Ultrastructural and immunohistochemical findings in oral hairy leukoplasia*

Xiaolin Zhang¹, Angelika Langford ², Jürgen Becker ², Jörg-Peter Rabanus ², Hans-Dieter Pohle ³, Peter Reichart ², and Hans Gelderblom ⁴

¹ Institute of Stomatology, Beijing Medical University, Haidian Weigongcun, Beijing, P.R. China
² Freie Universität Berlin, Abteilung für Zahnärztliche Chirurgie/Oralchirurgie Nord, Föhren Strasse 15, D-1000 Berlin 65
³ II. Medizinische Klinik, Universitätsklinikum Rudolf Virchow, Augustenburger Platz 1, D-1000 Berlin 65
⁴ Robert Koch-Institut des Bundesgesundheitsamtes, Nordufer 20, D-1000 Berlin 65

Summary. Three cases of HL from the lateral border of the tongue of male homosexual AIDS patients were investigated by thin section electron microscopy. Keratinocytes contained condensed chromatin in their pyknotic nuclei and a few organelles in the oedematous cytoplasm. Chromatin was in close association to the nuclear membrane and showed a punched-out appearance. Particles typical of the herpes virus group were abundant in the upper two thirds of the epithelium in all three cases. Virus particles were seen frequently in the nuclei of the ballooned keratinocytes, but rarely in cells containing Candida albicans. Viral nucleocapsids were observed budding at the inner nuclear membrane, thereby acquiring the prospective viral envelope. Complete, enveloped virions were found in the endoplasmic reticulum and in the extracellular space. These virions were identified immunohistochemically as Epstein-Barr virus (EBV) using two monoclonal antibodies directed against EBV capsid and membrane antigen, respectively. Candida albicans was observed in the stratum corneum and in the upper layer of the stratum spinosum. Special cytoplasmic tubular structures arranged in parallel bundles were found in koilocytotic cells in addition to characteristic membrane structures composed of undulating convoluted membranes. Epithelial basement membranes were always intact.

Key words: Hairy leukoplakia – Electron microscopy – Epstein-Barr virus – Candida albicans

Introduction

Oral hairy leukoplasia (HL), a new clinical entity, was first described by Greenspan et al. (1984) as pathognomonic for the HIV-infection. HL is characterized by whitish plaques at the lateral border of the tongue which cannot be rubbed off. The lesion, mainly observed in HIV-infected homosexual males, was also reported in heterosexual HIV-infected individuals, haemophiliacs, a transfusion recipient (Greenspan et al. 1986; Rindum et al. 1987), and intra-venous (iv) drug abusers (Reichart et al. 1986, 1987). A survival analysis showed that the probability of developing AIDS in patients with HL was 40% during 16 months and 83% during 31 months (Greenspan et al. 1987).

Light microscopic investigations (Greenspan et al. 1984; Greenspan et al. 1985; Eversole et al. 1986) indicated, that the lesion is characterized by parakeratosis, acanthosis, koilocytosis and lack of inflammation in the subepithelial tissue. Using electron microscopy (EM) particles of the herpes virus group were found in HL (Greenspan et al. 1984, 1985; Belton and Eversole 1986; Konrad 1986). Immunofluorescence studies demonstrated Epstein-Barr virus (EBV) antigen, and a nucleic acid-hybridization procedure revealed EBV viral DNA in a high copy number (Greenspan et al. 1985; Löning et al. 1987).

Purpose of the present study was to describe ultrastructural features of HL and to identify the virion particles using monoclonal antibodies against EBV core and membrane antigens.

Materials and methods

From July 1984 to July 1987 among 195 HIV seropositive individuals a total of 61 patients (male n = 59, female n = 2; homo-
bisexual \( n = 55 \), i.v. drug abusers \( n = 5 \), blood transfusion recipient \( n = 1 \) with the clinical diagnosis of HL have been observed. The average age of HL patients was 34.2 years. At the time of serodiagnosis four HIV positive individuals were clinically symptomless, 18 patients showed ARC-, and 39 patients AIDS symptoms. For the present study biopsies of HL were obtained from 3 male homosexual patients (age 39, 46 and 83 years), showing different AIDS manifestations (multiple Kaposi sarcoma: \( n = 1 \), opportunistic infections: \( n = 2 \)). All three patients died within 2.8 months after biopsy. Biopsies were taken from the lateral margin of the tongue (Fig. 1) under local anaesthesia (Ultracain DS\(^{\circ} \)). Prior to biopsy all three patients had been treated for two weeks with topical (Mikonazole), two patients additionally with systemic antimycotic therapy (Ketokonazole).

Tissues were divided and one part was fixed in 2.5% glutaraldehyde in PBS for 3 hours. After washing with PBS specimens were postfixed in 1% OsO\(_4\) for 1 h at 4 °C, and afterwards treated for 1 h at room temperature within 1% uranyl acetate. After dehydration in a graded series of ethanol, specimens were infiltrated by 3 changes of propylene oxide and embedded in Epon 812 following routine techniques (Gelderblom et al. 1974). Semithin sections (0.5–1 \( \mu m \) in thickness of each specimen were cut, stained with toluidine blue, and evaluated light microscopically for pathognomonic changes. After finding suspecte areas in light microscopy, ultrathin sections (40–60 nm) were cut. These were mounted on bare grids, poststained with lead citrate, stabilized with carbon and examined using a Zeiss EM 10A at 60 kV.

The other part of the biopsy was fixed in dimethylsuberimidate (DMS, Hassel and Hand, 1974) for 2 h, washed in PBS and infiltrated stepwise with 5%, 10%, 1.2 M and 2.3 M sucrose in PBS. Biopsies were deep frozen in liquid nitrogen. Semithin cryosections of 0.5 \( \mu m \) were cut using the Reichert FC4 cryo attachment (Reichert AG, Wien). Sections were incubated with monoclonal antibodies directed against EBV capsid (VCA) and membrane antigen (MA) (dilution 1:1000 in PBS). Immunobinding was visualized using a modification of the alkaline-phosphatase-mouse-anti-alkaline-phosphatase technique (APAAP; Becker et al. 1987). Controls included the use of second and third step antibodies, as well as normal tissues of HIV seronegative persons.

**Results**

Common features in all cases were hyperkeratosis, parakeratosis and acanthosis of epithelium. Above the basal cell layer an increased degree of intracellular oedema of spinous cells was noted. The balloononed keratinocytes often lost their chromatin pattern and sometimes basophilic intranuclear inclusions were observed. Candida albicans was found within epithelial cells of the superficial layers. Infiltration by inflammatory cells was not apparent, neither in the lamina propria of the mucosa nor in the epithelium.

In all cases, balloononed keratinocytes were seen in clusters within the spinous layer of the epithelium on electronmicroscopy. These cells were increased in size and contained only few organelles, including degenerated mitochondria and abundant intermediate sized keratin fibrils. Foci of condensed chromatin with a punched-out appearance were found in pyknotic nuclei mainly in close association with the nuclear membrane (Fig. 2). Candida albicans was frequently observed in the stratum corneum as well as in the upper layers of the stratum spinosum (Fig. 3).

Virus particles of the herpes virus group were present in the upper spinous layer mainly in and around koilocytotic cells, and in the intercellular space of flattened cells of the superficial layers.