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

R. RICCARDI, ScD, N. PIMPINELLI, MD, G. FICARRA, MD, L. BORGOGNONI, MD, D. GAGLIOTI, MD, D. MILO, MD, and P. ROMAGNOLI, MD

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-

From the Department of Human Anatomy and Histology, Second Dermatology Clinic, and Chair of Maxillofacial Surgery, University of Florence, Florence, Italy; and the Division of Infectious Diseases, National Health Service, District n 10/D of Florence, Florence, Italy. Accepted for publication December 8, 1989.

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

Address correspondence and reprint requests to R. Riccardi, ScD, Department of Human Anatomy and Histology, Section Histology "E. Allara", Viale Pieraccini, 6, I - 50139 Firenze (Italia).

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entiation and mutual interactions of immune response cells within HL, 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 paraplast 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]

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

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 × 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 specificities[24] are listed in Table 1.

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 HL specimens, a sparse infiltrate of CD2+, CD3+, CD8+, CD1−, 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 Clone, Birmingham, United Kingdom; ODS, Ortho Diagnostic Systems, Raritan, NJ; BD, Becton Dickinson, Sun Valley, CA; DP, Dakopatts, Copenhagen, Denmark.

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

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

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

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 HL, 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 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 skin[31-41] 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 monocytes[42-44] 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 nonkeratinocyte 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-

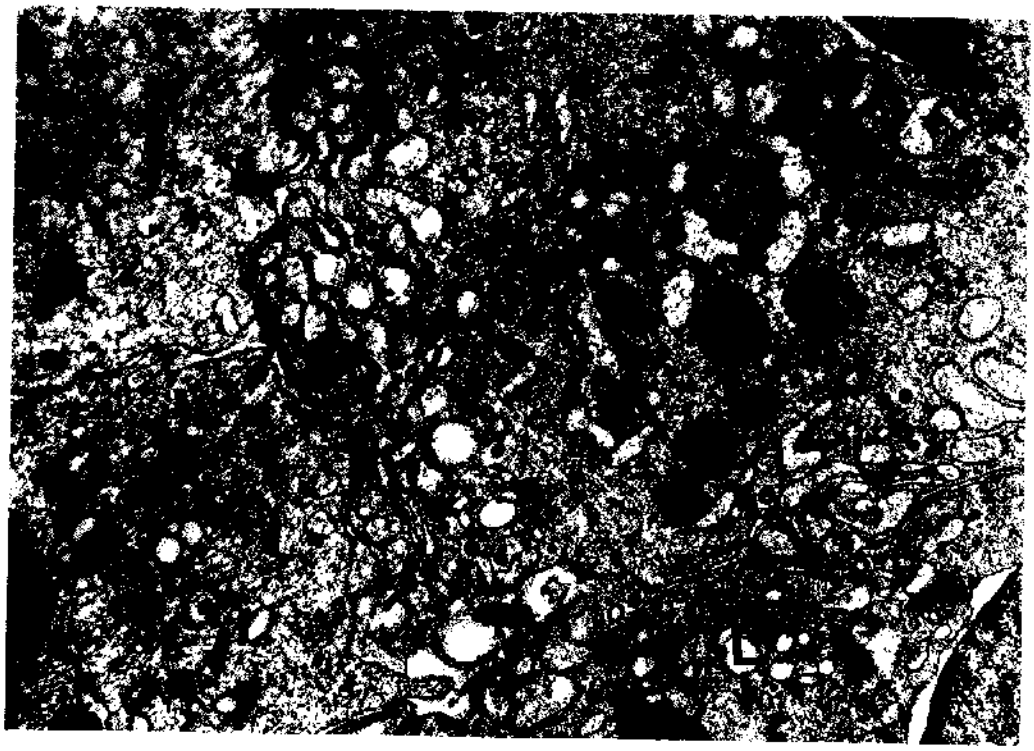

FIGURE 6. Lamina propria of HL. Nonlymphoid cell in close contact with two lymphocytes (L) which were recognized at lower magnification. Primary lysosomes, coated vesicles (arrowhead), and narrow invaginations of the plasma membrane (arrows) are evident. (Electron microscopy; magnification × 16,560.)

