyperpigmentation have been reported (4). The purpose of the present study was to describe in detail the ultrastructural findings from oral hyperpigmentations seen in six HIV-infected patients. While in two patients the lesions could be related to systemic medication with clofazimine and ketokonazole, in the other four the etiology remained unknown.

Material and methods

Among 250 HIV-infected patients six presented with oral hyperpigmentations (five homosexuals, one woman i.v. drug abuser). Biopsies of oral mucosa with hyperpigmentation were obtained from the six patients. In addition to routine histology (H & E), tissues were fixed in 2.5% glutaraldehyde in PBS for 3 h. After washing with PBS, specimens were postfixed in 1% OsO4 for 1 h at 4°C, and afterwards treated for 1 h at room temperature in 1% uranyl acetate. After dehydration in a graded series of ethanol, specimens were infiltrated by three changes of propylene oxide and embedded in Epon 812 following routine techniques (5). Semithin sections (0.5–1.0 μm) of each specimen were cut, stained with toluidine blue and evaluated by light microscopy. Ultrathin sections (40–60 nm) were cut and mounted on bare grids, poststained with lead citrate, stabilized with carbon and examined using a Zeiss EM 10A at 60 KV.

Results

In six patients with oral hyperpigmentation the buccal mucosa (4), the hard palate (1), the gingiva (1) and the tongue were affected (Fig. 1). Five homosexuals were of an average age of 33.8 yr (range 27 to 47 yr), one woman, i.v. drug abuser was 29 yr old. One homosexual (CDC stage IVCl) had received clofazimine therapy (2 months), another (CDC stage IVD) had taken ketokonazole (3 months). Corticosteroids (homosexual, CDC stage IVCl) and antibiotics (woman, i.v. drug abuser, CDC stage IVCl) were also of relevance. The men were non-smokers. The average period of observation was 7 months.

LM – In the paraffin sections an in-

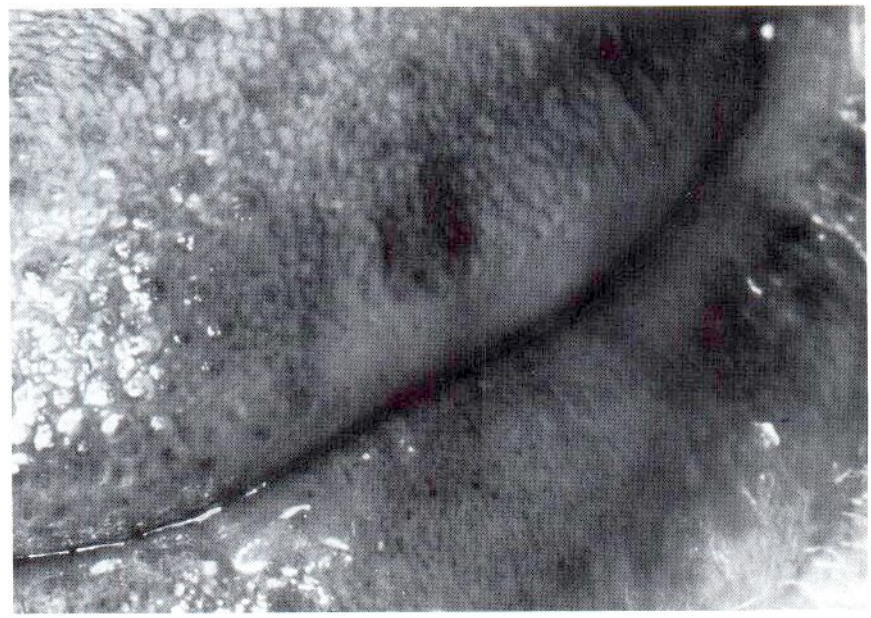

Fig. 1. Focal hyperpigmentation on lateral margin of tongue in an i.v. drug-abusing woman.

