Virchows Archiv A Pathol Anat (1991) 419:237–244

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunoelectron microscopy shows an atypical pattern and a quantitative shift of collagens type I, III and VI in oral Kaposi's sarcoma of AIDS

J. Becker[1], D. Schuppan[2], J.-P. Rabanus[1], H.R. Gelderblom[3], and P. Reichart[1]

[1] Department of Oral Surgery, Faculty of Dentistry, Free University of Berlin, Föhrer Strasse 15, W-1000 Berlin 65, Federal Republic of Germany
[2] Department of Medicine, Division of Gastroenterology, Steglitz Medical Center, Berlin, Federal Republic of Germany
[3] Robert Koch-Institut des Bundesgesundheitsamtes, Berlin, Federal Republic of Germany

Received March 5, 1991 / Received after revision April 22, 1991 / Accepted April 23, 1991

Summary. The localization of collagen types I, III and VI in normal human alveolar and palatal mucosa and in oral Kaposi's sarcoma (KS) was studied by light microscopy and cryo-immunoelectron microscopy. Normal oral mucosa revealed two different types of organization. The upper connective tissue stroma contained a loose reticular network mainly composed of collagen types III and VI, while collagen type I immunostaining predominated in the deeper stroma. Ultrastructurally, in the KS tumour stroma, a loose pattern of individual thin collagen fibrils was noted. These often fanned out at their ends showing a filamentous substructure. The fibrils consisted predominantly of collagen type I similar to individual fibrils of normal oral mucosa. However, there was a marked loss of thick fibre bundles of collagen types I and III in KS compared with normal oral mucosa, whereas collagen type VI was markedly increased and found preferentially in clusters and strands around cross-striated fibrils that often spanned the distance between single collagen fibres. The abundance of collagen type VI in a pattern similar to early stages of wound healing suggests that the KS stroma resembles an early organizational stage of the interstitial and vascular extracellular matrix subject to a high rate of collagen turnover. This character of the KS stroma appears to result from a continuous auto- and paracrine stimulation of cell growth and collagen synthesis and provides an excellent model to study the structural arrangement of collagen type VI in relation to the fibrillar collagen types I and III.

Key words: Collagen – Immunoelectron microscopy – Immunocryoultramicrotomy – Kaposi's sarcoma – AIDS

Offprint requests to: J. Becker

Introduction

Kaposi's sarcoma (KS) has recently attracted major attention due to its association with the acquired immunodeficiency syndrome (AIDS) (Reichart et al. 1987; Armes 1989). KS is classified into three stages clinically and morphologically. An abnormal vasculature with an inflammatory and spindle cell infiltration around a central vessel is the predominant feature of the early patch and plaque stages. In the advanced nodular stage of KS, typical aggregates of spindle cells together with a network of blood-filled spaces predominate (Kuntz et al. 1987; Holden 1989). The pathogenesis of KS is still not well understood. Recent data show that KS-derived cell lines display an overproduction of several cytokines (Nakamura et al. 1988) responsible for auto- and paracrine stimulation of cell growth and matrix production, indicating that KS is not a real neoplasm but a reactive hyperplasia.

The extracellular matrix (ECM) consists of four major components, namely collagens, glycoproteins, proteoglycans and elastin (Schuppan and Hahn 1987). Their enhanced remodelling, involving degradation of existing structures as well as the synthesis of new molecules, occurs in a variety of conditions such as tissue growth, wound healing, fibrosis and tumour invasion (Vaes 1985; van den Hoof 1983, 1988).

Most studies on the distribution of ECM proteins in KS have focused on basement membrane proteins due to the prominent vascular endothelial cell type. Staining for basement membrane proteins revealed a delicate and partly fragmented lining around sinusoid-like vascular spaces and an occasional diffuse interstitial fluorescence in the tumour stroma of advanced lesions (Kramer et al. 1985; Becker et al. 1987). Staining for basement membrane components was used to detect proliferating endo-

thelial cells in very early KS lesions (Autio-Harmainen et al. 1988; Green et al. 1988; Penneys et al. 1988).

Using light microscopy an overproduction of collagen types I, III, VI and fibronectin was demonstrated in the KS tumour stroma, while collagen type V was absent (Kramer et al. 1985; Becker et al. 1987). Since the ECM including the various types of collagen may be abnormally composed in KS and this ECM may modulate the phenotype of the adjacent cells, we studied the pattern of collagen types I, III and VI in oral KS at the ultrastructural level.

Materials and methods

Ten biopsies of clinically normally appearing alveolar mucosa covering impacted third molars and a further four biopsies of palatal mucosa (male, 5; female, 7; mean age 18 years, range 16–24 years) covering impacted canines were taken during surgical routine procedures for the removal of impacted teeth.

Four biopsies from the nodular type of KS at the hard palate from HIV-seropositive male homosexual patients (age 34, 45, 47, 51 years) were removed under local anaesthesia (Ultracain DS-forte) for diagnostic reasons. Immediately after removal the specimens were divided. One part was fixed in 10% formalin and processed for routine histology (H&E), one part was deep frozen in liquid nitrogen and cryostat sections of 4–6 µm in thickness were prepared as described previously (Becker et al. 1987). Another part of the biopsy was divided into cubes of about 2 × 2 mm edge length. The cubes were fixed in 2% paraformaldehyde (PFA) for 15 min at 4° C. After a washing in phosphate buffered saline (PBS) the cubes were transferred into solutions of 0.3 M sucrose in PBS at room temperature, followed by stepwise infiltration with 0.6 M, 1.2 M and 2.3 M sucrose in PBS. Sedimentation of the blocks indicated complete infiltration by the respective solution. After saturation in 2.3 M sucrose the specimens were mounted on aluminum carriers (Reichert, Vienna, Austria), shock-frozen at −210° C and transferred into the cryo-chamber of an FC-4 cryostat system (Reichert).

