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Morphology and Membrane Antigens of Nonlymphoid Accessory Cells in Oral Hairy Leukoplakia

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We have investigated the features and distribution of accessory cells (ACs) and the relationships of these cells to each other and to lymphocytes in the epithelium and lamina propria of oral hairy leukoplakia (HL), with the objective of better defining the differentiation and mutual interactions of immune-response cells within HL as a preliminary step to understanding the onset and significance of this lesion during human immunodeficiency virus (HIV) infection. Twenty-four HIV-infected patients with HL, two asymptomatic HIV-positive subjects, and three HIV-negative subjects were studied by immunohistochemistry; five HIV-positive patients with HL and three asymptomatic HIV-positive subjects were studied by electron microscopy. In both the epithelium and the lamina propria of HL, we found cells with the immunohistochemical and ultrastructural features of variably differentiated ACs; differences were found between the epithelium and lamina propria. In the lamina propria, ACs were characterized by dendritic shape, multiple contacts with lymphocytes, expression of CD1a antigen, and ultrastructural features of fully differentiated ACs. Conversely, in the epithelium ACs showed bluntly dendritic shape, low expression of CD1a, absent expression of HLA-DR, constant expression of CD11c and CD14 antigens, only occasional contacts with lymphocytes, and ultrastructural features of variably, but always incompletely, differentiated cells of monocyte-dendritic lineage. Seventy-nanometer wide intracisternal particles, closely resembling A particles described in retroviral infections, were found in the intraepithelial ACs in two patients with HL. The defective differentiation of ACs in the epithelium of HL—possibly influenced by the perturbation of the epithelial microenvironment induced by Epstein-Barr virus, and following the direct HIV infection of these cells—and the exceptional finding of close contacts with lymphocytes suggest that the lesional epithelium of HL may constitute a pathway for the entry

of foreign antigens which circumvent monitoring by ACs and can induce immune tolerance. The impairment of the local immune response in HL may contribute to the development of full blown, systemic immunodeficiency. *HUM PATHOL* 21:897-904. © 1990 by W.B. Saunders Company.

Langerhans cells are accessory cells (ACs) of the immune system which express on their plasma membrane CD4 antigen^{1,2} and are therefore susceptible to infection by human immunodeficiency virus (HIV), which leads to a reduction of Langerhans cells bearing DR antigens, and possibly to a functional impairment of these cells in the skin.³⁻⁸ Langerhans cells are possible hosts of HIV in the earliest phases of infection,⁹ and have been shown to be capable of *in vivo* and *in vitro* production of HIV.¹⁰⁻¹² In the oral epithelia, CD1a+ Langerhans cells have been found to be progressively reduced in number, from normal subjects (ie, subjects not infected with HIV), through asymptomatic HIV-infected subjects and patients with acquired immunodeficiency syndrome (AIDS)-related complex, to full-blown AIDS patients, but these differences in number were not significant; the numbers of CD1a+ cells in the lamina propria of the same biopsies were generally similar among the different groups of subjects.^{13,14}

Hairy leukoplakia (HL) is a lesion of the lateral margins of the tongue. Recently identified in patients infected by HIV, it was first recognized in male homosexuals, and later also in hemophiliacs and drug addicts of both sexes.¹⁵⁻¹⁸ Epstein-Barr virus has been identified in the spinous epithelial cells of HL; this virus may have a causal role in the lesion.¹⁶⁻²² Hairy leukoplakia has a relevant, unfavorable prognostic value.¹⁵ A decrease in the number of CD1a+ Langerhans cells has been described in the lesional epithelium of HL, compared with adjacent, apparently healthy mucosal areas.¹³

In view of the unfavorable prognostic value of HL in AIDS and of the role of Langerhans cells in stimulating immune responses, we have investigated, with light microscopic immunohistochemistry and electron microscopy, the features and distribution of Langerhans cells and other ACs, and the relationships of these cells to each other and to lymphocytes in the epithelium and lamina propria of HL. The objective of this study was to better define the differ-

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Key words: accessory cells, acquired immunodeficiency syndrome, hairy leukoplakia, human immunodeficiency virus, Langerhans cells, oral mucosa.

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entiation and mutual interactions of immune response cells within IIL, as a preliminary step to understanding the onset and significance of this lesion during HIV infection.

MATERIALS AND METHODS

Patients and Biopsies

The subjects for this study belong to a previously described group of patients.^{16,18,21} They are 24 patients with HL, 92% of whom belong to the intravenous drug abusers risk group, with the remaining 8% belonging to other risk groups. All these subjects were serologically positive for HIV, and had clinically typical lesions on the lateral margins of the tongue.

Punch biopsies were obtained from each lesion at the time of diagnosis. Biopsies from the tongues of three subjects not infected with HIV, and from clinically healthy buccal and tongue mucosa of three HIV-infected patients, were also obtained. Tissue samples from lesions and control mucosa were in part formalin-fixed and embedded in paraffin for histologic examination. Part of each specimen was snap-frozen and stored at -80°C until preparation for immunohistochemistry; parts of five specimens from HL and three specimens from clinically healthy mucosa of HIV-infected patients were prepared for electron microscopy. The histologic examination (Fig 1) demonstrated the well-

known characteristic features of HL.^{16,19} The diagnosis of HL was confirmed by *in situ* DNA hybridization for Epstein-Barr virus in 20 cases,²¹ and ultrastructural demonstration of herpes-type virions in four cases.¹⁶

Light Microscopic Immunohistochemistry

Tissue specimens were snap-frozen and stored at -70°C until sectioned. Six-micrometer thick cryostat sections were air-dried, fixed in acetone for 10 minutes, and processed according to a previously described indirect immunoperoxidase method.²³ Normal human lymph nodes were likewise stained as positive controls. Sections incubated with only the primary antibody and sections incubated without any antibody were used as negative controls. For a quantitative analysis, the stained cells were counted in five consecutive microscopic fields of the lamina propria and in five consecutive microscopic fields of the epithelium (comprising the basal membrane) at a magnification $\times 250$. The results in the lamina propria were expressed as percentage of stained cells; those in the epithelium were expressed as the number of stained cells overlying 100 basal cells. Only cells whose nucleus was contained in the plane of the section were considered. The antibodies used and their specificities²⁴ are listed in Table I.

