Ultrastructural findings in clinically uninvolved oral mucosa of patients with HIV infection

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Zhang X, Langford A, Gelderblom H, Reichart P: Ultrastructural findings in clinically uninvolved oral mucosa of patients with HIV infection. J Oral Pathol Med 1989; 18: 35–41.

Twelve biopsies of clinically normal oral mucosa taken from HIV seropositive patients have been investigated by means of light- and electron microscopy. Vascular abnormalities were found in all biopsies, regardless of the clinical stage of the HIV infection. In particular slit-like vascular channels, sparseness of intercellular junctions and swollen, protruded endothelial cells with an increased quantity of Weibel-Palade bodies were noticed. These findings were similar to those described in lesions of early stage Kaposi's sarcoma.

Key words: AIDS; HIV; mouth, structure; oral mucosa; vascular abnormalities.

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Accepted for publication October 29, 1988.

On the basis of severe virus-induced defects in the hosts immune system, HIV induces a variety of immunological disorders and clinical symptoms. Kaposi's sarcoma (1) is one of the diseases that fullfills the CDC-surveillance criteria for the diagnosis of AIDS, which is the ultimate manifestation of this lentivirus infection. About 95% of AIDS-associated KS occur in male homo- or bisexual men, independent of the clinical staging of the Walter Reed classification (2, 3). There has been an unexplained steady decline in the percentage of patients with AIDS, presenting with KS, decreasing from 21% before 1984 to the current level of 14% (4).

The histopathologic pattern of KS is characterized by an increase in capillary growth: angiogenesis is manifested by multiple, often densely packed vascular, slit-like channels lined by enlarged endothelial cells. In later stages of KS extravasation and intervascular spindle-shaped cells become more prominent (5, 6). Recently, atypical vascular proliferation with vessels lined by cuboidal endothelial cells protruding into the lumina has been described in cutaneous nodules of cat-scratch disease occurring in the course of HIV-infection (7–9). Similar findings have been reported to occur in clinically normal skin of AIDS-patients (10, 11).

It was the purpose of the present study to examine the histology and ultrastructure of clinically uninvolved oral mucosa of patients with different clinical manifestations of HIV infection.

Material and methods

Biopsies were taken from clinically normal oral mucosa of 10 HIV-seropositive men and two women (average age: 34.7 y; homo-/bisexual men: $n=7$; i.v. drug abusers: $n=5$). Four patients were clinically asymptomatic and three patients

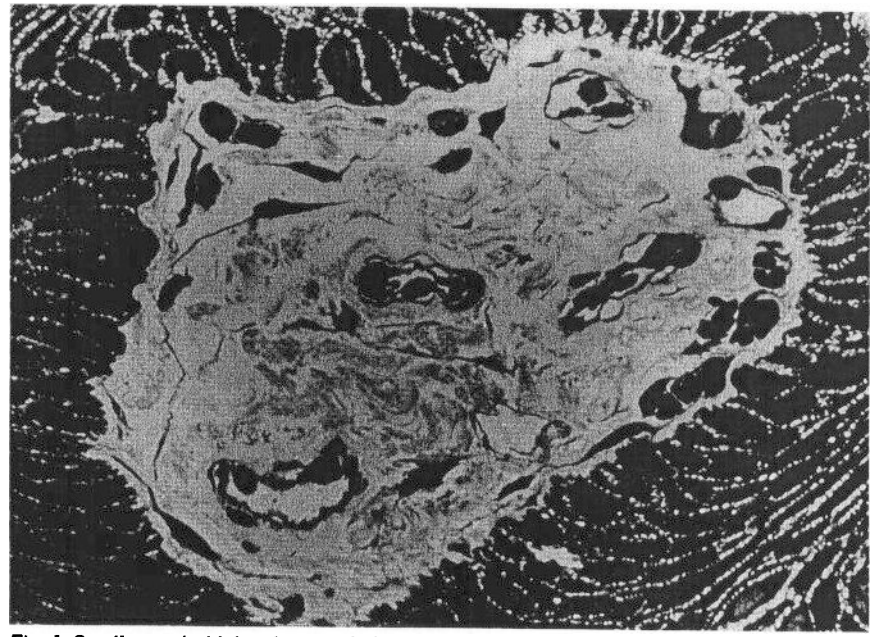

Fig. 1. Swollen, cuboidal and protruded endothelial cells of blood vessels within subepithelial connective tissue. Toluidine blue × 800.

