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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 *

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Summary. Twelve oral AIDS-associated Kaposi sarcomas (KS) were studied for the distribution of extracellular matrix components using indirect immunofluorescence. Staining for basement membrane (BM) components revealed two distinct patterns of distribution: a delicate and partly fragmented lining of BMs around sinusoid-like vascular spaces or an occasional diffuse interstitial fluorescence in the tumour stroma; or an irregular broad rim of fluorescence in walls of larger blood vessels. These findings support a vascular cell origin of the endothelial- and spindle cell component in KS. The tumour stroma was almost completely negative for collagen type V and undulin, whereas an intensive fluorescence was noted for procollagens type I, III and collagen type VI. In areas adjacent to KS a loss of procollagens type I and III, collagens type V, VI and undulin was noted. An intimal sheath of collagen type V was usually absent from blood vessels of the tumour or the peritumourous connective tissue. Immunohistochemical findings indicate that the preexisting interstitial connective tissue matrix is destroyed during tumour invasion and that subsequently procollagens type I, III and collagen type VI are synthesized de novo by cells of the tumour stroma.

Key words: AIDS – Kaposi sarcoma – Collagen – Extracellular matrix – Basement membrane

Kaposi sarcoma (KS) is a rare multicentric angiosarcoma, which was originally described by Moricz Kaposi in 1872 in elderly people of Mediterranean or Eastern Askenazi Jewish origin. In recent years, KS has been observed with increasing incidence in association with the acquired immunodeficiency syndrome (AIDS). About 30% of all AIDS-patients, primarily homosexual men, develop KS (Friedman-Kien 1984). While oral non-AIDS KS is extremely rare, 50% of AIDS-patients with KS develop oral manifestations. In 76% of all cases oral KS is first located on the hard palate (Green et al. 1984; Reichart et al. 1987). The clinical appearance and histology of idiopathic KS from Europe or Africa and of cases associated with AIDS are identical (Mc Nutt et al. 1983; Leu and Odermatt 1985). Histologically, KS is characterized by two predominant tumour cell types, the major variable being the stage of development of the lesion (Murray and Lothe 1962; O'Connell 1977). One main component in KS consists of vascular channels lined by endothelial cells containing enlarged nuclei of irregular shape which often proliferate in a preformed vascular lumen or invade the surrounding tissue through the particularly destroyed vascular reticulin sheath. The ultrastructure of these cells shows all characteristics of normal endothelial cells, i.e. Weibel-Palade bodies, multivesicular bodies, tight junctions and basement membranes (Leu and Odermatt 1985; Langford-Kuntz et al. 1987). The second component is represented by intervascular spindle-shaped stromal cells with blunt-ended, enlarged nuclei. Only a minor fraction of these cells possesses the characteristics of endothelial cells such as Weibel-Palade bodies, tight junctions and basement membranes; the majority resembles undifferentiated mesenchymal cells (Leu and Odermatt 1985).

The connective tissue consists of numerous different collagens, which form a family of structurally related proteins (for review see: Bornstein and

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Sage 1980; Schuppan and Hahn 1987). Collagens type I and III, as the typical fibril forming collagens, are the major constituents of all interstitial connective tissues. Collagen type IV is only found in basement membranes where it forms a transitory scaffold for the other basement membrane constituents (Timpl et al. 1981). Collagen type V is a fine fibrous component not of basement membranes, but of the interstitial connective tissue and of the intima of arteries and veins (Becker et al. 1986a; Schuppan et al. 1986a).

Collagen type VI is a minor but ubiquitous component of the interstitial connective tissue, especially of the normal oral mucosa (Becker et al. 1986a). It forms microfibrils (von der Mark et al. 1984) and appears to be the major constituent of the 100 nm periodic filaments observed in interstitial connective tissues (Bruns et al. 1986).

Undulin, a recently discovered glycoprotein of the interstitial connective tissue with a Mr of ca. 620 kD is composed of a large globular head and a ca. 70 nm long tail and has been found in a variety of dense connective tissues, where it appears to be associated with bundles of mature collagen fibrils (Schuppan et al. 1987).