Electron Microscopy

Specimens were fixed with 2% glutaraldehyde and 2.5% formaldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, followed by 1% OsO_4 in 0.1 mol/L phosphate buffer, pH 7.4, and were embedded in Epon 814. Sections were stained with uranyl acetate and bismuth tartrate,²⁵ and observed in Siemens Elmiskop I and 102 electron microscopes at 80 kV.

RESULTS

Immunohistochemistry

In the lamina propria of 22 out of 24 IIL specimens, a sparse infiltrate of CD2+, CD3+, CD8+, CD4-, CD25-, CD30-, HLA-DR- lymphoid cells, and CD1a+ dendritic cells (Fig 2) was found; the finding of CD4+ cells was occasional. In these cases,

TABLE 1. Monoclonal Antibodies Used

Monoclonal Antibody	Cluster of Differentiation	Source	Specificity ²⁴
T11	CD2	CC	E-rosette receptor
OKT3	CD3	ODS	Mature T-cells
T4	CD4	CC	Helper/inducer T-cells
OKT8	CD8	ODS	Suppressor/cytotoxic T-cells
Leu-14	CD22	BD	B cells
OKT6	CD1a	ODS	Langerhans cells, T-zone accessory cells
OKM1	CD11b	ODS	Monocytes, macrophages
Leu-M5	CD11c	BD	Monocytes, macrophages
Leu-M3	CD14	BD	Monocytes, macrophages
Anti-HLA DR		BD	Class II molecules
Anti-IL2 receptor	CD25	BD	Activated cells
Ki-1	CD30	DP	Activated cells

Abbreviations: CC, Coulter Clonc, Birmingham, United Kingdom; ODS, Ortho Diagnostic Systems, Raritan, NJ; BD, Becton Dickinson, Sun Valley, CA; DP, Dakoparis, Copenhagen, Denmark.



FIGURE 1. Hairy leukoplakia. Classic histopathologic features (acanthosis, parakeratosis, and large pale-staining cells—so called balloon-cells) are shown. (Hematoxylin-eosin stain; magnification $\times 300$.)

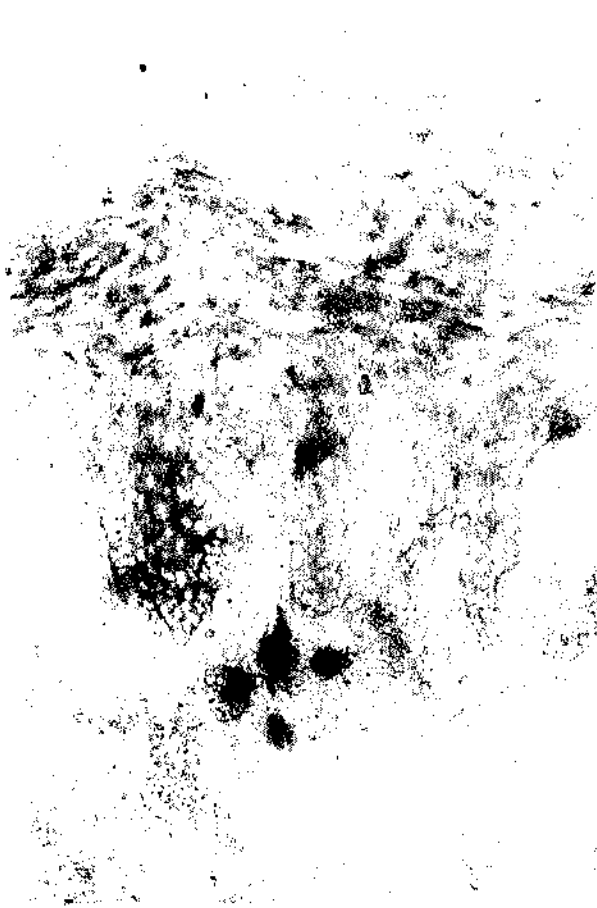


FIGURE 2. CD1a+ dendritic cells in the lamina propria of HL. Note the virtual absence of CD1a+ cells in the epithelium. (Immunoperoxidase stain; magnification $\times 360$.)



FIGURE 3. Epithelium of HL: only one CD1a+ dendritic cell is evident. (Immunoperoxidase stain; magnification $\times 360$.)

a large number of HLA-DR+ vascular structures were found. No appreciable infiltrate was found in the lamina propria of the control biopsies from HIV-positive and HIV-negative subjects, except for small clusters of T cells, with CD4/CD8 ratios varying from 1:1 to 3:1, and CD1a+ dendritic cells. The number of CD11b+, CD11c+, and CD14+ cells in the lamina propria was fairly constant among all the specimens, with no relevant difference between HL specimens and other groups of specimens.