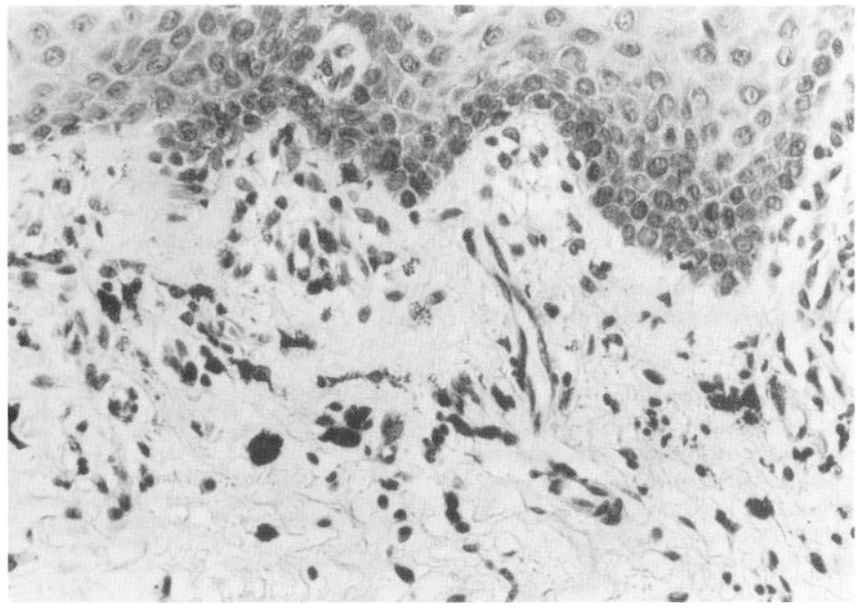

Fig. 2. Accumulation of pigment granules in subepithelial connective tissue and in some basal cells. (H&E ×190)

crease in the amount of pigment granules could be found in the oral mucosa with hyperpigmentation. Pigmentation was seen in some epithelial cells of the basal cell layer but considerably more was found extracellulary in scattered foci within the subepithelial mucosa or contained within macrophages. With hematoxylin and eosin stain the pigment was golden brown. A mild chronic inflammatory infiltrate was found in the subepithelial connective tissue (Fig. 2).

EM – Melanocytes were detectable and situated in the basal layer of the oral epithelium. Desmosomes between the melanocytes and adjacent keratinocytes were not observed. Hemidesmosomes could not be seen between basal cells and the basal membrane. Some epithelial basal cells lacked tonofilaments, however, contained melanosomes in the cytoplasm (Fig. 3). Several sections revealed dendritic processes of melanocytes extending into interkeratinocytic spaces (Fig. 4). Premature melanosomes with coating membranes were found in the cytoplasm of superficial epithelial cells (Fig. 5). Macrophages containing aggregations of melanosomes were observed in the lamina propria of the oral mucosa with hyperpigmentation. These melanosomes appeared to be contained by lysosomes (Fig. 6). Occasionally, some elongated cells, rich in rough-endoplasmic reticulum, and therefore probably representing fibroblasts were found in the lamina propria. These cells contained a number of phagosomes filled with extremely dense material representing melanosomes (Fig. 7).

Discussion

Dermal and mucosal acquired hyperpigmentation may be caused by several endogenous and exogenous factors: hormonal disturbance during pregnancy, systemic disorders like Addison's disease or neurofibromatosis and prolonged administration of different drugs like clofazimine, minocycline (7) or ketokonazole (8). Hyperpigmented lesions of the tongue and of finger- and toe-nails have been reported in heroin addicts as fixed drug eruptions (9). Exogenous hyperpigmentation of the oral mucosa may result from exposure to heavy metal, from tobacco smoking (smoker's melanosis) (10), from amalgam tattooing, or from tobacco or betel nut chewing (11, 12).

In this study, hyperpigmentation in two cases could be related to systemic medication with clofazimine and ketokonazole, the etiology of hyperpigmentation in the other cases remained unknown (4).