The immunohistochemical distribution of the basement membrane components collagen type IV and laminin in non-AIDS KS has been described by Bendelac et al. (1985). In a further study the distribution of collagens type I and IV, laminin and fibronectin (Kramer et al. 1985) in KS associated with AIDS has been investigated. Both studies suggested an endothelial origin for tumour cells in KS.

The aim of the present study was to analyse the pattern of a broad spectrum of extracellular matrix proteins which are known to be present in vessel walls in oral KS to get further insight into the histogenesis and interplay between tumour cells and the extracellular matrix.

Material and methods

Procollagens type I and III (pNI and pNIII), lacking the carboxyterminal propeptide, were purified from newborn monkey and bovine skin respectively (Becker et al. 1986b). The 7-S long domain of type IV collagen was isolated from a mixture of type IV collagen fragments after pepsin and collagenase digestion of human placenta followed by ion exchange and molecular sieve chromatography (Risteli et al. 1980; Becker et al. 1986a; Schuppan et al. 1986b). The NC1 domain of collagen type IV was obtained from collagenase-digested human placenta (Schuppan et al. 1986b). Human laminin fragment P1 was isolated from pepsin digested placenta and finally freed from contaminating collagens by collagenase digestion and molecular sieve chromatography (Risteli and Timpel 1981; Becker et al. 1986a). Collagens type V and VI were isolated after pepsin digestion as described previously (Schuppan et al. 1985 and 1986a; Becker et al. 1986a, b). Undulin was extracted and purified from newborn monkey skin (Schuppan et al. 1987).

Antibodies were raised in rabbits with the exception of antibodies against the 7-S domain of collagen type IV (from goat) and the NC1 domain of collagen type IV (monoclonal mouse antibody). Details of the characterization of the affinity purified antibodies as monospecific by sensitive radioimmunoassays, western blotting or blocking experiments were described previously.

Biopsies of 12 oral KS from homosexual men were examined (mean age 40 years, range 31–56 years). All tumours were localized at the hard palate. Immediately after removal tissues were orientated and one portion was snap frozen in liquid nitrogen. A second portion was fixed in formalin and processed for routine histology (H & E).

For immunofluorescence studies cryostat sections (4 µm) were air-dried at room temperature for 2 h and fixed in chloroform-acetone for 5 min at 5° C. Procollagens type I and III, collagens type V, VI and Undulin were examined after double staining. The tissue sections were first incubated with the monoclonal antibody to the NC1 domain of collagen type IV followed by FITC-conjugated goat-anti-mouse IgG (Jackson, Avondale, PA, USA). The same sections were then incubated with purified antibodies against the above mentioned proteins, followed by TRITC- conjugated goat-anti-rabbit IgG (Jackson, Avondale, PA, USA). The distribution of the 7-S domain of collagen type IV and of laminin was examined by single immunofluorescence followed by a TRITC conjugated rabbit-anti goat or goat-anti-rabbit-IgG, respectively (Jackson, Avondale, PA, USA). After each step sections were washed three times in PBS (pH 7.2). Immunostaining was visualized with a Leitz Orthoplan microscope and photographed with Agfapan 400 films.

Results

H & E stain: Nine KS represented late tumour stage lesions consisting of well demarcated nodules or lesions with a diffuse infiltration of the lamina propria. Three KS represented an early stage with focal proliferations of thin walled, dilated vessel like channels. Immunohistochemical findings are summarized in Table 1.