Ultra-thin sections were obtained with glass knives at −90° C using a cutting speed of 0.5 mm/s as described previously (Rabanus et al. 1991). Sections were collected on droplets of 2.3 M sucrose in PBS held in a platinum loop and transferred to 300 mesh hexagonal copper grids coated by a film of Pioloform F (0.1% in chloroform; Wacker Chemie, Munich, FRG). Carbon reinforcement and hydrophilization of the grids by a glow discharge were performed in an Edwards 306 coating unit (Edwards, Sussex, England).

Isolation of the antigens, antiserum production and affinity purification of antibodies have been described elsewhere (Becker et al. 1986a, b; Schuppan et al. 1985, 1986; Geerts et al. 1990). Collagen types I and III were isolated from neutral salt-extracted monkey and bovine skins, respectively. Human collagen type VI was purified from pepsin digests of human placenta. The purity of all preparations was checked by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, amino acid analysis, western blotting and rotary shadowing electron microscopy. Hyperimmune sera raised in rabbits were affinity purified and the resulting antibodies were tested for monospecificity by radioimmunoassay and western

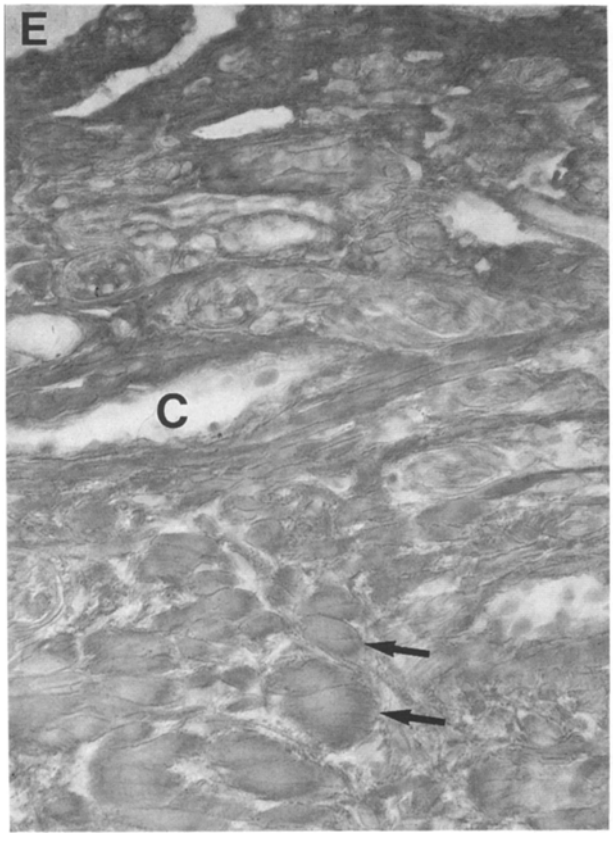

Fig. 1. Normal palatal mucosa (*E*, epithelium; *C*, capillary vessel). The most intense staining for collagen type III was observed in the upper connective tissue stroma close to the subepithelial basement membrane. In the deeper stroma staining was most pronounced at the margin of collagen fibril bundles (*arrows*). × 1040

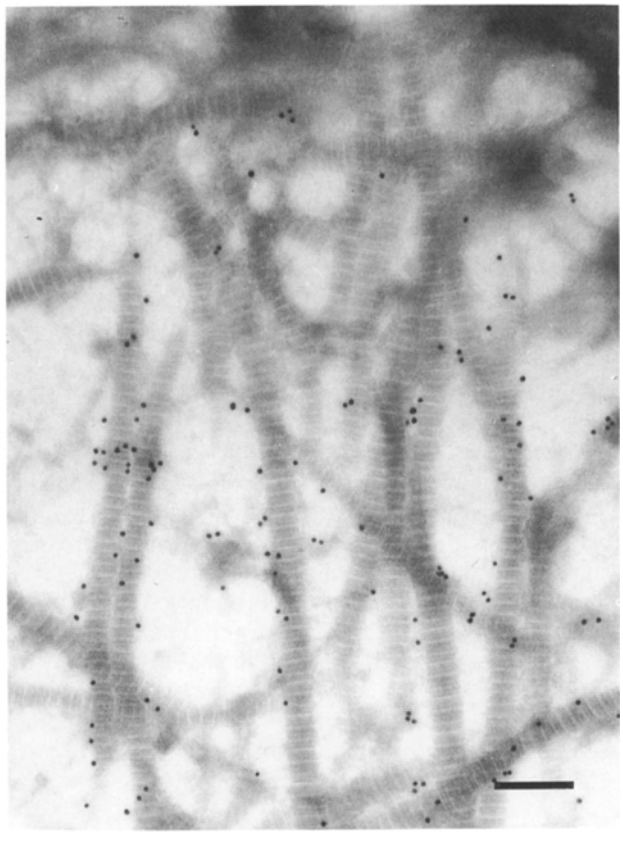

Fig. 2. Normal palatal mucosa: loose reticular network of individual cross-striated fibrils in the upper connective tissue close to the subepithelial basement membrane. Most fibrils are stained by the monoclonal antibody to collagen type III. *Bar* = 200 nm. × 50000