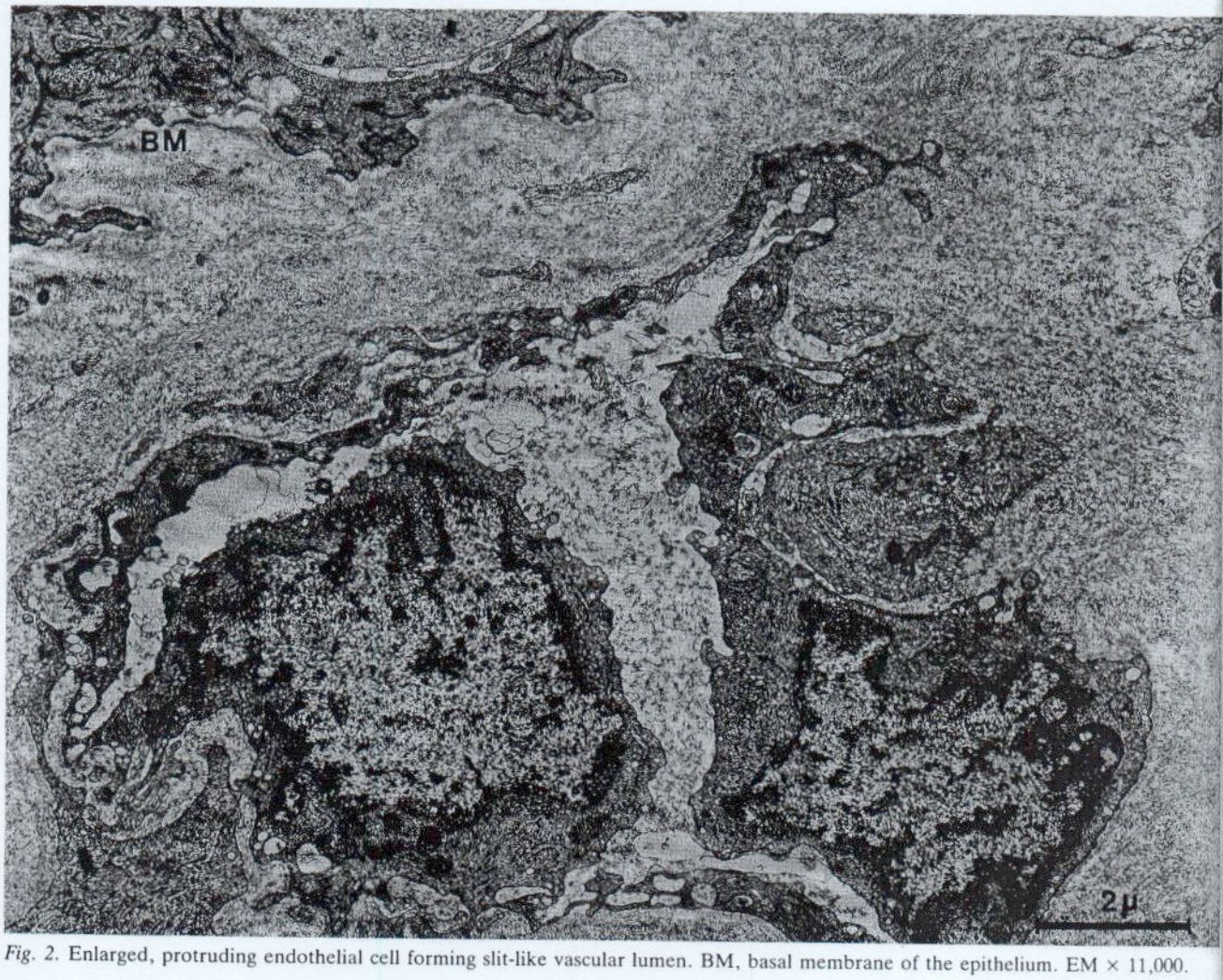

Fig. 2. Enlarged, protruding endothelial cell forming slit-like vascular lumen. BM, basal membrane of the epithelium. EM × 11,000.

showed ARC symptoms. Five biopsies were taken from AIDS patients, two of whom suffered from disseminated KS.