For collagen type IV (NC1-domain) two distinct patterns of distribution were observed. A deli-

Table 1. Distribution of extracellular matrix proteins in normal oral mucosa and KS

	Normal oral mucosa	Adjacent connective tissue of KS	KS tumour stroma
Procollagen type I	+	+−	+
Procollagen type III	+	+−	+
Collagen type V	+	−	−
Collagen type VI	+	+−	+
Undulin	+	−	−

(+: Intense fluorescence of the entire connective tissue; +−: Weak fibrous staining due to strong degradation; −: Not present)

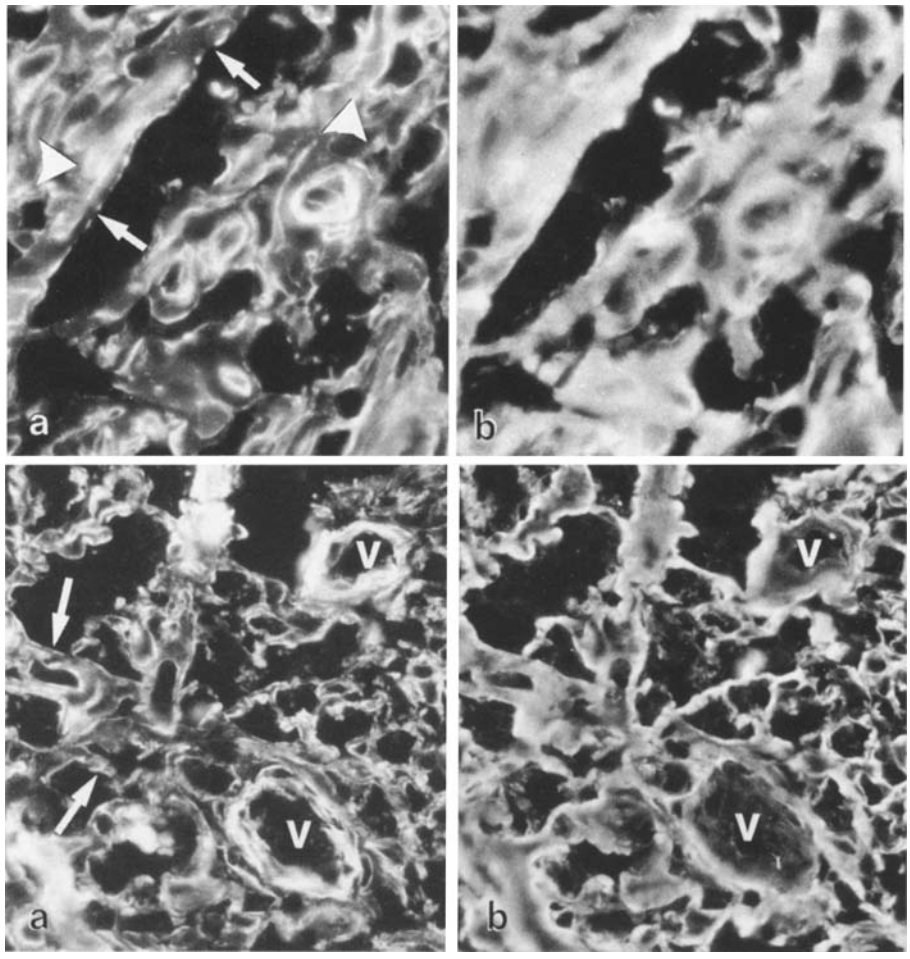

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Fig. 1a. NC1: Fragmented delineation of numerous tumour vascular channels (*arrows*). Diffuse interstitial staining in KS tumour stroma (*arrowheads*). × 1040. **b** Procollagen type I (double staining to Fig. 1a): Intense fine fibrous staining of KS tumour stroma. × 1040

Fig. 2a. NC1: Larger vessels (*V*) with a broad rim of fluorescence of the subendothelial BM and the BM around individual smooth muscle cells of the media. Faint, non-continuous delineation of tumour vessels (*arrows*). Diffuse interstitial staining of the tumour stroma (*arrowheads*). × 830. **b** Procollagen type III (double staining to Fig. 2a): Intense fibrous fluorescence in the entire KS tumour stroma, pronounced staining in the adventitia of blood vessels (*V*). × 830