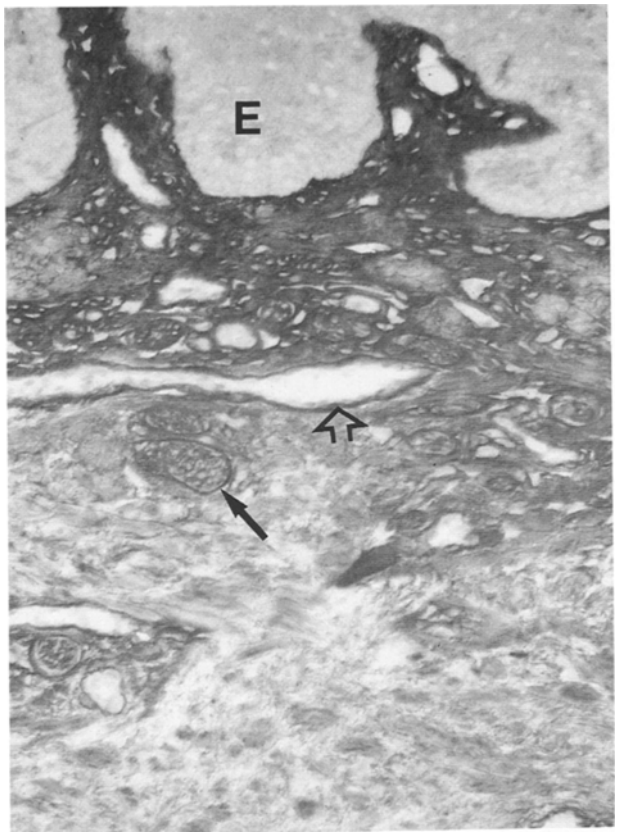

Fig. 3. Normal palatal mucosa: collagen type VI was most pronounced in the subepithelial connective tissue, around nerves (*arrow*) and capillar vessel walls (*arrowhead*). In the deeper connective tissue stroma only a faint staining was noted between the fibril bundles. ×425

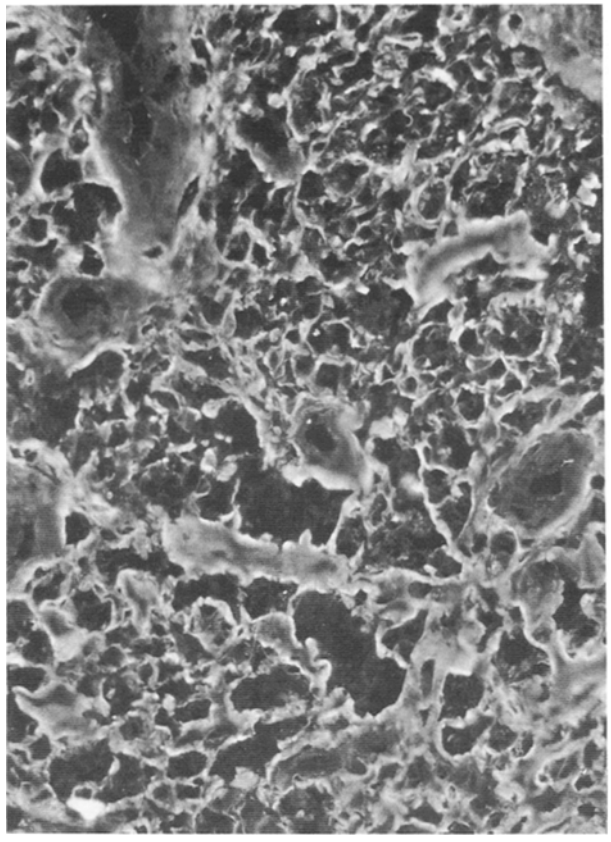

Fig. 4. Kaposi's sarcoma (KS) tumour stroma: when compared with normal palatal mucosa a diffuse interstital staining for procollagen type III was observed in the KS tumour stroma. ×650

blotting. The monoclonal antibody against collagen types III was purchased from Heyl Pharma (Berlin, FRG).

Antibody incubation, immunogold labelling and stabilization of ultra-thin sections were performed according to the method described previously (Tokuyasu 1980; Griffiths et al. 1984; Gelderblom et al. 1985; Rabanus et al. 1991). In brief, ultra-thin sections were pre-incubated with 0.02% L-lysine (Merck, Darmstadt, FRG) in PBS for 10 min followed by primary antibodies at 30–100 µg/ml for 10 min and finally by goat anti-rabbit IgG gold conjugate (auroprobe GAR or GAM 5 nm, dilution 1:60; Janssen, Beerse, Belgium) for 20 min at room temperature. Washing between each step was carried out on 5 droplets of PBS containing 0.1% bovine serum albumin and 0.02% L-lysine for 20 min. After immunolabelling sections were fixed in 2% glutaraldehyde (Sigma, Munich, FRG), pH 7, and contrasted with 2% uranyl acetate, pH 7.2 for 5 min and finally in uranyl acetate oxalate, pH 4, for 5 min. Immunostaining was documented with a Zeiss 10 A electron microscope (Zeiss, Oberkochen, FRG) and Scientia film 23 D 56 (Agfa-Gevaert, Leverkusen, FRG). Controls using second antibodies only were included in every study.

For light microscopy cryostat sections of normal oral mucosa and oral KS were fixed by immersion in ice-cold acetone for 20 min. The tissue slices were incubated with 10–20 µl antibody