Within the epithelium of 21 out of 24 HL specimens, CD2+, CD3+, CD8+, CD4-, CD25-, CD30-, and HLA-DR- lymphoid cells were seen in small numbers, while this finding was exceptional in all the other specimens. Only two CD4+ cells were observed in the epithelium among all the examined specimens. The number of CD1a+ dendritic cells in the epithelium of HL was always scarce (Fig 3). This number was much lower than in the apparently unaffected adjacent mucosa from the same subjects (Fig 4), and in the epithelium of clinically normal mucosa of HIV-positive and HIV-negative individuals. These cells were always HLA-DR- in HL, and sometimes HLA-DR+ in the other cases. CD11c+, CD14+, CD11b+/-, CD1a-, and HLA-DR- cells were observed in the epithelium of HL only, being mainly

located in the basal layer (Fig 5). Cells with similar immunologic features were never detected in the control biopsies from HIV-positive and HIV-negative subjects.

Quantitative data are given in Table 2.

Electron Microscopy

In the lamina propria of HIL, variable numbers of infiltrating cells were seen in clusters of two or more. In these cell clusters, lymphocytes and nonlymphoid cells were regularly associated with each other. Nonlymphoid cells had an oval, indented, pale nucleus with a thin, peripheral condensed rim, a well-developed Golgi apparatus, many smooth vesicles throughout all the cytoplasm, and many pits on the plasma membrane. The rough endoplasmic reticulum was variably developed; primary lysosomes were invariably found in the cytoplasm of these cells, and sometimes secondary lysosomes as well. These cells came into close contact with each other and lymphocytes over extended areas; in these areas the unit membranes of adjacent cells were about 20 nm distant from each other (Fig 6). Some mast cells were contained in this infiltrate.

In the lamina propria of apparently healthy areas of HIV-infected patients (with or without HL),



FIGURE 4. Perilesional lingual mucosa of patient with HL. Large numbers of CD1a+ dendritic cells are documented. (Immunoperoxidase stain; magnification $\times 360$.)

some infiltrating cells were seen with features resembling those described above for the cells in the lamina propria of areas of HL.

In the epithelium of IIL, an appreciable number of lymphocytes, sometimes gathered in groups of two to five, were seen. A small number of nonlymphoid, nonkeratinocyte cells were also found within the basal epithelial layer. All these cells had an oval, indented nucleus and pale chromatin with a thin, peripheral condensed rim; they were sometimes roundish with poorly developed organelles, and sometimes, on the contrary, they were dendritic in shape (but with relatively large and poorly branched dendrites) and contained a well-developed Golgi apparatus and many smooth vesicles (Fig 7). Typical or rudimentary Birbeck granules were never seen, despite careful examination. Only exceptionally were these cells seen in contact with lymphocytes (Fig 7). Round particles, about 70 nm in diameter, formed by a dense core bounded by a unit membrane, were seen within the endoplasmic reticulum of the above-described nonlymphoid, nonkeratinocytic, intraepithelial cells of two patients (Fig 9, top).

In the epithelium of apparently healthy areas of HIV-infected patients (with or without HL), some lymphocytes, sometimes gathered in groups of two to



FIGURE 5. Scattered CD11c+ cells in the basal epithelium of HL. (Immunoperoxidase stain; magnification $\times 360$.) (Inset) Note the dendritic shape of one of these cells (arrow) at higher magnification. (Magnification $\times 1,400$.)

five, and nonlymphoid cells were seen. These latter cells were located in the basal and suprabasal layers, were often in contact with lymphocytes, and had an ovoid body and thin, long, cytoplasmic branches (Fig 8). They contained well-developed smooth endoplasmic reticulum and Golgi apparatus, some cisternae of rough endoplasmic reticulum, numerous primary lysosomes, and sometimes Birbeck granules as well (Fig 9, bottom left and right).

DISCUSSION

In both the epithelium and lamina propria of HL, we have found cells with the immunohistochemical and ultrastructural features of ACs. Differences in the features of these cells were found between the epithelium and the lamina propria.

In the lamina propria, these cells were characterized by dendritic shape, clustering with lymphocytes, and expression of CD1a antigen. By electron microscopy, these cells were well-recognized because of their localization, shape, and relationship to lymphocytes. They were characterized by an indented pale nucleus with a thin peripheral rim of condensed chromatin, a

TABLE 2. Quantitative Assessment of Immunostained Cells

Monoclonal Antibody	Epithelium*		Lamina Propria†	
	HL	HIV +/- ‡	HL	HIV +/- ‡§
Anti-CD2	5-14	2-4/1-4	54-72	61-68/67-75
Anti-CD3	5-11	0-3/0-3	49-67	52-60/58-69
Anti-CD4	—	—	0-4	15-27/34-41
Anti-CD8	4-9	0-2/0-3	44-61	18-33/15-24
Anti-CD25	—	—	—	2-8/4-17
Anti-CD30	—	—	—	—/1-4
Anti-HLA Dr	—	2-4/3-9	—	5-12/11-19
Anti-CD1a	1-3	9-16/11-21	8-23	11-27/7-16
Anti-CD11b	1-4	—	6-9	5-9/2-8
Anti-CD11c	5-8	—	8-12	6-12/4-10
Anti-CD14	3-7	—	6-11	5-8/3-8
Anti-CD22	—	—	—	—

* Immunostained cells in the epidermis overlying 100 basal cells.

† Immunostained cells per 100 infiltrating cells.

‡ HIV-positive controls: either lingual mucosa from HIV-infected subjects without HL or perilesional, clinically healthy mucosa from patients with HL.