cate and partly fragmented lining of BMs around sinusoid-like vascular spaces (Figs. 1a, 2a, 4a) was associated with an occasional diffuse, interstitial fluorescence in the tumour stroma of advanced lesions (Figs. 2a, 4a). In contrast, larger vascular structures could easily be differentiated from the faintly delineated tumour vessel-like channels due to a broad rim of fluorescence (Figs. 2a, 3a, 6a). The walls of these vessels showed often a multilamellar, rugged BM (Figs. 3e, 6a). In areas surrounding the KS-tumour nearly all larger blood vessels revealed a staining pattern (Fig. 3a) which was comparable to that found in normal oral mucosa. Patterns obtained with antibodies to the 7-S domain of collagen type IV or the laminin P1 fragment were identical (not shown), although staining for laminin was less intensive than that for 7-S or NC1.

The tumour stroma was strongly positive for procollagen type I, and the fluorescence often touched the basement membrane of the tumour-vessels (Fig. 1b). In peritumourous areas, especially in the subepithelial connective tissue, procollagen type I was less prominent than in normal oral mucosa, whereas it was pronounced around larger vessels.

The distribution of procollagen type III was comparable to that of procollagen type I, although the pattern consisted of rather course fibers (Fig. 2b).

Collagen type V showed a fine-fibrous staining pattern in the peritumourous interstitial connective