The endogenous pigments most commonly contributing to the color of the oral mucosa are melanin and hemoglobin. Melanin is produced by specialized pigment cells called melanocytes which are situated in the basal layer of the oral epithelium and the epidermis. Melanocytes lack desmosomes and tonofilaments but possess long dendritic processes that extend between the keratinocytes, often passing through several layers of cells. Melanin pigment is synthesized within the melanocytes as small structures called melanosomes (7). The cell shown in Fig. 3 was a

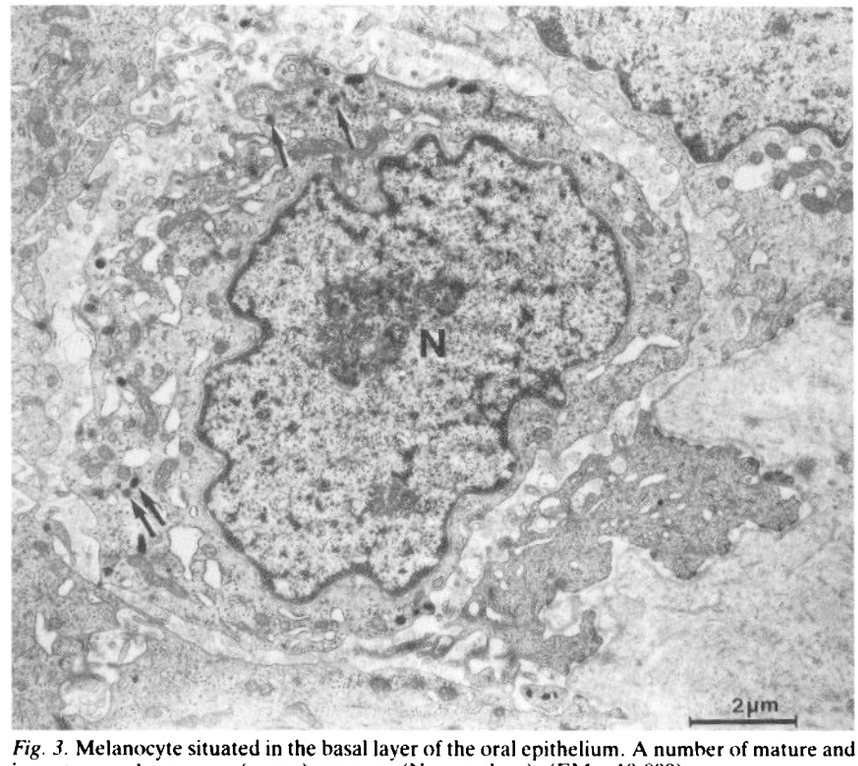

Fig. 3. Melanocyte situated in the basal layer of the oral epithelium. A number of mature and immature melanosomes (arrow) are seen (N = nucleus). (EM ×10,000)