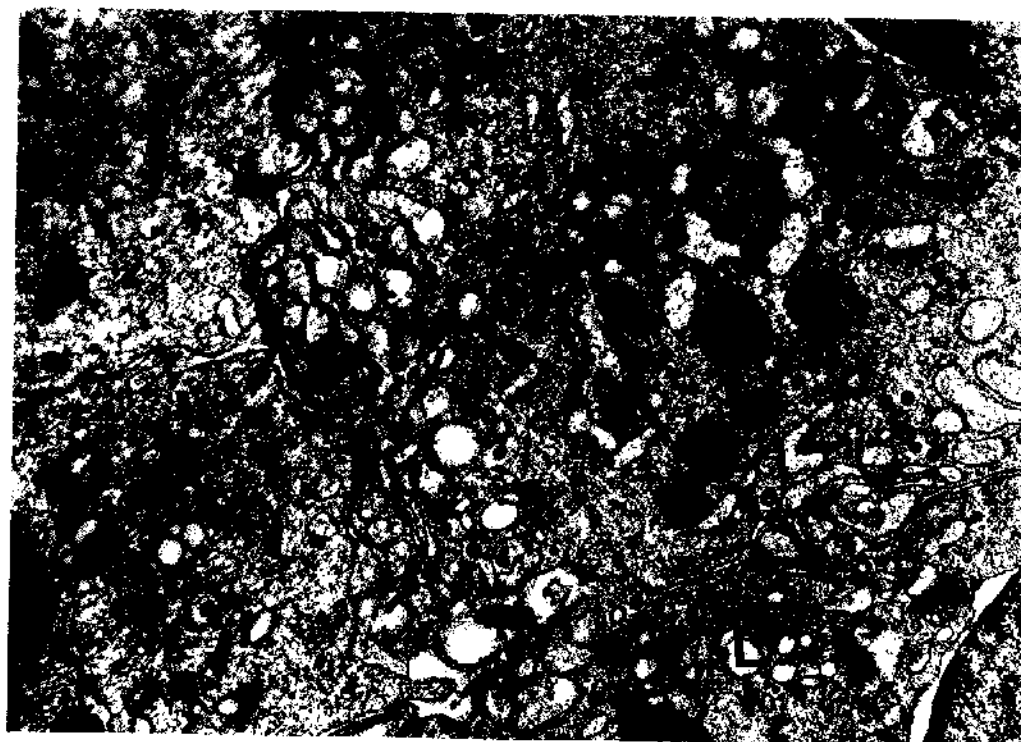
§ Appreciable infiltrate only focally found.

well-developed Golgi apparatus, and many smooth vesicles and primary lysosomes. All these features meet the criteria of fully differentiated ACs of secondary lymphoid organs (interdigitating reticulum cells)²⁶⁻³⁰ and of lymphoid T-cell infiltrates of the skin³¹⁻⁴¹ and mucous membranes.^{36,38} The expression of CD1a antigen, the shape, the ultrastructure, and the extended and close contacts with each other and with lymphocytes are shared by the ACs observed in the lamina propria of HL and those which are known to be functionally operating in the immune response.²⁹ Therefore, the morphologic equivalent of a cell-mediated immune response, ie, clusters of T

cells and fully differentiated ACs in close apposition to each other, occurs in the lamina propria of HL. However, the presence of these clusters may not necessarily imply that immune responses in these areas are normal because the antigenic phenotype of T cells—which are predominantly CD8+—does not correspond to that usually found in cutaneous and mucosal immune responses where CD4+ lymphocytes predominate.^{36,38}

In the epithelium, nonlymphoid nonkeratinocytic cells were characterized by bluntly dendritic shape, localization restricted to the basal layers, low or absent expression of CD1a antigen, absence of HLA-DR expression, constant expression of CD11c and CD14 antigens, and lack of clustering with lymphocytes. The CD11c and CD14 antigens are strongly expressed by monocytes⁴²⁻⁴⁴ and tissue macrophages,^{44,45} while CD11c antigen only is expressed, even though to a lesser extent, by Langerhans cells.⁴⁶ The CD1a antigen, on the contrary, is strongly expressed by Langerhans cells and T-zone ACs in dermatopathic lymphadenitis^{47,48} and in the skin,^{37,40,41} but not by monocytes and macrophages. By electron microscopy some of the nonlymphoid nonkeratinocytic cells in the epithelium were roughly similar to those found in the lamina propria, but they showed relatively large and poorly branched dendrites, contained few if any primary lysosomes, and contacted lymphocytes only exceptionally; others had a roundish shape and poorly developed organelles, therefore resembling monocytes. We interpret all these cells as variably, but always incompletely, differentiated cells of monocyte-dendritic lineage.^{26,28,29,47} The incomplete differentiation of ACs and the virtual absence of CD4+ T cells in the lesional epithelium of HL indi-

FIGURE 6. Lamina propria of HL. Nonlymphoid cell in close contact with two lymphocytes (L) which were recognized at lower magnification. Primary lysosomes, coated vesicles (arrowhead), and narrow invaginations of the plasma membrane (arrows) are evident. (Electron microscopy; magnification $\times 16,560$.)