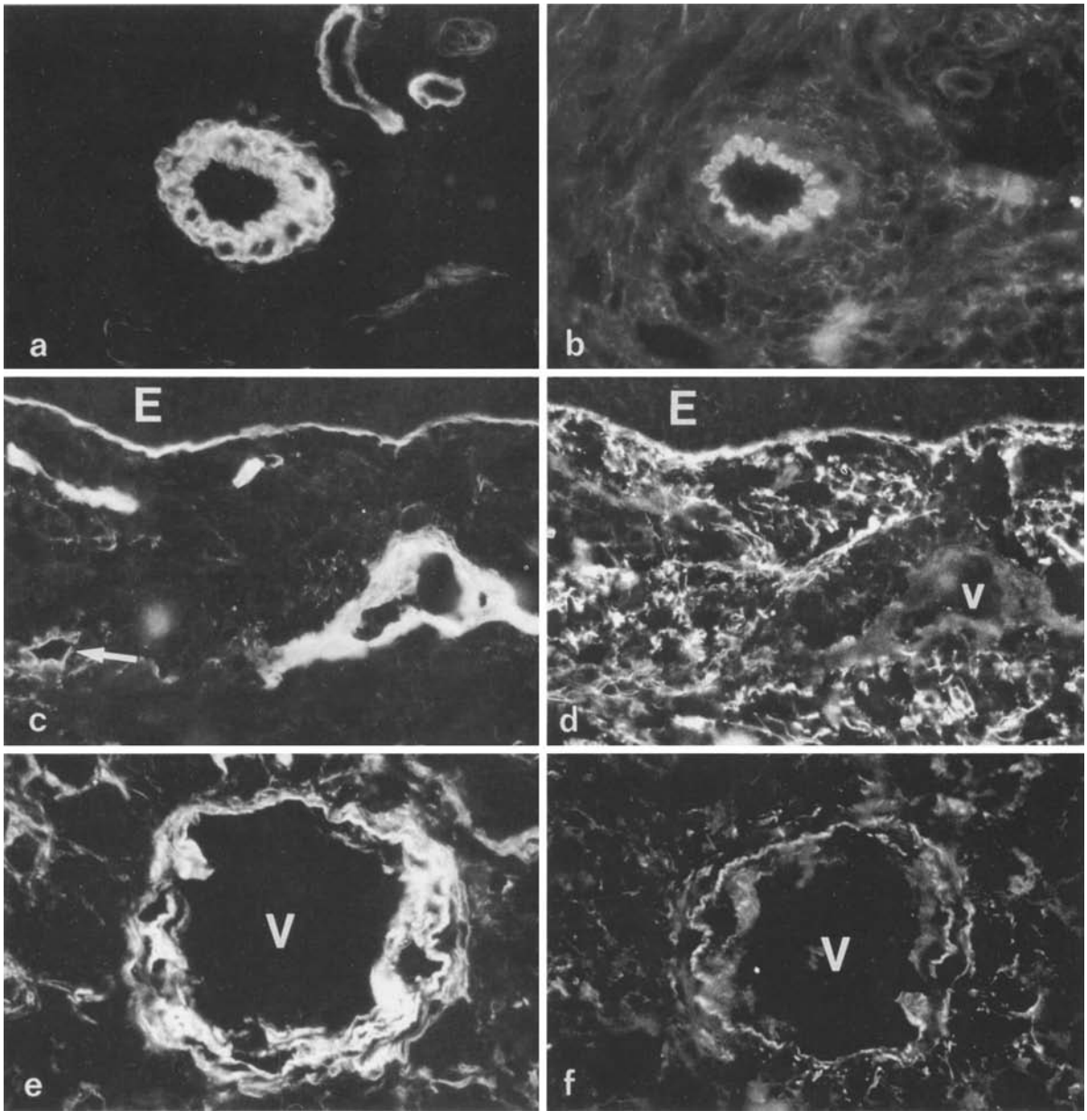

Fig. 3a. NC1: Intense fluorescence of the subendothelial BM and around individual smooth muscle cells of the media of a peritumourous blood vessel. ×830. **b** Collagen type V (double staining to Fig. 3a): Unilamellar sheath in the intima of a peritumourous blood vessel. The surrounding interstitial connective tissue at the tumour periphery is completely negative for type V collagen. ×830 **c** NC1: Intense fluorescence of a blood vessel in the subepithelial connective tissue. An atypical vascular proliferation (*arrow*) is delineated by faint BM labelling (*E*=epithelium). KS tumour periphery. ×830. **d** Collagen type V (double staining to Fig. 3c): Fragmented, disorganized labelling of collagen type V in the subepithelial connective tissue (*E*=epithelium). The intima of the blood vessel is completely negative. KS tumour periphery. ×830. **e** NC1: Multilamellar irregular staining of a blood vessel (*V*) in KS tumour. ×830. **f** Collagen type V (double staining to Fig. 3e): Faint, interrupted sheath of collagen type V in the media and adventitia of a blood vessel in KS. ×830

tissue of KS (Fig. 3d), whereas it was almost completely lost from the interstitial connective tissue of the tumour itself (Fig. 3b, 3f). In ten out of 12 biopsies the larger, possibly preexisting vessels in the tumour stroma or of the peritumourous connective tissue did not contain the typical fibrous staining pattern for collagen type V in the intima (Fig. 3d) which is found in normal oral mucosa. In some instances, however, larger vessels in the tumour or in the peritumourous connective tissue revealed a faint and interrupted rim of fluorescence for type V collagen, which appeared to be pushed