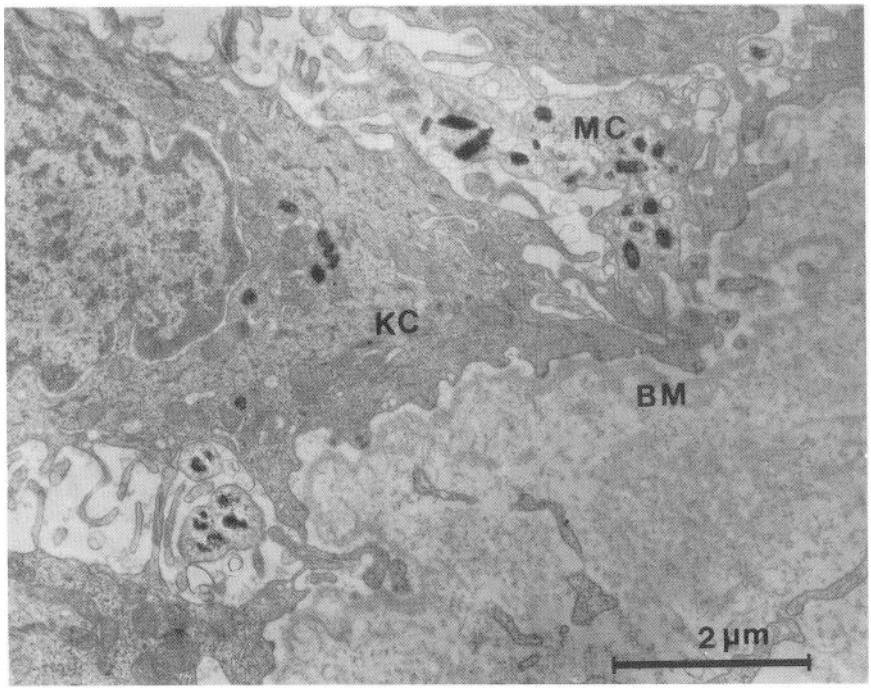

Fig. 4. Two processes of melanocyte extending between keratinocytes. Melanosomes are found in cytoplasm of the process (MC, melanocyte. KC, keratinocyte. BM, basal membrane). (EM ×14,300)

typical melanocyte containing melanosomes.

Two types of fixed macrophages may be found in the lamina propria of the oral mucosa, namely the melanophage and the siderophage. The melanophage, which is common in the pigmented oral mucosa, is a macrophage which has ingested melanin granules extruded from melanocytes within the epithelium. The siderophage is a phagocytic cell containing hemosiderin derived from blood cells that have been extravasated into the tissues. Hemosiderin can persist within the cells for some time and the resultant brownish color may appear clinically as a bruise (13). In the present study melanosomes could be found in the cytoplasm of some phagocytes within the connective tissue (Fig. 6). These phagocytes therefore were melanophages and no siderophages.

In the present investigation premature melanosomes have been observed in the superficial epithelium. It appeared that the premature melanosomes were transferred by the cytoplasmic processes of the melanocytes to keratinocytes, however, the mechanism for this transfer is unknown (14). The presence of premature melanosomes, especially in the superficial layer of the epithelium, might however suggest that the function of melanocytes may be activated during HIV infection. The demonstration of HIV structural proteins in oral mucosa by immunohistochemistry as well as an increase of labelled CD4- and CD8-positive cells in the connective tissue stroma was an indication of a high continuous antigenic stimulation of the local immune system due to the presence of opportunistic infection (myocotic, viral, bacterial) (15). While during this process activation of melanocytes may occur simultaneously, it may also be assumed that due to an increased turnover rate of the oral epithelium during HIV infection premature melanosomes appear in epithelial layers where usually they are not found. Further studies on activation of melanocytes and epithelial turnover rates in HIV infection are needed.

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References

1. Esposito R. Hyperpigmentation of skin in patients with AIDS (Letter). *Br Med J* 1987; **294**: 840 (only).
2. Glasgow BJ, Steinsapir KD, Anders K, Layfield LJ. Adrenalin pathology in the acquired immunodeficiency syndrome. *Am J Clin Pathol* 1985; **84**: 594–7.
3. Chernosky ME, Finley VK. Yellow nail syndrome in patients with acquired immunodeficiency disease. *J Am Acad Dermatol* 1985; **13**: 731–6.
4. Langford A, Pohle H-D, Gelderblom H, Zhang X, Reichart P. Oral hyper-