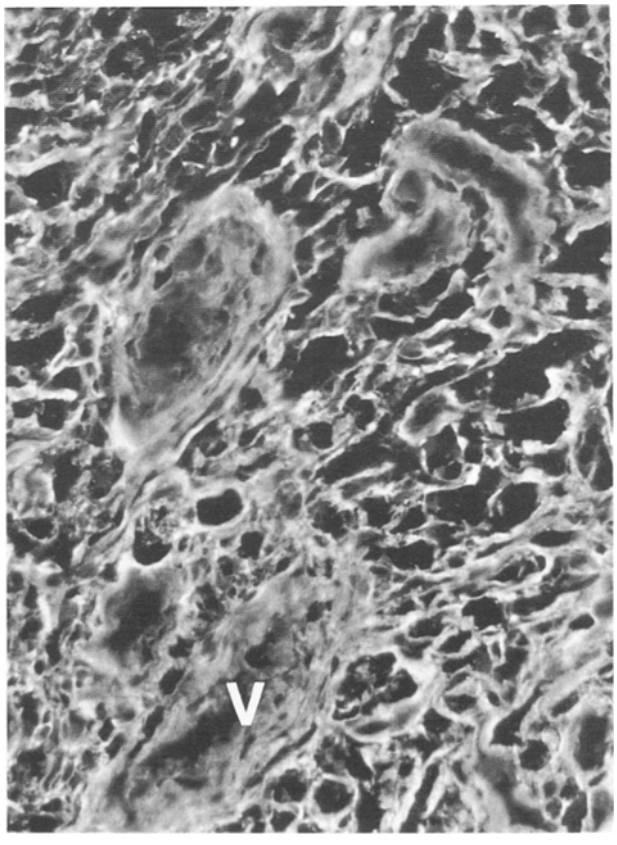

Fig. 5. KS tumour stroma: diffuse interstitial staining for collagen type VI in the stroma. Label was pronounced when compared with the deeper normal palatal mucosa. Blood vessels (*V*) showed a broad rim of fluorescence. ×800

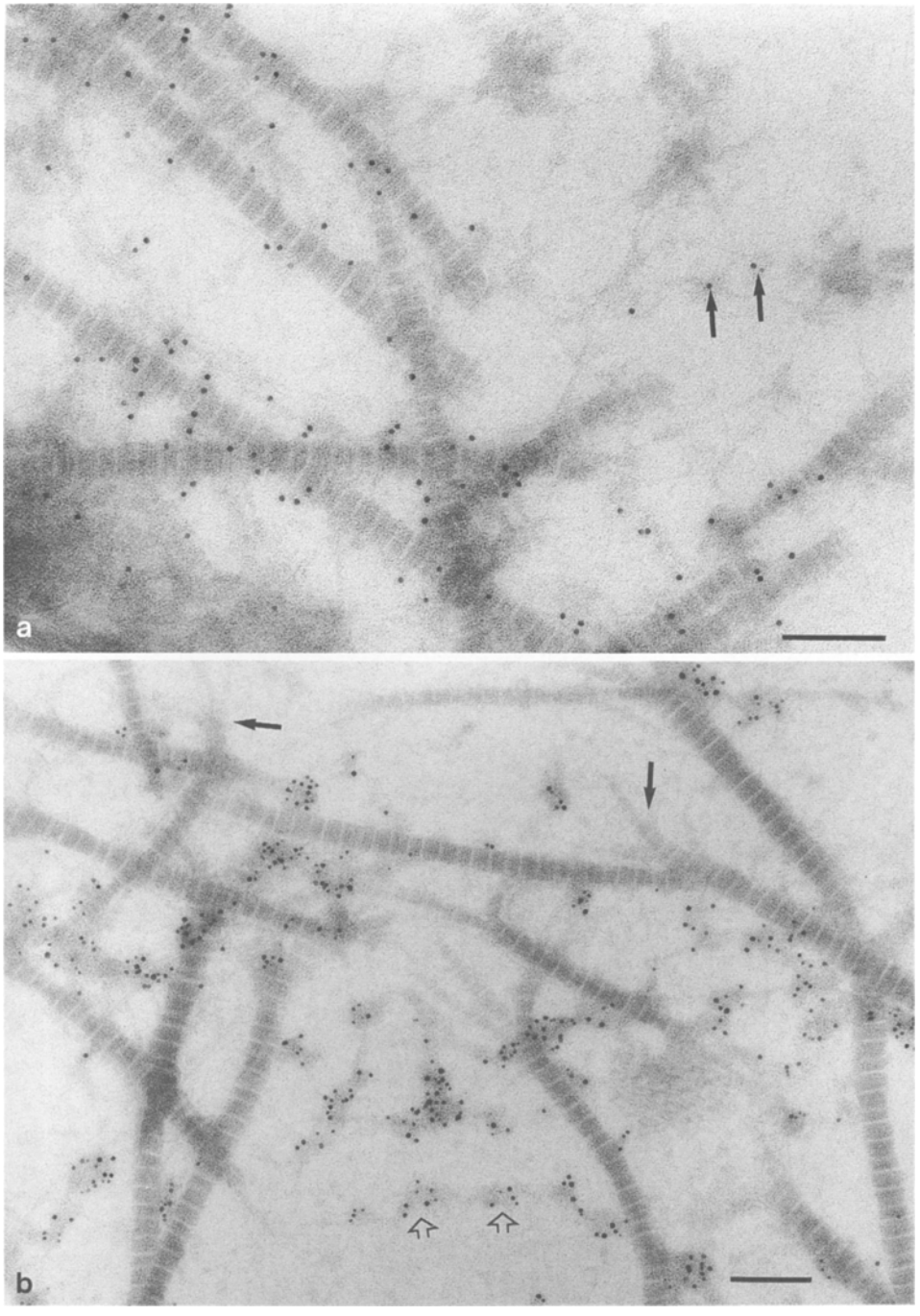

Fig. 6. a KS tumour stroma: label for collagen type I was found on single cross-striated fibrils and occasionally on microfibrils (*arrows*). *Bar* = 100 nm. × 190000. **b** KS tumour stroma: label for collagen type VI was pronounced on globular protuberances (*arrowhead*). The cross-striated fibrils were never organized into bundles, often fanning out their ends (*arrows*), and exhibited the relatively small diameter of 20–40 nm. *Bar* = 100 nm. × 145000

solution [0.5 (APAAP) – 5 μg/ml (indirect immunofluorescence)] for 30–60 min. Immunostaining was visualized using indirect immunofluorescence or the APAAP technique as described previously (Becker et al. 1987, 1988).