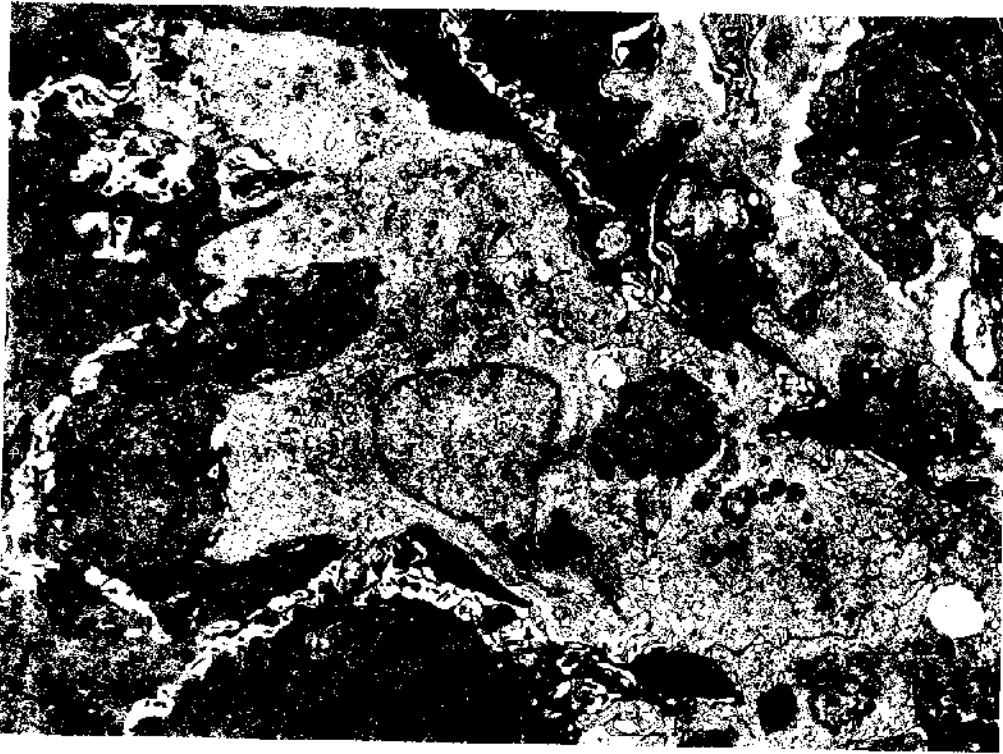


FIGURE 7. Epithelium of HL. Nonlymphoid nonkeratinocytic cell with large and poorly branched dendrites in the basal layer. This figure documents the occasional finding of apposition to lymphocytes (arrows). The arrowheads indicate the intracisternal particles, shown at higher magnification in Fig 9, top. (Electron microscopy, magnification $\times 5,045$.)

cate that an efficient immune response cannot occur in this tissue, and suggest that defects in the differentiation of intraepithelial ACs play a major role in the defective intraepithelial immune response.

To the best of our knowledge, this is the first report on the presence of CD11c+ and CD14+ cells in the epithelium of HL, and on the ultrastructure of

ACs in the epithelium and lamina propria of HL and clinically normal mucosa of HIV-positive subjects. The findings concerning the density and localization of CD1a+ cells in the epithelium and lamina propria of HL and control specimens are in agreement with those of other investigators,¹³ showing a relevant decrease in the number of these cells in the lesional

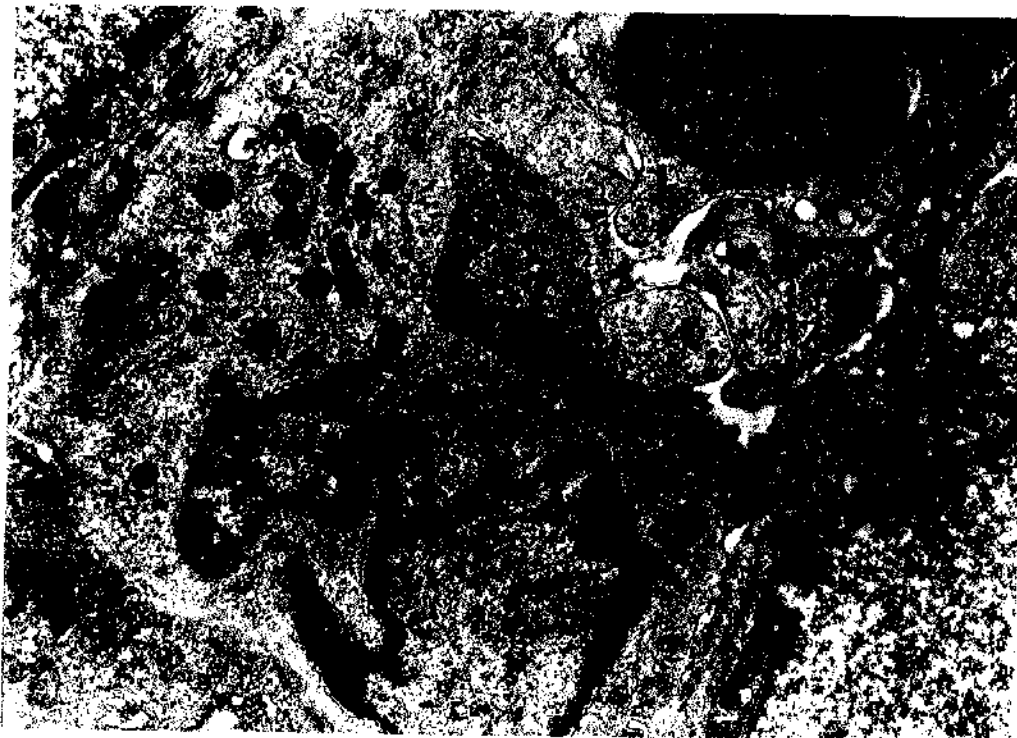


FIGURE 8. Epithelium of clinically healthy mucosa of HIV-positive patient. Nonlymphoid nonkeratinocytic cell in close contact (arrow) with a lymphocyte (L) in the basal layer. (Electron microscopy, magnification $\times 11,800$.)