Tissues were divided for light and electron microscopy. For light microscopy specimens were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Sections were cut at 5 µm and stained with H&E. Semithin sections (0.5–1 µm in thickness) were cut from Epon-embedded specimens and stained with toluidine blue. For transmission electron microscopy (TEM) biopsies were cut into 1 mm cubes, fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. After en bloc staining in 1% uranyl acetate and stepwise dehydratation in ethanol, cubes were embedded in Epon 812, according to standard methods (12). Ultrathin sections (40–50 nm) were mounted on bare grids, poststained with lead citrate, stabilized with carbon and examined using a Zeiss EM 10A at 60 KV.

Results

Light microscopy

All cases revealed a high number of capillary vessels within the subepithelial connective tissue. Endothelial cells, lining the lumina of the vessels, were often enlarged to cuboidal shape protruding into the lumina and showed small amounts of cytoplasm. The lumina of some capillaries were reduced to slit-like spaces (Fig. 1).

Electron microscopy

In 10/12 biopsies capillaries of the subepithelial connective tissue were lined by enlarged endothelial cells, often occluding the capillary lumen (Fig. 2). Some of the endothelial cells showed highly irregular, cuboidal shape. In 9/12 biopsies loss of intercellular junctions between neighbouring endothelial cells, presence of intercellular gaps (Fig. 3) and interruption of the basal membranes was observed (Fig. 4). Weibel-Palade bodies (WPB), characteristic for endothelial cells, were frequently detected (Table 1). They showed a rod-like shape with a diameter of 150 nm and longitudinal striations with a periodicity of 20 nm (Fig. 5). Aggregates of tubulo-reticular structures with a diameter of 30 nm were found in the endoplasmic reticulum of endothelial cells (11/12 biopsies) (Fig. 6). Another type of intracytoplasmic structure was noted in one of the endothelial cells; it was characterized by tube-like projections and contained a few long microtubuli, thus resembling the cilia of respiratory epithelial cells. These cilia-like structures seemed to insert within the cytoplasm of the endothelial cell, while the projection extended out of the cell surface (Fig. 7). The connective tissue surrounding vessels contained many collagen fibers. In 4/12 biopsies multiple extravasated erythrocytes were noted (Fig. 8). Infil-