Results

Ultrastructurally, the healthy human alveolar and palatal mucosa displayed two patterns of organization. The deeper connective tissue stroma was mainly composed of densely packed and large bundles of thick, cross-striated collagen fibrils, whereas the connective tissue close to the subepithelial basement membrane mainly showed a loose meshwork of individual cross-striated fibrils in connection with a reticular network of microfibrils.

Both by light and electron microscopy collagen type I immunolabel was most prominent in the deeper layers of the connective tissue stroma in association with dense fibril bundles. In contrast, collagen type III dominated in the loose subepithelial connective tissue (Figs. 1, 2) with a much lower staining intensity in the deeper layers, where it seemed to be located at the periphery of fibril bundles (Fig. 1). Like type III, collagen type VI was expressed at a higher level in the upper connective tissue

Fig. 7. Hard palate at the KS tumour periphery: label for collagen type I was limited to bundles of large (diameter 60–90 nm) cross-striated fibrils in the deeper connective tissue. *Bar* = 100 nm. × 120000

as compared to the deeper stroma, where only a mild interstitial labelling between individual fibril bundles was noted. Staining for collagen type VI was most intensive in the adventitia of capillary vessel walls and around nerve fibres (Fig. 3). At the ultrastructural level the antibodies for collagen type VI decorated unbanded microfilaments of 10–15 nm diameter, which apparently formed bridges between cross-striated fibres in the subepithelial connective tissue.

By light microscopy an intense, diffuse interstitial staining between the vascular spaces of the KS tumour stroma was noted for collagen types I, III and IV (Fig. 4). Labelling for collagen types III and IV was pronounced in the adventitia of blood vessels (Fig. 5). Ultrastructurally, the KS tumour stroma consisted of a loose network of individual cross-striated fibrils positive for collagen type I embedded in a matrix of microfilamentous structures (Fig. 6a). These collagen fibrils exhibited a relatively small diameter of 20–40 nm, were never organized into bundles and often fanned out at their ends (Fig. 6b). The density of gold particles in the tumour stroma reactive for collagen type I was of similar intensity when compared with that of the adjacent normal palatal connective tissue that was characterized by a high proportion of dense fibril bundles (Fig. 7). Equally, label for collagen type III on the cross-striated fibrils of the tumour stroma (Fig. 8) was comparable to that of the surrounding normal connective tissue. The abundance of collagen type VI adjacent to and between individual cross-striated fibrils of the KS tumour stroma contrasted with the scarcity of label at the periphery in the adjacent deeper connective tissue stroma (Fig. 9). In general, label on the microfilaments was mainly located on globular protuberances which often showed a repeat distance of 100–110 nm (Fig. 10). These microfilaments were only observed in proximity to collagen fibrils (Fig. 9).

Whereas the structural preservation of the cross-striated fibrils and the microfilaments was excellent, that of inflammatory and tumour cells was less satisfactory, probably due to the mild fixation in PFA. Immunolabelling was specific, since all controls using only the second antibodies revealed a low background of only 5–8 gold particles on the original negatives (8.6 cm × 8 cm) at a primary magnification of 1:25000.

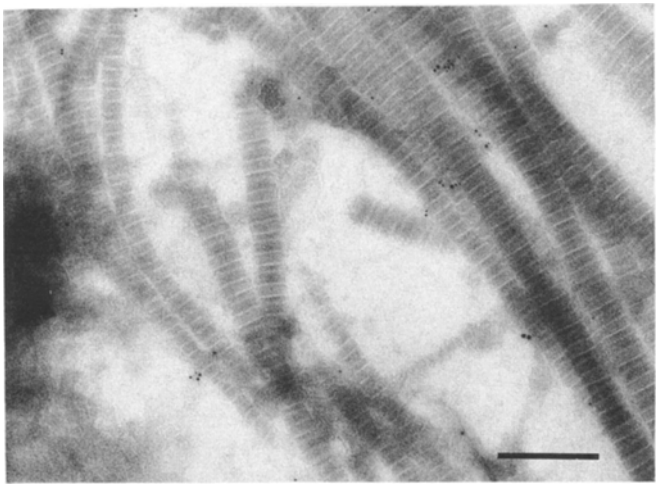

Fig. 8. KS tumour stroma: only individual cross-striated fibrils in the tumour stroma were positive for collagen type III. *Bar* = 200 nm. × 85000

Discussion

Normal oral mucosa shows two major types of morphological organization accompanied by a different composition of ECM proteins, as demonstrated by previous biochemical and immunohistological studies on the distribution of collagen types I, III and VI (Ballard and Butler 1974; Chavrier et al. 1984; Becker et al. 1986a; Rabanus et al. 1991). Our data confirm that the upper connective tissue is composed of a loose reticular network of individual cross-striated fibrils interwoven with a microfilamentous network of collagen type VI. The deeper layers are characterized by bundles of densely