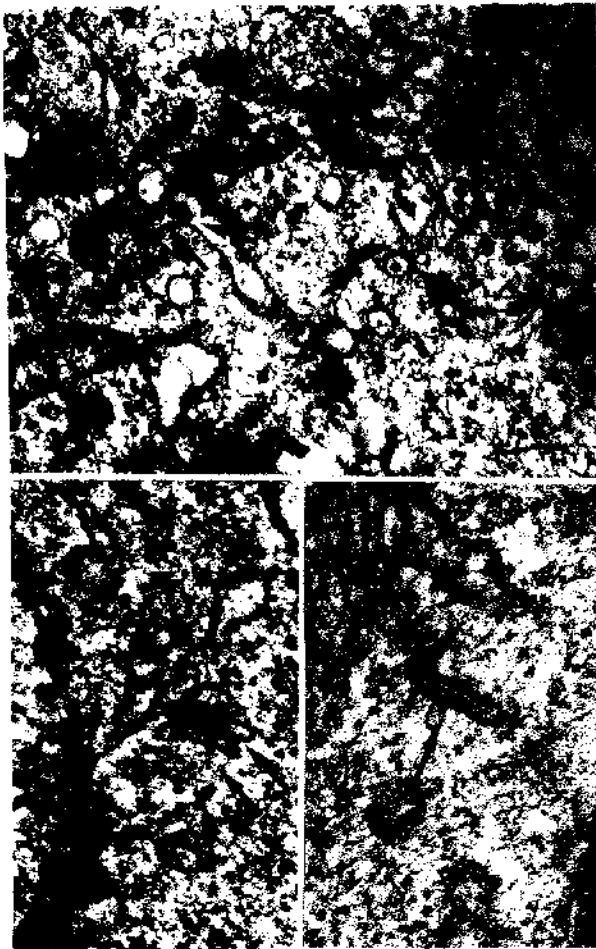


FIGURE 9. (Top) Epithelium of HL. Round particles with a dense core (arrows) in the endoplasmic reticulum of the nonlymphoid nonkeratinocytic cell shown in Fig 7. (Electron microscopy; magnification $\times 35,000$.) (Bottom left and right) Epithelium of clinically healthy mucosa of HIV-positive patient. Birbeck granules of different sites (arrows) from two nonkeratinocytic cells. (Electron microscopy. Magnifications: left, $\times 5,500$; right, $\times 70,000$.)

epithelium of HL compared with the adjacent epithelium of clinically normal mucosa and of HIV-positive and HIV-negative controls.

The intracisternal particles, about 70-nm wide, found in the intraepithelial ACs of two patients resemble intracisternal A particles described in retroviral infections⁴⁹; this finding is in line with the reported infection of these cells by HIV.^{4,6,9}

It is impossible to state exactly what initiates this peculiar alteration of the lesional epithelium known as HL. On one hand, the perturbation of the epithelial microenvironment, possibly induced by Epstein-Barr virus,⁵⁰ along with other putative local factors may have a direct effect on the differentiation of ACs as suggested by various authors in different studies.^{40,51-58} On the other hand, the direct HIV infection of ACs¹⁰⁻¹² may contribute to the perturbation of the local immune response, thus facilitating the expression of Epstein-Barr virus within keratinocytes.

In any case, it may be inferred from the data

presented here that the lesional epithelium of HL represents a pathway for the entry of foreign antigens, which may induce immune tolerance because they circumvent monitoring by Langerhans cells.⁵⁹ Since IIL often heralds full-blown AIDS,¹⁵ the demonstration of a defective intraepithelial differentiation of ACs in this condition, achieved in this research, leads us to stress the importance of alterations of ACs as an early step in the development of immunodeficiency.^{4,9,10} Moreover, the results of this study as well as those of other investigations^{14,60} show the importance of taking into account differences in the degree of impairment of local immune response among different regions of the body in order to understand the clinical manifestations of AIDS.