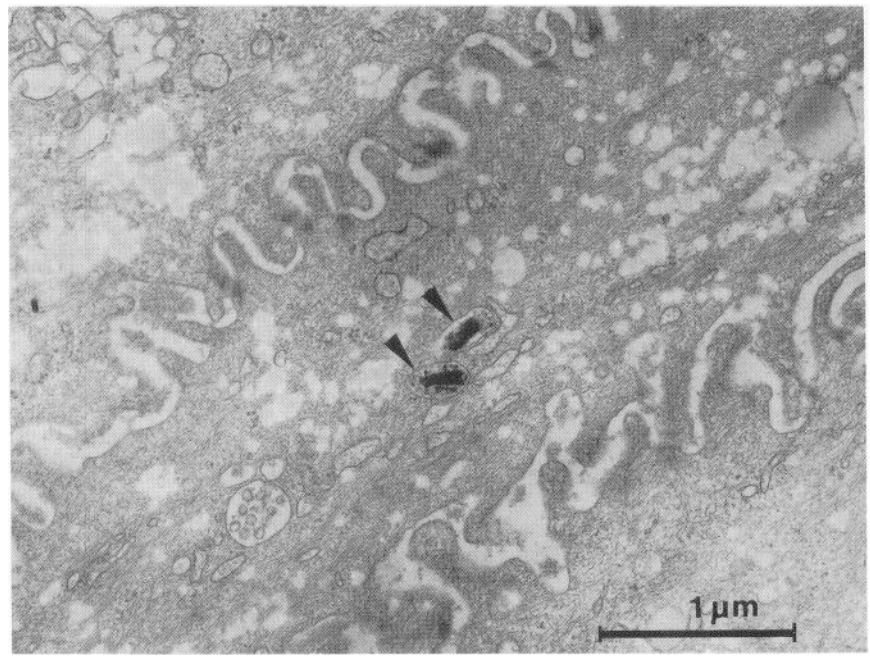

Fig. 5. Premature melanosomes with coating-membrane (arrowheads) are found in cytoplasm of cells of keratinized layer. (EM ×29,600)

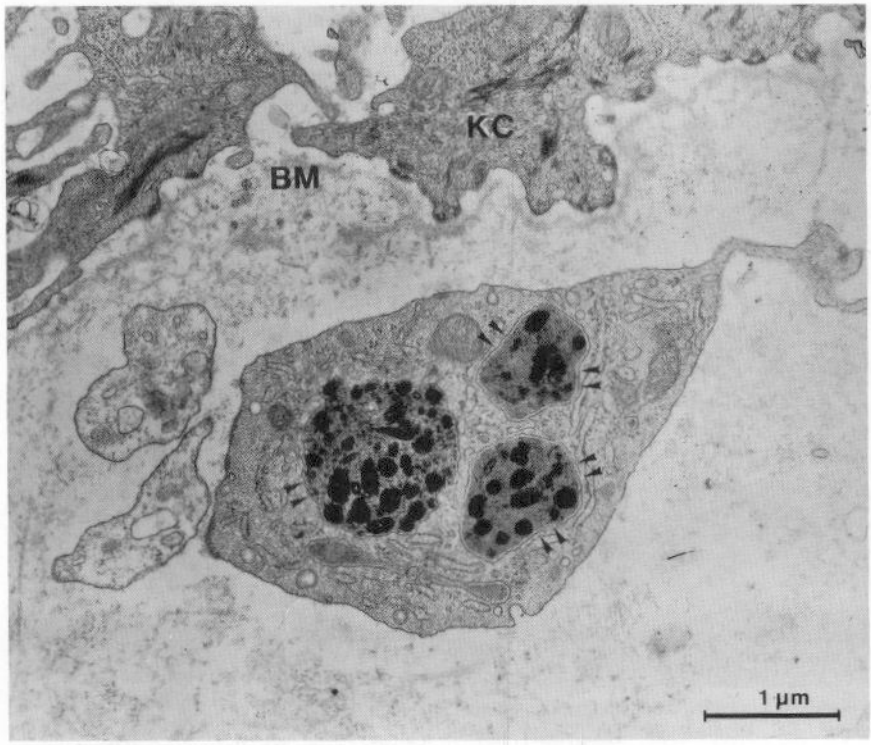

Fig. 6. Portion of macrophage observed in the subepithelial connective tissue which contained some melanosomes. (EM ×25,200)