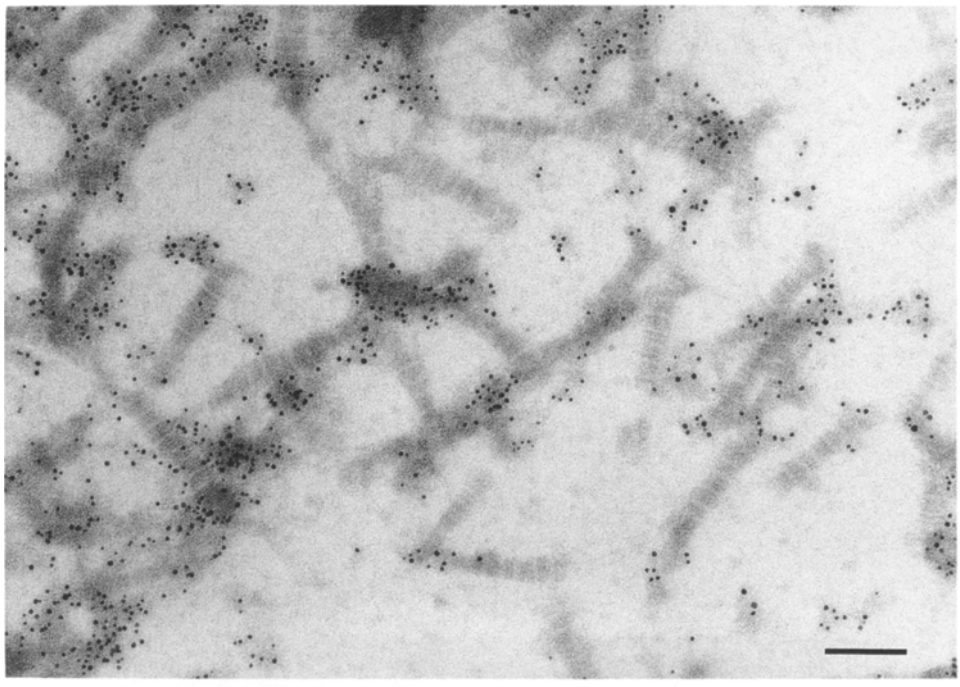

Fig. 9. KS tumour stroma: a dense meshwork of collagen type VI was observed in the vicinity of single cross-striated main fibrils. *Bar* = 100 nm. × 135000

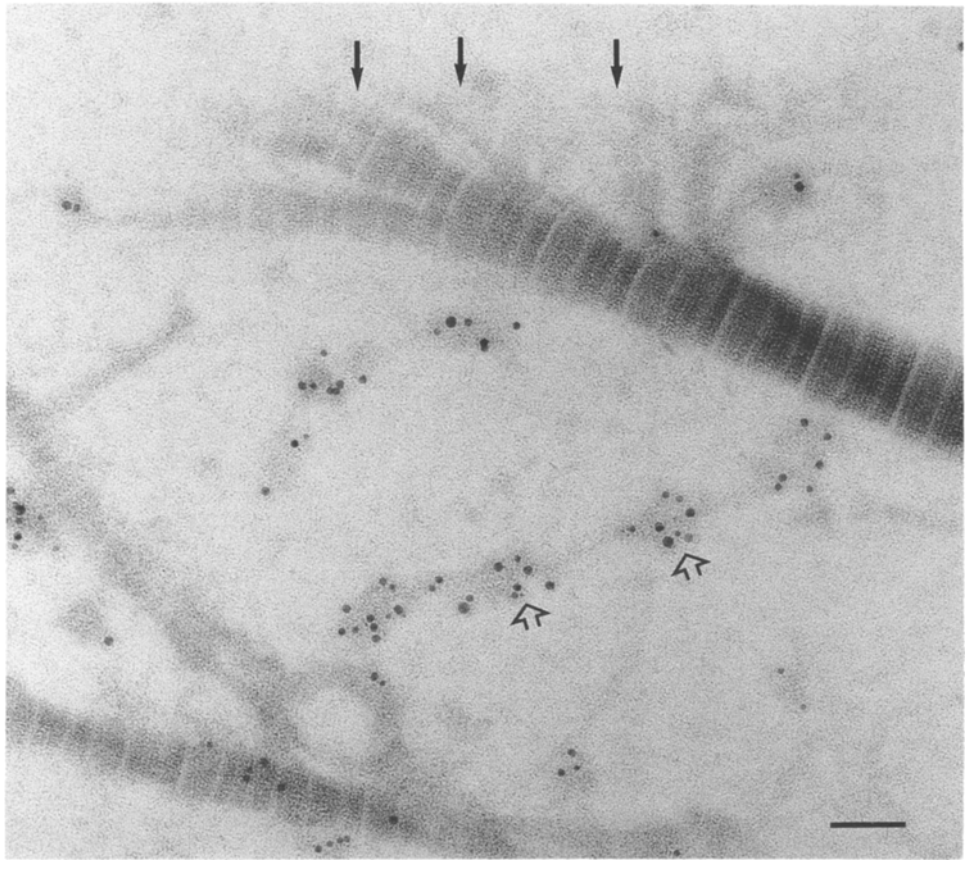

Fig. 10. KS tumour stroma: label for collagen type VI was pronounced on large globular protuberances (*arrowheads*). A cross-striated fibril is split at the end showing the microfilamentous substructure (*arrows*). *Bar* = 50 nm. × 250000

packed collagen fibres and a striking loss of collagen type VI immunoreactivity. The finding that the three chains of collagen type VI harbour several von Willebrand factor A domains with a potential to bind to fibrillar collagen and sequences within the collagenous domain with the recognition motif (Arg-Gly-Ser) for cellular integrin receptors (Chu et al. 1989; Bonaldo et al. 1990) supports the suggested function of collagen type VI as a mediator of cellular attachment to fibrillar collagens. Whereas in the tumour stroma of oral KS collagen