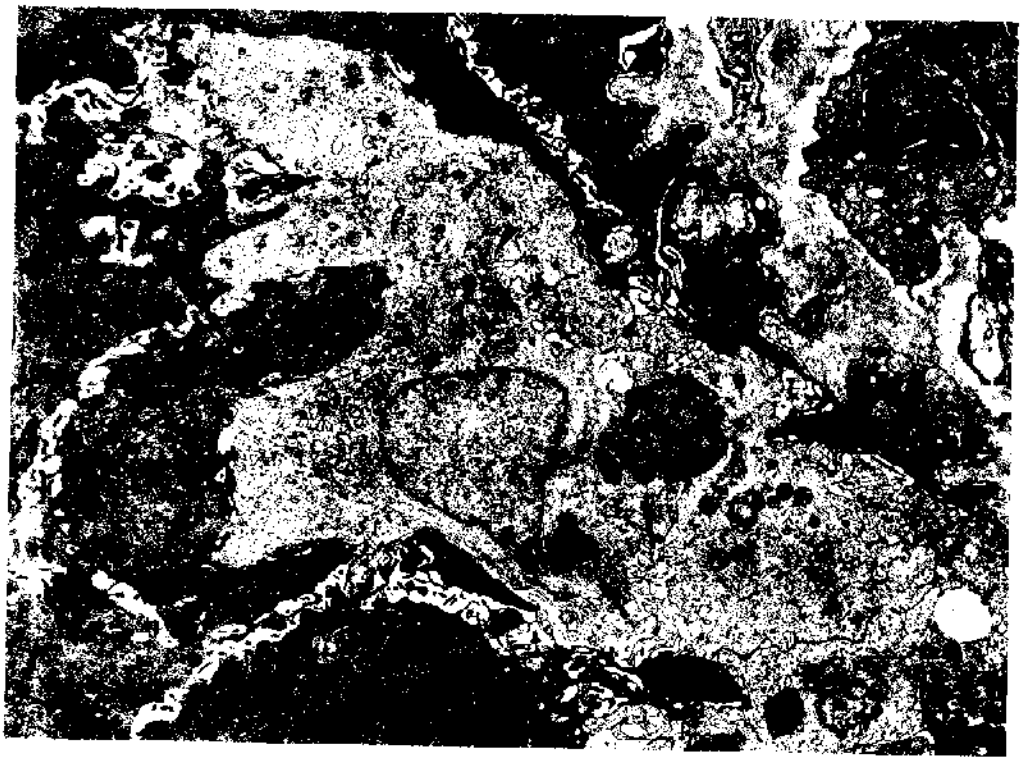

FIGURE 7. Epithelium of HL. Nonlymphoid nonkeratinocytic cell with large and poorly branched dendrites in the basal layer. This figure documents the occasional finding of apposition to lymphocytes (arrows). The arrowheads indicate the intracisternal particles, shown at higher magnification in Fig 9,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