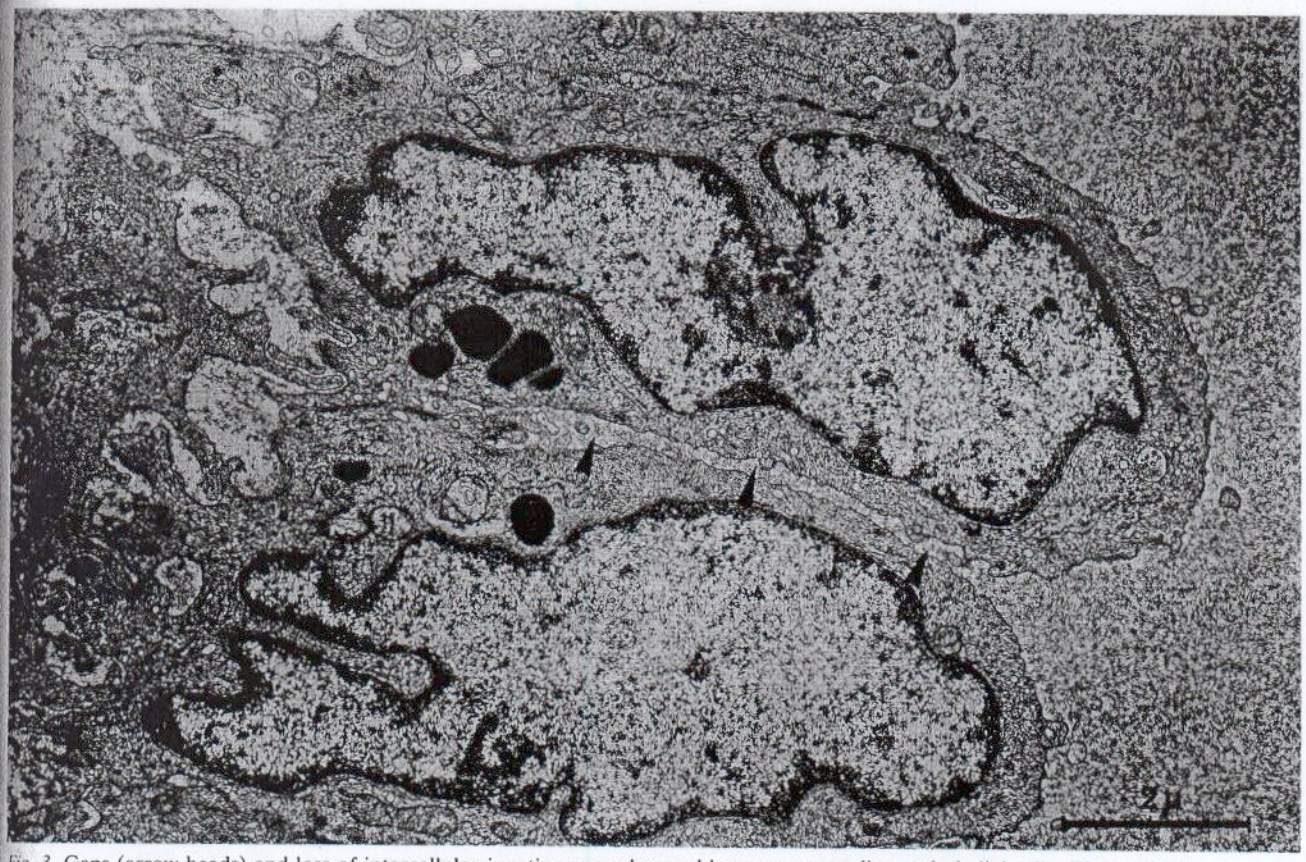

Fig. 3. Gaps (arrow heads) and loss of intercellular junctions are observed between protruding endothelial cells. EM × 12,000.

tration by lymphocytes and/or macrophages was not observed. The ultrastructural findings of the biopsies are summarized in Table 1.

Discussion

Kaposi's sarcoma (KS) in HIV-infected patients has become a rather frequent manifestation of a previously rare tumor (12).