type VI was concentrated around fibrils preferentially reactive for collagen type I, in the upper connective tissue stroma of normal oral mucosa it was rather found to be associated with fibrils reactive for collagen type III. Studies of the ultrastructure of the vasculature and of tumour cells in classical KS as well as in KS of AIDS (Daimont-Waldo et al. 1977; Leu and Odermatt 1985; Kuntz et al. 1987) have revealed that the dissection and degradation of collagen fibres is an early event associated with the proliferating tumour cells (Autio-Harmainen et al. 1988). Similar findings were reported for angiosarcoma (Rosai et al. 1976) and lymphangiosarcoma (Salm 1963). Our studies confirmed a rarefication and thinning out of the fibrillar collagen matrix in KS, but in addition showed that collagen type VI deposition appeared to be strikingly increased compared to normal oral mucosa. These ultrastructural changes may be related to the conspicuous inflammatory reaction in KS tumour stroma accompanied among other cells by macrophages (Leu and Odermatt 1985), which are believed to play a central role in connective tissue remodelling by releasing cytokines and connective tissue degrading enzymes such as stromelysin and collagenases upon immunological or inflammatory stimuli (Woolley 1984; Vaes 1985; Nicholson et al. 1989). Whereas an earlier light microscopic study suggested that the intensity or distribution of collagen type I between the KS tumour stroma and the adjacent normal tissue was essentially identical (Kramer et al. 1985), we observed a quite different ultrastructural pattern. In the tumour periphery label for collagen type I was only found on mature cross-striated fibrils organized in bundles but label in KS tumour stroma was mainly associated with single thin fibrous and few microfilamentous structures. At present it is impossible to decide whether these filaments are fragments of degraded collagen fibrils or whether they represent incompletely assembled de novo synthesized collagen type I.

By light microscopy collagen types I, III and VI were observed in identical locations in the KS tumour stroma both in the present and in a previous study (Becker et al. 1987). Ultrastructurally, however, label for collagen type III was less intense compared with that for collagen type I, thus reflecting much better the original proportion of both proteins in the deeper connective tissue stroma of the hard palate. The results clearly demonstrated that immunoelectron microscopy when compared with indirect immunofluorescence might allow a more precise semi-quantitative evaluation of individual proteins. Application of such a semi-quantitative assessment suggests that collagen type VI may be a major component of the KS tumour stroma.

To date the cell type in KS responsible for the abundant production of collagen type VI has not been defined. During the early stages of wound healing in human alveolar bone, sprouting vascular endothelial cells are surrounded by a dense rim of both collagen types IV and VI, whereas interstitial staining for these collagens in the adjacent granulation tissue is absent. This indicates that in wound healing the endothelial or directly adjacent mesenchymal cells are the most probably sources of these proteins (Becker et al. 1989). Its abundant presence during wound healing in human alveolar bone, its high level of expression in Schwann cells (Peltonen et al. 1990) and its deposition in close vicinity to a variety of other cells (Bruns et al. 1986) together with our finding of its excessive deposition in KS tumour stroma suggests that collagen type VI plays an important role in the early structural organization of the ECM during wound healing and neovascularization.

Thus, ultramicrotomy, largley by avoiding labelling artefacts such as those resulting from high or low antibody penetration occurring in conventional methods such as pre-embedding immunoelectron microscopy (Fleischmajer et al. 1981), may allow more objective information on the supramolecular organization and quantitative derangements of ECM proteins in pathological conditions such as KS of AIDS.

Acknowledgements. This study was supported in part by the Bundesministerium für Forschung und Technologie (grant II-071-88) and the Deutsche Forschungsgemeinschaft (Be 1017/1-3).

References

Armes J (1989) A review of Kaposi's sarcoma. Adv Cancer Res 53:73–87

Autio-Harmainen H, Karttunen T, Apaja-Sarkkinen M, Dammerrt K, Risteli L (1988) Laminin and type IV collagen in different stages of Kaposi's sarcoma and other vascular lesions of blood vessel or lymphatic vessel origin. Am J Surg Pathol 12:469–476

Ballard JB, Butler WT (1974) Proteins of the periodontium: biochemical studies on the collagen and non-collagenous proteins of human gingiva. J Oral Pathol 3:176–184

Becker J, Schuppan D, Hahn EG, Albert G, Reichart P (1986a) The immunohistochemical distribution of collagens type IV, V, VI and of laminin in the human oral mucosa. Arch Oral Biol 31:179–186

Becker J, Schuppan D, Benzian H, Bals T, Hahn EG, Cantaluppi C, Reichart P (1986b) Immunohistochemical distribution of collagen types IV, V and VI and of procollagens type I and III in human alveolar bone and dentine. J Histochem Cytochem 34:1417–1429

Becker J, Schuppan D, Reichart P (1987) The extracellular matrix in oral Kaposi sarcoma (AIDS): the immunohistochemical distribution of collagens type IV, V, VI, of procollagens type I and III, of laminin and of undulin. Virchows Arch [A] 412:161–168

Becker J, Ulrich P, Kunze R, Gelderblom H, Langford A, Reichart P (1988) 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

Becker J, Schuppan D, Selle C, Bunte T, Reichart P (1989) Immunhistochemische Untersuchungen zum Aufbau der extrazellulären Bindegewebsmatrix während der Wundheilung im Knochen. Z Zahnärztl Implantol V:1205–1210

Bonaldo P, Russo V, Bucciotti F, Doliana R, Colombatti A (1990) Structural and functional features of the α3 chain indicate a bridging role for chicken collagen VI in connective tissues. Biochemistry 29:1245–1254

Bruns RR, Press W, Engvall E, Timpl R, Gross J (1986) Type VI collagen in extracellular, 100 nm periodic filament and fibrils: identification by immunoelectron microscopy. J Cell Biol 103:393–404

Chavrier C, Couble ML, Magloire H, Grimaud JA (1984) Connective tissue organization of healthy human gingiva. J Periodont Res 19:221–229

Chu ML, Pan TC, Conway D, Kuo HJ, Glanville RW, Timpl

R, Mann KH, Deutzmann R (1989) Sequence analysis of α1(VI) and α2(VI) chains of human type VI collagen reveals internal triplication of globular domains similar to the A domains of von Willebrand factor and two α2(VI) chain variants that differ in the carboxy terminus. EMBO J 8:1939–1946