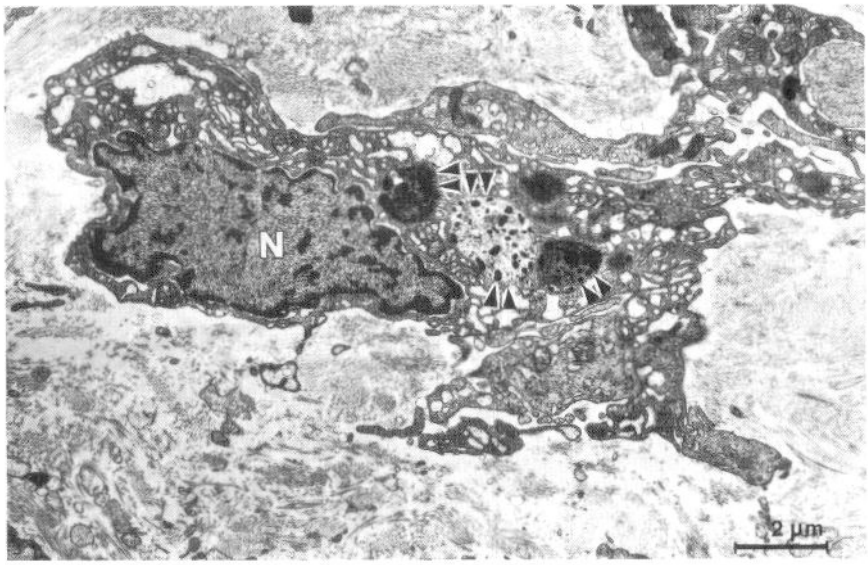

Fig. 7. Fibroblasts containing melanosomes in phagocytic bags are located in the subepithelial connective tissue (N, nucleus). (EM ×9,400)

pigmentation in HIV-infected patients. Oral Surg 1988; **67**: 301–7.

5. GELDERBLOM HR, OGURA H, BAUER H. On the occurrence of oncornavirus-like particles in Hela cells. *Cytobiol* 1974; **8**: 339–44.
6. CHOUNG R, GOLDBERG MH. Oral hyperpigmentation associated with Addison's disease. *J Oral Maxillofac Surg* 1983; **41**: 680–2.
7. ARGENYI ZB, FINELLI L, BERGFELD WF, MCMAHON JT, RATZ JL, PETROFF N. Minocycline-related cutaneous hyperpigmentation as demonstrated by light microscopy, electronmicroscopy. *J Cutan Pathol* 1987; **14**: 176–80.
8. BAES H, VAN CUTSEM J. Primary cutaneous cryptococcosis. *Dermatologica* 1985; **171**: 357–61.
9. WESTERHOF W, WOLTERS EC, BROOKBAKKER JTW, BOELSEN RE, SCHIPER MEI. Pigmented lesions of the tongue in heroin addicts-fixed drug eruption. *Br J Dermatol* 1983; **109**: 605–10.
10. HEDIN CA. Smokers melanosis. Occurrence and localization in the attached gingiva. *Arch Dermatol* 1977; **113**: 1533–8.
11. DUMMETT CO, BARENS G. Pigmentation of the oral tissues: a review of the literature. *J Periodontol* 1967; **38**: 369–78.
12. GIANSANTI JS, TILLERY D, OLANSKY S. Oral mucosal pigmentation resulting from antimalaria therapy. *Oral Surg* 1971; **31**: 66–9.
13. TEN CATE AR. *Oral histology, development, structure and function.* The C. V. Mosby: London, 1980; p. 363–5, 373.
14. RHODIN JAG. *Histology*. 2nd ed., New York: Oxford University Press, 1977; p. 486.
15. BECKER J, ULRICH P, KUNZE R, GELDERBLOM H, LANGFORD A, REICHART P. Immunohistochemical detection of HIV structural proteins and distribution of T-lymphocytes and Langerhans cells in the oral mucosa of HIV infected patients. *Virchows Arch A* 1988; **412**: 413–9.