The histopathology of the early stages of KS is characterized by a con-

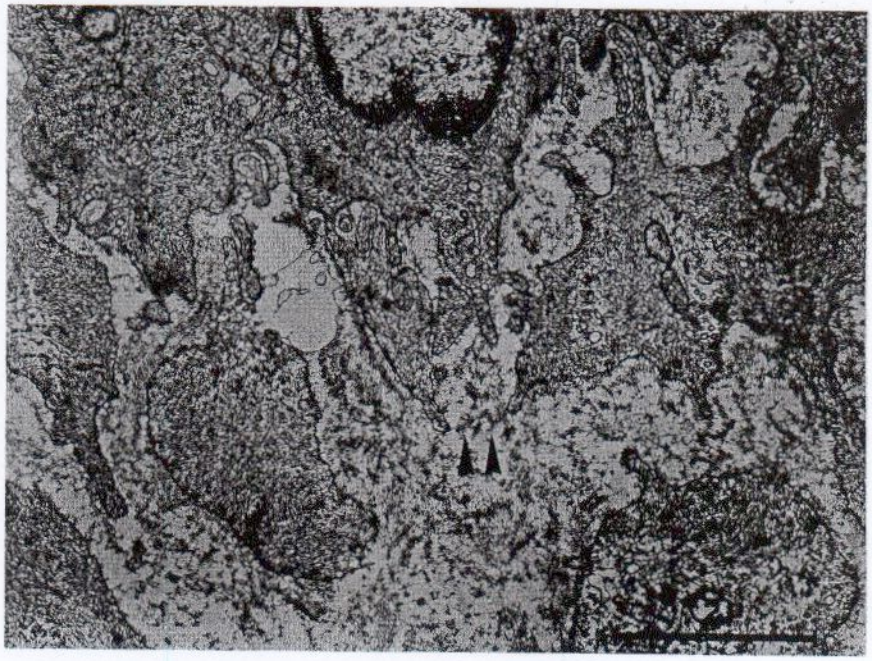

Fig. 4. Interruption and incomplete formation of basal membranes of vessel-like structure (arrow head). EM × 13,000.

Table 1. Electron microscopic findings in biopsies taken from 12 HIV-1 seropositive persons.

Biopsy	Swollen endothelial cells	Loss of junction	Interruption of BM	W-P body	Tubular structure	"Cilia"	Extravasated RBC
1	+	−	−	+	+	−	−
2	+	+	+	+	+	−	−
3	+	−	+	+	+	−	+
4	+	+	+	+	+	−	−
5	+	+	+	−	+	−	+
6	+	+	+	+	−	−	−
7	−	+	−	−	+	+	+
8	−	−	+	+	+	−	−
9	+	+	+	+	+	−	−
10	+	+	+	+	+	−	+
11	+	+	+	−	+	−	−
12	+	+	−	−	+	−	−

spicuous increase in capillary growth and lateron by the predominance of spindle-shaped cells. Furthermore, hemosiderin, eosinophilic bodies and extravasated erythrocytes as well as plasma cells and lymphocytes were frequently observed in KS lesions (5, 6, 14), the proportion of each component varying with the clinical stage of the lesion. While the histological appearance of late-stage KS lesions, clinically presenting as nodules, is dominated by spindle cells with interspersed blood-filled clefts, early KS lesions, clinically appearing as macules, are composed predominantly of endothelium-lined, vessel-like structures (6, 15). The appearance of cuboidal endothelial cells with enlarged nuclei and partially incomplete formation of the basal membranes has been considered to be the earliest ultrastructural hint for neoplastic growth (5).

The occurrence of vascular channels with proliferating endothelial cells, however, is also a characteristic pattern of vascularization in healthy tissue, but in addition it can be often noted in benign tumors such as hemangioma, pyogenic granuloma and dermatofibroma (15). The main ultrastructural differences between benign vascular growth and KS lesions are described as the reduction of dendritic pericytes in vessels, frequent discontinuities in the endothelial lining, and necrosis of individual endothelial cells (15). While some of these features can be found in very limited regions of the benign vascular neoplasms, they are observed regularly throughout KS lesions.

An epithelioid angiomatosis-like entity, occurring in HIV-infected patients, has been described recently (7–9). These cutaneous vascular lesions, clinically resembling KS, consist of proliferating capillaries lined by swollen, cuboidal endothelial cells and a mixed interstitial inflammatory infiltrate, composed of neutrophils, plasma cells and lymphocytes. The presence of clusters of bacteria, identified as cat scratch disease bacillus (CSD) characterized this lesion as a reactive, inflammatory vascular proliferation.

Abnormal vascular formation (pre-Kaposi's sarcoma) has been reported as well in clinically uninvolved skin of a homosexual patient with AIDS and Kaposi sarcoma (10). Ultrastructural studies in these cases revealed protrusion of endothelial cells, vascular slit-like channels, sparseness of intercellular junctions, and extravasated erythrocytes (10, 11). All of these phenomena, common and essential changes of early KS stage (5, 6, 15), were also found in our study of uninvolved oral mucosa. The cells lining the vascular spaces revealed characteristics of endothelial cells such as Weibel-Palade bodies, tight junctions, desmosomes and basal membranes. Basal membranes are considered to be extracellular matrices, separating epithelial or endothelial cells from the underlying connective tissue. They play an important role in vessel permeability, in endothelial cell attachment and vascular tissue organization and seem to act as a barrier to cellular migration (16, 17). Partly interrupted basal membranes, leading to extravasation of erythrocytes, have been found around endothelial cells in early KS lesions (6, 14, 18) as well as in clinically uninvolved skin in AIDS patients(11). Similar phenomena were seen in this study in biopsies of clinically uninvolved oral mucosa.