Daimont-Waldo E, Vuletin JC, Kaye GI (1977) The ultrastructure of vasculature tumors: additional observations and a review of the literature. Pathol Annu 12:279–308

Fleischmajer R, Timpl R, Tuderman L, Raisher L, Wiestner M, Perlish JS, Graves PN (1981) Ultrastructural identification of extension aminopropeptides of type I and III collagens in human skin. Proc Natl Acad Sci USA 78:7360–7364

Green TL, Meyer JR, Daniels TE, Greenspan JS, Souza Y de, Kramer RH (1988) Kaposi's sarcoma in AIDS: basement membrane and endothelial cell markers in late stage lesions. J Oral Pathol 17:266–272

Geerts A, Schuppan D, Lazeroms S, Zanger R de, Wilde A de, Wisse E (1990) Presence of hybrid interstitial fibrils containing type I and III collagen in the space of Disse of the rat liver. Hepatology 12:233–241

Gelderblom HR, Kocks C, L'age-Stehr J, Reupke H (1985) Comparative immunoelectronmicroscopy with monoclonal antibodies on yellow fever virus-infected cells: pre-embedding labelling versus immunocryoultramicrotomy. J Virol Methods 10:225–239

Griffiths G, McDowall A, Back R, Dubochet J (1984) On the preparation of cryosections for immunohistochemistry. J Ultrastruct Res 89:65–78

Holden CA (1989) Histogenesis of Kaposi's sarcoma and angiosarcoma of the face and scalp. J Invest Dermatol 93:119S–124S

Hoof A van den (1983) Connective tissue changes in cancer. In: Hall DA, Jackson DS (eds) International review of connective tissue research. Academic Press, New York, pp 395–427

Hoof A van den (1988) Stromal involvement in malignant growth. Adv Cancer Res 50:159–196

Kuntz AA, Gelderblom HR, Winkel T, Reichart P (1987) Ultrastructral findings in oral Kaposi's sarcoma. J Oral Pathol 16:372–379

Kramer RH, Fuh GM, Hwang CBC, Conant MA, Greenspan JS (1985) Basement membranes and connective tissue proteins in early lesions of Kaposi's sarcoma associated with AIDS. J Invest Dermatol 84:516–520

Leu HJ, Odermatt B (1985) Multicentric angiosarcoma (Kaposi's sarcoma). Light and electron microscopic findings of idiopathic cases in Europe and Africa and of cases associated with AIDS. Virchows Arch [A] 408:29–41

Nakamura S, Salahuddin SZ, Biberfeld P, Ensoli B, Markham PD, Wong-Staal F, Gallo RC (1988) Kaposi's sarcoma cells: long-term culture with the growth factor from retrovirus-infected $CD4^+$ T cells. Science 242:426–429

Nicholson R, Murphy G, Breathnach R (1989) Human and rat malignant-tumor-associated mRNAs encode stromelysin-like metalloproteinases. Biochemistry 28:5195–5203

Peltonen J, Jaakkola S, Hsiao LL, Timpl R, Chu M-L, Uitto J (1990) Type VI collagen: in situ hybridization and immunohistochemistry reveal abundant mRNA and protein levels in human neurofibroma, schwannoma and normal peripheral nerve tissue. Lab Invest 62:487–492

Penneys NS, Bernstein H, Leonardi C (1988) Confirmation of early Kaposi's sarcoma by polyclonal antibody to type IV collagen. J Am Acad Dermatol 19:447–450

Rabanus JP, Gelderblom HR, Schuppan D, Becker J (1991) Distribution of collagens type V and VI in the normal oral mucosa: an immunoelectronmicroscopical study using ultrathin frozen sections. J Periodont Res (1991) 26:138–143

Reichart PA, Gelderblom HR, Becker J, Kuntz A (1987) The HIV infection: virology, etiology, origin, immunology, precautions and clinical observations in 110 patients. Int J Oral Maxillofac Surg 16:129–153

Rosai J, Summer HW, Major MC, Kostianowsky M, Perez-Mesa C (1976) Angiosarcoma of the skin: a clinicopathologic and fine structural study. Hum Pathol 7:83–109

Salm R (1963) The nature of the so-called postmastectomy lymphangiosarcoma. J Pathol Bacteriol 85:445–456

Schuppan D, Hahn EG (1987) Components of the extracellular matrix (collagens, elastin, glycoproteins and proteoglycans). In: Wolf JR, Berry M, Sievers J (eds) Epithelial-mesenchymal interactions in neural development. NATO ASI Ser H5:3–29

Schuppan D, Rühlmann T, Hahn EG (1985) Radioimmunoassay for human type VI collagen and its application to tissue and body fluids. Anal Biochem 149:238–247

Schuppan D, Becker J, Boehm H, Hahn EG (1986) Immunofluorescent localization of type V collagen as a fibrillar component of the interstitial connective tissue of human oral mucosa, artery and liver. Cell Tissue Res 243:535–543

Tokuyasu KT (1980) Immunocytochemistry on ultrathin frozen sections. J Histochem 12:381

Vaes G (1985) Macrophage secretory products and connective tissue remodeling: role of macrophage enzymes and of "matrix regulatory monokines". In: Dean RT, Stahl P (eds) Developments in cell biology. Butterworths, London, pp 99–117

Von der Mark H, Aumailley M, Wick G, Fleischmajer R, Timpl R (1984) Immunochemistry, genuine size and tissue localization of collagen VI. Eur J Biochem 142:493

Wooley DE (1984) Mammalian collagenases. In: Piez KA, Reddi AM (eds) Extracellular matrix biochemistry. Elsevier, New York, pp 119–157