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REFERENCES

1. Wood GS, Warner NL, Warnke RA: Anti-Leu 3/T4 antibodies react with cells of monocyte/macrophage and Langerhans lineage. *J Immunol* 131:212-216, 1983
2. Groh V, Jani M, Harrer AB, et al: Leu 3/T4 expression on epidermal Langerhans cells in normal and diseased skin. *J Invest Dermatol* 86:115-120, 1986
3. Belsito DV, Sanchez MR, Baer RL, et al: Reduced Langerhans cell Ia antigen and ATPase activity in patients with acquired immunodeficiency disease. *New Engl J Med* 310:1279-1282, 1984
4. Armstrong JA, Dawkins RL, Horne R: Retroviral infection of accessory cells and the immunological paradox in AIDS. *Immunol Today* 6:97-98, 1985
5. Oxholm P, Helweg-Larsen S, Permin H: Immunohistological skin investigations in patients with the acquired immune deficiency syndrome. *Acta Pathol Microbiol Immunol Scand [A]* 94:113-116, 1986
6. Tschachler E, Groh V, Popovic M, et al: Epidermal Langerhans cells. A target for HTLV-III/LAV infection. *J Invest Dermatol* 88:233-237, 1987
7. Gielen V, Schmitt D, Dezutter-Dambuyant C, et al: Epidermal Langerhans cells are immunophenotypically modified in HIV-infected patients, in Thivolet J, Schmitt D (eds): *The Langerhans Cell*. London, UK, Libbey, 1988, pp 431-440
8. Dreno B, Milpied B, Bignon JD, et al: Prognostic value of Langerhans cells in the epidermis of HIV patients. *Br J Dermatol* 118:481-486, 1988
9. Boylston AW, Francis ND: Does it matter which cells are infected by the human immunodeficiency virus type 1? *J Pathol* 156:93-96, 1988
10. Braathen LR, Ramirez G, Kunze ROF, et al: Langerhans cells in mucous membranes and skin may be the primary target cells for HIV, in Thivolet J, Schmitt D (eds): *The Langerhans Cell*. London, UK, Libbey, 1988, pp 441-448
11. Ray A, Schmitt D: La cellule de Langerhans: Antigenes CD1 et granule de Birbeck. *Pathol Biol (Paris)* 36:846-853, 1988
12. Rappersberger K, Gartner S, Schenk P, et al: Langerhans' cells are actual site of HIV-1 replication. *Intervirology* 29:185-194, 1988
13. Daniels TE, Greenspan D, Greenspan JS, et al: Absence of Langerhans cells in oral hairy leukoplakia, an AIDS associated lesion. *J Invest Dermatol* 89:178-182, 1987
14. Becker J, Ulrich P, Kunze R, et al: 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]* 412:413-419, 1988
15. Greenspan D, Greenspan JS, Hearst NC, et al: Relation of oral hairy leukoplakia to infection with the human immunodeficiency virus. *J Invest Dermatol* 89:178-182, 1987