In the present study aggregates of tubular structures (TBS) were regularly detected within the cytoplasm of endothelial cells. Their origin and nature is not yet resolved. TBS, contiguous with the endoplasmic reticulum, have been found in the vascular endothelial cells

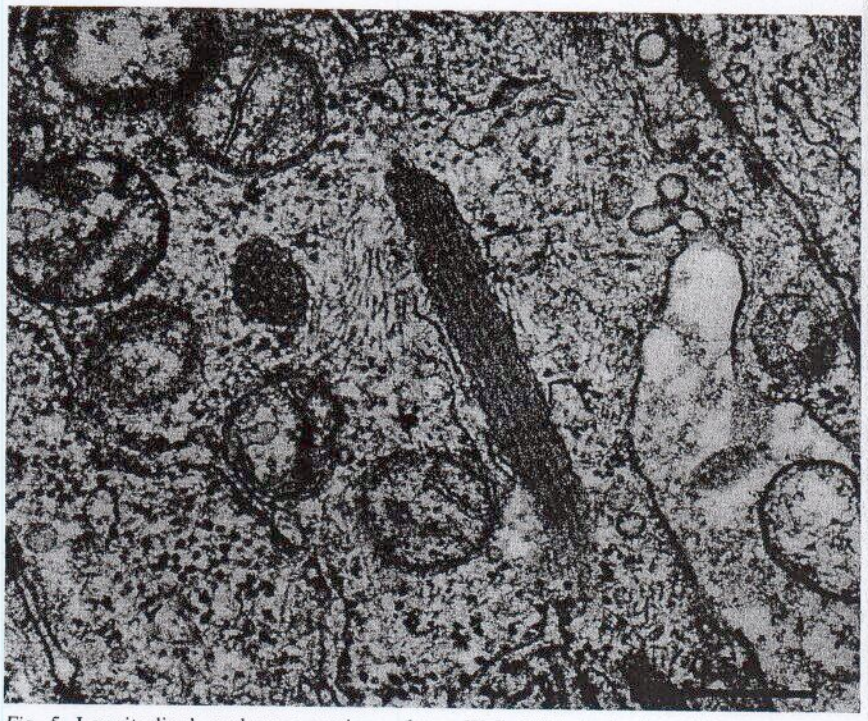

Fig. 5. Longitudinal- and cross sections of two Weibel-Palade bodies within cytoplasm of endothelial cell. EM × 48,000.