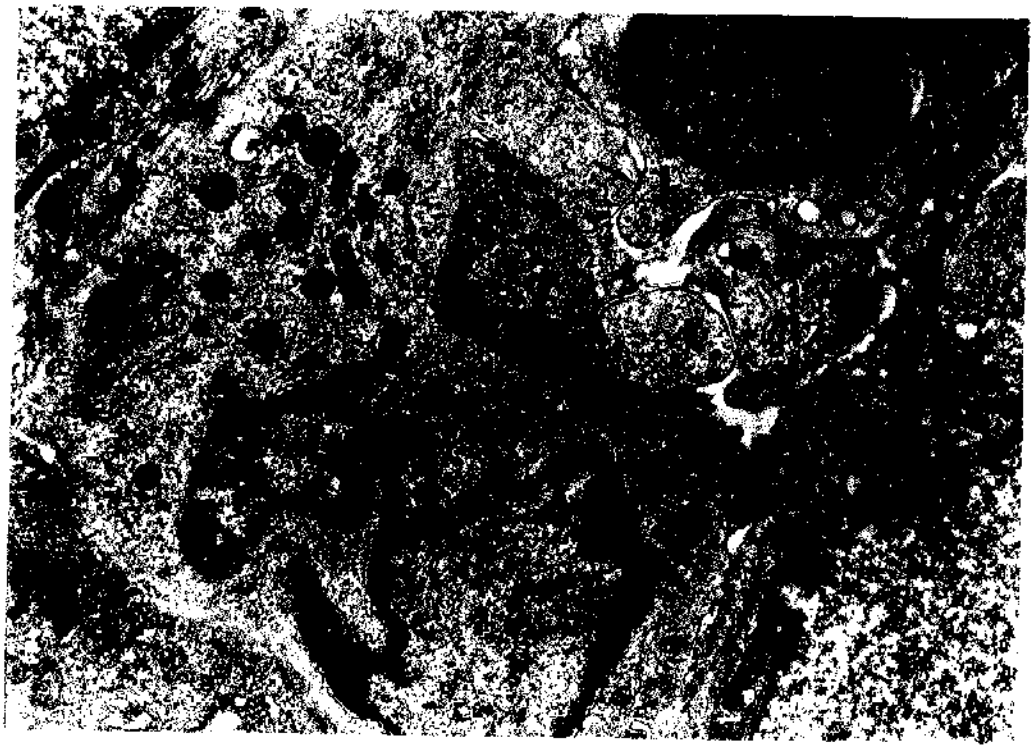

FIGURE 8. Epithelium of clinically healthy mucosa of HIV-positive patient. Nonlymphoid nonkeratinocytic cell in close contact (arrow) with a lymphocyte (L) in the basal layer. (Electron microscopy; magnification × 11,800.)

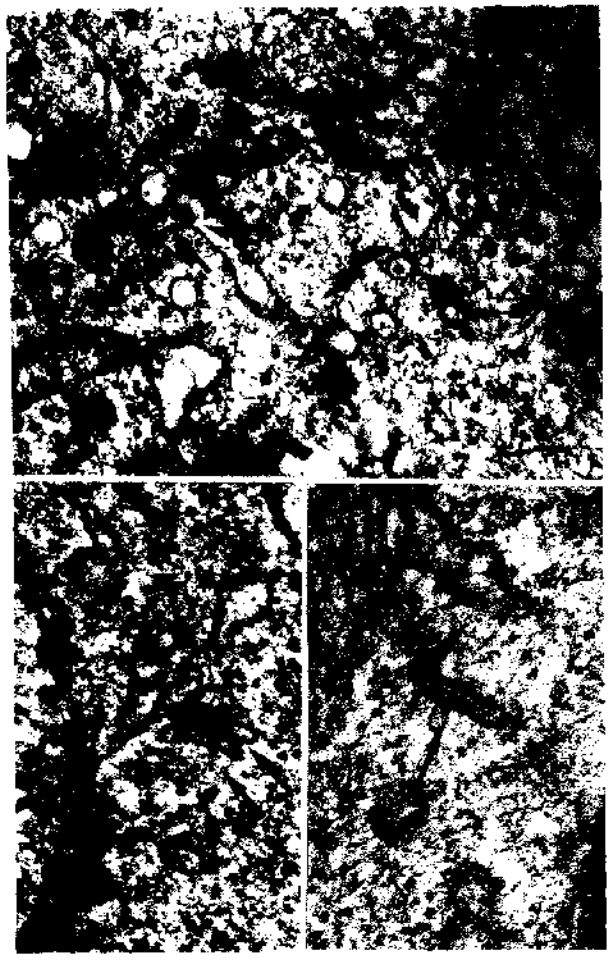

FIGURE 9. (Top) Epithelium of HL. Round particles with a dense core (arrows) in the endoplasmic reticulum of the nonlymphoid nonkeratinocytic cell shown in Fig 7. (Electron microscopy; magnification × 35,000.) (Bottom left and right) Epithelium of clinically healthy mucosa of HIV-positive patient. Birbeck granules of different sites (arrows) from two nonkeratinocytic cells. (Electron microscopy. Magnifications: left, × 5,500; right, × 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 infections[49]; 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 ACs[10-12] 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 HL 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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