- ciency virus and the risk of developing AIDS. *J Infect Dis* 155:475-481, 1987
16. Ficarra G, Barone R, Gaglioti D, et al: Oral hairy leukoplakia among HIV-positive drug abusers: A clinicopathologic and electron microscopic study. *Oral Surg* 4:421-426, 1988
 17. Alessi A, Cusini M, Zerboni R: Mucocutaneous manifestations in patients infected with human immunodeficiency virus. *J Am Acad Dermatol* 19:290-297, 1988
 18. Barone R, Ficarra G, Gaglioti D, et al: Prevalence of oral lesions among HIV-infected intravenous drug abusers and other risk groups. *Oral Surg* 1989 (in press)
 19. Greenspan D, Greenspan JS, Conant M, et al: Oral "hairy" leukoplakia in male homosexuals: Evidence of association with both papillomavirus and a herpes-group virus. *Lancet* 2:831-834, 1984
 20. Ficarra G: AIDS e odontoiatria. Milan, Italy, Masson, 1987
 21. Ficarra G, Barone R, Gaglioti D, et al: Oral hairy leukoplakia: Clinicopathologic, ultrastructural and DNA hybridization findings in IV drug abusers. *Oral Surg* 66:568, 1988
 22. Zhang X, Langford A, Becker J, et al: Ultrastructural and immunohistochemical findings in oral hairy leukoplakia. *Virchows Arch [A]* 412:533-542, 1988
 23. Pimpinelli N, Romagnoli P, Moretti S, et al: Non-lymphoid accessory cells in the cutaneous infiltrate of B cell lymphomas. An immunohistochemical and ultrastructural study. *Br J Dermatol* 118:353-362, 1988
 24. Chan JKC, Ng CS, Hui PK: A simple guide to the terminology and application of leukocyte monoclonal antibodies. *Histopathology* 12:461-480, 1988
 25. Riva A: A simple and rapid staining method for enhancing the contrast of tissues previously treated with uranyl acetate. *J Microsc* 19:105-108, 1974
 26. Veldmann JE: Histophysiology and electron microscopy of the immune response. Doctoral thesis, University of Groningen, Groningen, The Netherlands, 1970
 27. Kaiserling E, Lennert K: Die interdigitierende Reticulumzelle im menschlichen Lymphknoten. *Virchows Arch [B]* 16:51-61, 1974
 28. Hoefsmit ECM: Mononuclear phagocytes, reticulum cells and dendritic cells in lymphoid tissues, in Van Furth R (ed): *Mononuclear Phagocytes in Immunity, Infection and Pathology*. Oxford, England, Blackwell, 1975, pp 129-146
 29. Fossum S, Ford WL: The organization of cell population within lymph nodes: Their origin, life history and functional relationships. *Histopathology* 9:469-499, 1985
 30. Fossum S, Vaaland JL: The architecture of rat lymph nodes. I. Combined light and electron microscopy of lymph node cell types. *Anat Embryol* 167:229-246, 1983
 31. Goos M: T-cell specific microenvironment in mycosis fungoides. *Arch Dermatol Res* 255:215-218, 1976
 32. Goos M, Kaiserling E, Lennert K: Mycosis fungoides: Model for T-lymphocyte homing to the skin? *Br J Dermatol* 94:221-222, 1976
 33. Braun-Falco O, Smoekel C, Wolff HH: The ultrastructure of mycosis fungoides, of Sezary's syndrome and of Worsinger Kolopp's disease (pagetoid reticulosis). *Bull Cancer* 64:191-208, 1977
 34. Holden CA, Morgan EW, MacDonald DM: The cell population in the cutaneous infiltrate of mycosis fungoides: In situ studies using monoclonal antisera. *Br J Dermatol* 106:385-392, 1982
 35. McMillan EM, Beeman K, Wasik R, et al: Demonstration of OK16-reactive cells in mycosis fungoides. *J Am Acad Dermatol* 6:880-887, 1982
 36. Giannotti B, De Panfilis G, Manara GG, et al: Macrophage-T-lymphocyte interaction in lichen planus. *Arch Dermatol Res* 275:35-40, 1983
 37. Smolle J, Kaudewitz P, Burg G, et al: Significance of non-lymphoid (accessory) cells in malignant lymphomas and pseudolymphomas of the skin. *Br J Dermatol* 113:677-685, 1985
 38. De Panfilis G, Rowden G: Quantitative studies on nonlymphoid mononuclear cell subpopulations in cutaneous infiltrates. I. Stage-related changes of dendritic nonlymphoid mononuclear cells, Langerhans cells and macrophages in lichen planus lesions. *Am J Dermatopathol* 8:44-52, 1986
 39. Romagnoli P, Moretti S, Fattorossi A, et al: Dendritic cells in the dermal infiltrate of Sezary syndrome. *Histopathology* 10:25-36, 1986
 40. Drijkoningen M, De Wolf-Peeters C, Degreef II, et al: Epidermal Langerhans cells, dermal dendritic cells, and keratinocytes in viral lesions of skin and mucous membranes: An immunohistochemical study. *Arch Dermatol Res* 280:220-227, 1988
 41. Bani D, Moretti S, Pimpinelli N, et al: Interdigitating reticulum cells in the dermal infiltrate of mycosis fungoides. An ultrastructural and immunohistochemical study. *Virchows Arch [A]* 412:451-458, 1988
 42. Schwarting R, Stein H, Wang CY: The monoclonal antibodies S-HCL 1 (Leu 14) and S-HCL 3 (Leu M5) allow the diagnosis of hairy cell leukemia. *Blood* 65:974-983, 1985
 43. Goyert SM, Ferrero EM, Scrementis SV, et al: Biochemistry and expression of myelomonocytic antigens. *J Immunol* 137:3909-3916, 1987
 44. Hogg N, Morton LA: Myeloid antigens: New and previously defined clusters, in McMichael AJ (ed): *Leukocyte Typing III. White Cell Differentiation Antigens*. Oxford, England, Oxford, 1988, pp 576-588
 45. Hogg N, Takacs L, Palmer DG, et al: The p 130, 95 molecule is a marker of human mononuclear phagocytes: Comparison with expression of class II molecules. *Eur J Immunol* 16:240-248, 1985
 46. De Panfilis G, Manara GC, Ferrari C, et al: Reactivity of Langerhans cells with anti CD11c monoclonal antibody, in Thivolet J, Schmitt D (eds): *The Langerhans Cell*. London, UK, Libbey, 1988, pp 149-156
 47. Goordyal P, Isaacson PG: Immunocytochemical characterization of monocytic colonies of human bone marrow: A clue to the origin of Langerhans cells and interdigitating reticulum cells. *J Pathol* 146:189-195, 1985
 48. Ralfkiaer E, Stein H, Plesner T, et al: In situ immunological characterization of Langerhans cells with monoclonal antibodies: Comparison with other dendritic cells in skin and lymph nodes. *Virchows Arch [A]* 403:401-412, 1984
 49. Dalton AJ, Haguenu F: Introduction [to RNA tumor viruses], in Dalton AJ, Haguenu F (eds): *Ultrastructure of Animal Viruses and Bacteriophages: An Atlas*. San Diego, CA, Academic, 1973, pp 255-260
 50. Purilo DT, Kipscomb H, Krueger G, et al: Role of Epstein-Barr virus in AIDS. *Adv Exp Med Biol* 187:53-65, 1985
 51. Morris HB, Catter KC, Sykes C, et al: Langerhans' cells in human cervical epithelium: Effects of wart virus infection and intraepithelial neoplasia. *Br J Obstet Gynecol* 90:412-420, 1983
 52. Tav SK, Jenkins D, Maddox P, et al: Subpopulations of Langerhans' cells in cervical neoplasia. *Br J Obstet Gynecol* 94:10-15, 1987
 53. Viau J, Haftek M, Chardonnet Y: Langerhans cells and human papillomavirus infections, in Thivolet J, Schmitt D (eds): *The Langerhans Cell*. London, UK, Libbey, 1988, pp 449-460
 54. Ford GP, Friedman PS, Ross J: Langerhans cells in autosomal dominant ichthyosis vulgaris. *Br J Dermatol* 109:309-311, 1983
 55. Kolde G, Happle R: Langerhans cell degeneration in X-linked dominant ichthyosis: A quantitative and ultrastructural study. *Arch Dermatol Res* 277:245-247, 1985
 56. Smolle J, Soyer HP, Eball R, et al: Langerhans cell density in epithelial skin tumors correlates with epithelial differentiation but not with the peritumoral infiltrate. *J Invest Dermatol* 87:477-479, 1986
 57. Sudo S, Saito A, Morohashi M: Immunohistochemical study of Langerhans cells in skin tumors. *Dermatologica* 174:76-83, 1987
 58. Pimpinelli N, Bani D: Langerhans cells and epidermal microenvironment. *Am J Dermatopathol* 11:188-189, 1989
 59. Hoefsmit ECM, Duijvestijn AM, Kamperdijk EWA: Relation between Langerhans cells, veiled cells and interdigitating cells. *Immunobiology* 161:255-265, 1982
 60. Santucci M, Pimpinelli N, Moretti S, et al: Classic and immunodeficiency-associated Kaposi sarcoma: Clinical, histologic, and immunologic correlations. *Arch Pathol Lab Med* 112:1214-1220, 1988