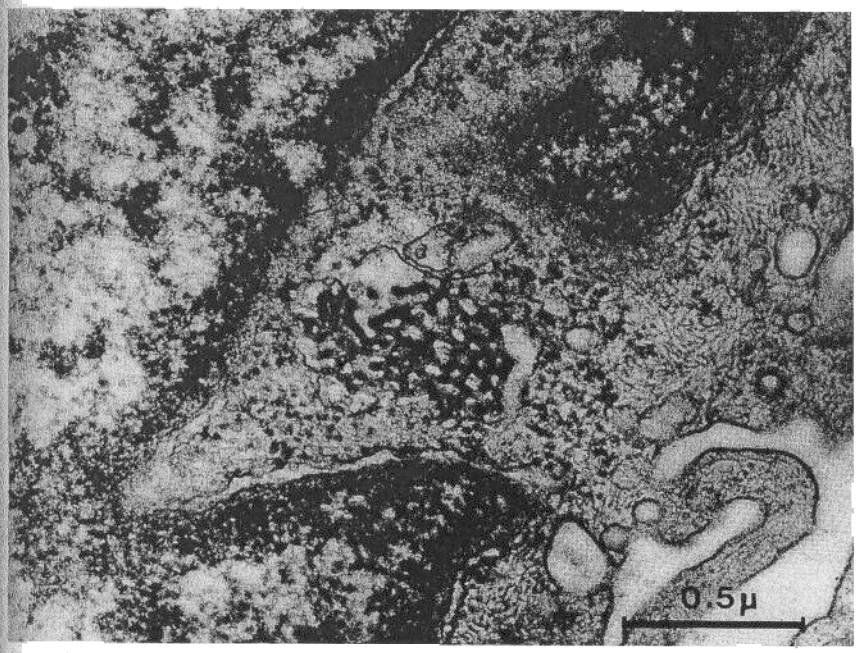

Fig. 6. Cross section of tubular structures within cytoplasm of endothelial cell. EM × 46,000.

or lymphocytes of patients with disease conditions of apparently indenpendant pathogenesis. These include multi-system autoimmune diseases, neoplasia, and virus infections (19). Besides blood cells and different tissues of AIDS patients (19, 20), epithelial cells in hairy leukoplakia infected with Epstein-Barr virus also contained tubular inclusions (20, 21). These cytoplasmic structures may be a morphologic indicator of interferon production, explaining their appearance in such different entities as viral infections, collagen-, vascular- and autoimmune diseases. Production of alpha and beta interferon, induced by viral infection in HIV-infected patients, enhances histocompatibility antigen expression, activates natural killer activity, suppresses blastogenesis (22) and may thus contribute to self-perpetuating immunologic dysfunctions.

WBP are characterized as rod-shaped structures in the cytoplasm of endothelial cells (23). Their occurrence is increased in rapidly proliferating capillaries during wound healing as well as during tumor angiogenesis (24). Therefore, the frequent finding of these cytoplasmic organelles indicates a dramatic proliferation of the endothelial cells in the examined specimens. Since an inflammatory infiltrate was lacking in all the examined specimens, it is unlikely that the activation and proliferation of endothelial cells occurred due to inflammation, but may be mediated by HIV-specific or non-specific cytokines.

Different etiologic factors have been discussed for Kaposi's sarcoma (25, 26). The relatively high incidence of KS in homo- or bisexual men, compared with other high risk groups (3), may point to the etiologic role of different cofactors or sexually transmitted systemic pathogens, capable of infecting the reticulo-endothelial system. The etiologic relationship of HIV (27) and lateron of CMV (28) to KS has been discussed. In vitro experiments have shown the association of tumor growth factors with the proliferation of atypical endothelial cells in KS (29, 30). Therefore it has been proposed, that the development of localized or distant foci of endothelial hyperplasia may be the reaction to an angiogenesis factor, or to the production of different cytokines and growth factors (30). The spontaneous regression of KS, reported in HIV-seronegative patients after discontinuation of immunosuppressive therapy and occasionally in HIV infected patients (25) may indicate that KS is a potentially controllable, reactive hyperplasia (31). Whether the proliferation of endothelial cells seen in clinically uninvolved skin (11) and oral mucosa, as observed in this study, may represent a reactive, perhaps protective process and/or whether it may precede the later development of KS is unknown. The report that features of Castleman's disease, a non-neoplastic atypical lymphoproliferative disorder associated with intrafollicular capillary proliferation and hyperplastic, hyalinized endothelium, preceeded the later occurrence of KS in HIV infected patients, may point to this possibility (32).

The ultrastructural findings in clinically uninvolved oral mucosa of pa-

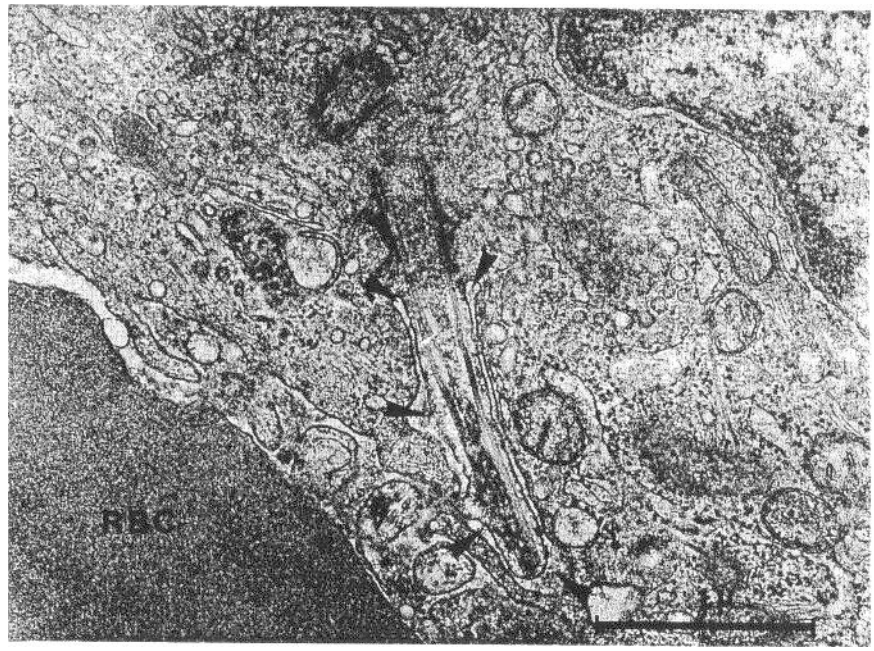

Fig. 7. Cilium-like structure inserting within cytoplasm of endothelial cell. Finger-shaped projection extends outside of cell (arrow head). RBC, red blood cell. EM × 28,000.

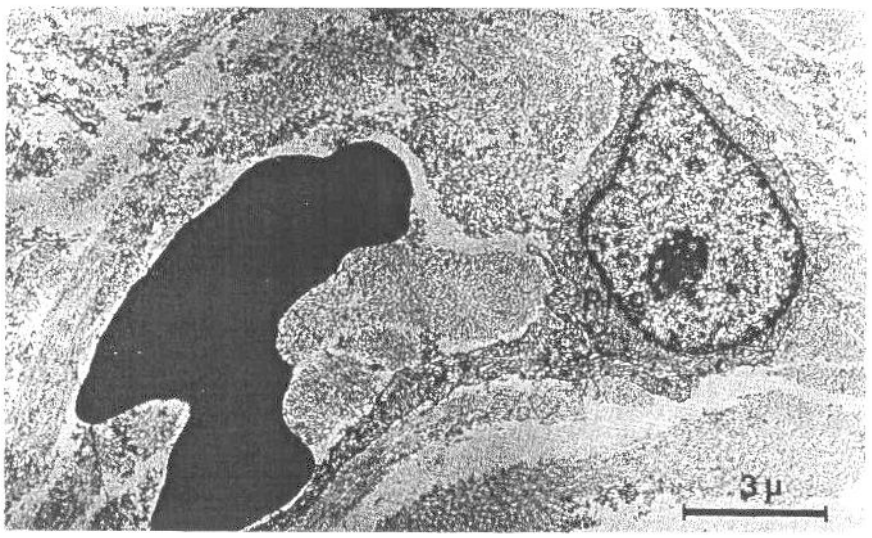

Fig. 8. Extravasated erythrocytes within collagen rich connective tissue in close association to phagocyte (PHC). EM × 6,000.

tients with HIV infection were comparable with those alterations in clinically normal skin of patients suffering from AIDS. The readily detectable presence of vascular abnormalities such as increased angiogenesis, protruded swollen endothelial cells, slit-like vascular channels, sparseness of intercellular junctions, increase in quantity of WPB and tubular structures as well as extravasated erythrocytes may be regarded as an indication that during HIV infection blood vessels undergo changes which probably involve the entire vascular system. Therefore, a general principle either represented by specific HIV-induced factors or additional viral infections may induce and support the dysregulation of vascular neogenesis.

Further histologic studies and a close clinical follow-up of these patients are needed to evaluate the pathogenetic relevance of these present ultrastructural findings.

Acknowledgment – Supported by a grant from Bundesministerium für Forschung und Technologie (Nr. II/071/88).

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