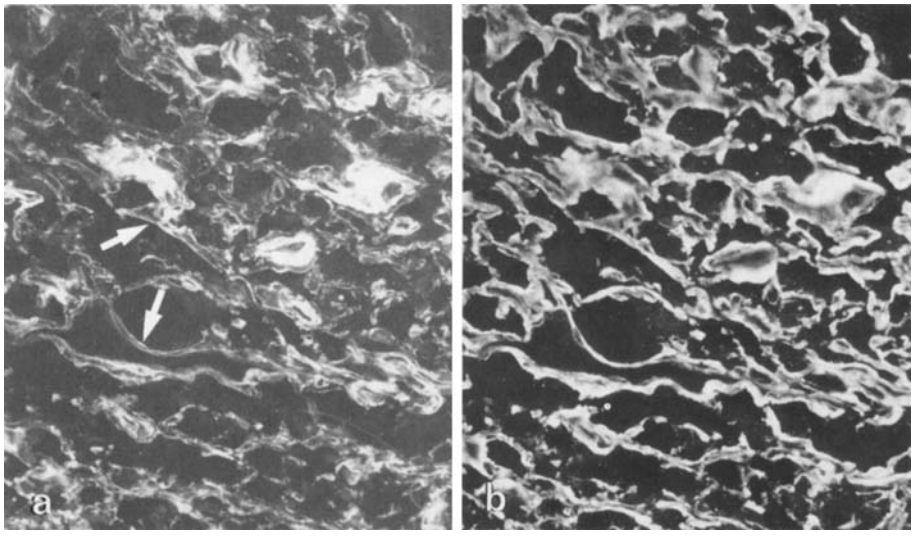

Fig. 4a. NC1: Distinct BM delineation of numerous tumor vascular channels (*arrows*). Larger blood vessels contrast due to their broad rim of fluorescence. ×520. **b** Collagen type VI (double staining to Fig. 4a): Intense amorphous staining in the entire KS tumour stroma. ×520

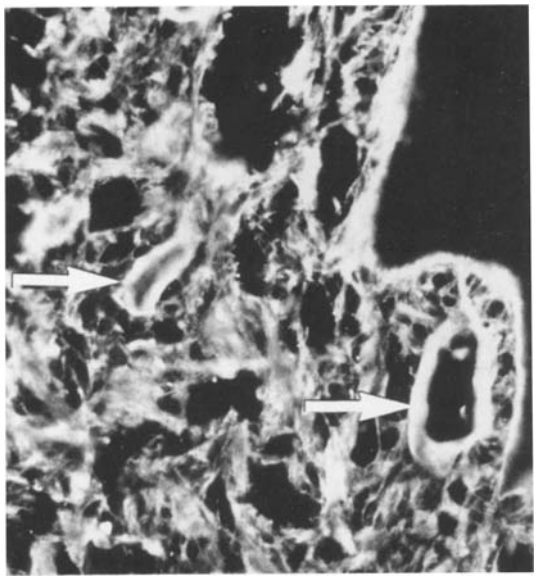

Fig. 5. Collagen type VI: Loss of collagen type VI in the subepithelial connective tissue. Pronounced staining in the adventitia of blood vessels (*arrows*). ×830

to the medial or even adventitial side of the vessel (Fig. 3f). Two biopsies with early lesions showed very few (2–4/section) vessels with the typical intimal staining for type V collagen (Fig. 3b).

Collagen type VI revealed an amorphous interstitial pattern and seemed to be codistributed with procollagens type I and III. Staining was pronounced in the adventitia of blood vessels (Figs. 4b, 5) as observed previously in normal oral mucosa. As for procollagens type I and III the tumour stroma was strongly positive for collagen type VI (Fig. 4b), whereas in the peritumourous subepithelial connective tissue an intense loss of collagen type VI was noted (Fig. 5).

The typical staining pattern for undulin, consisting of undulating, parallely aligned uniform fibers (Fig. 6c) was only rarely found in the connective tissue adjacent to tumour areas. Here, parallely aligned fibers were still present, but appeared to be unusually fragmented. The tumour stroma proper was almost completely negative for undulin and only diffuse deposits of immunoreactive material around vessel walls were observed as a broad rim of fluorescence for collagen type IV (Fig. 6b). Many – but not all – vessels in the peritumourous connective tissue had retained their perivascular sheath of undulin fibers.

Discussion

The histogenesis of KS has been discussed for many years. The consensus obtained from numerous studies is that the cells lining the vascular spaces are of vascular origin, from the presence of Weibel-Palade bodies, factor VIII related antigen and BM material (Millard and Heryet 1985). However, the origin of the spindle cells of KS remains controversial, because they do not, or only occasionally contain factor VIII related antigen. Rutgers et al. (1986) first demonstrated that both cell components of KS exhibit a differential expression of endothelial-cell associated antigens. The presence of two cell components with a differential expression of endothelial surface markers was explained earlier by the heterogeneity inherent in normal vascular endothelium (Knowles et al. 1984). We have demonstrated that blood vessel walls in connective tissue adjacent to KS contained

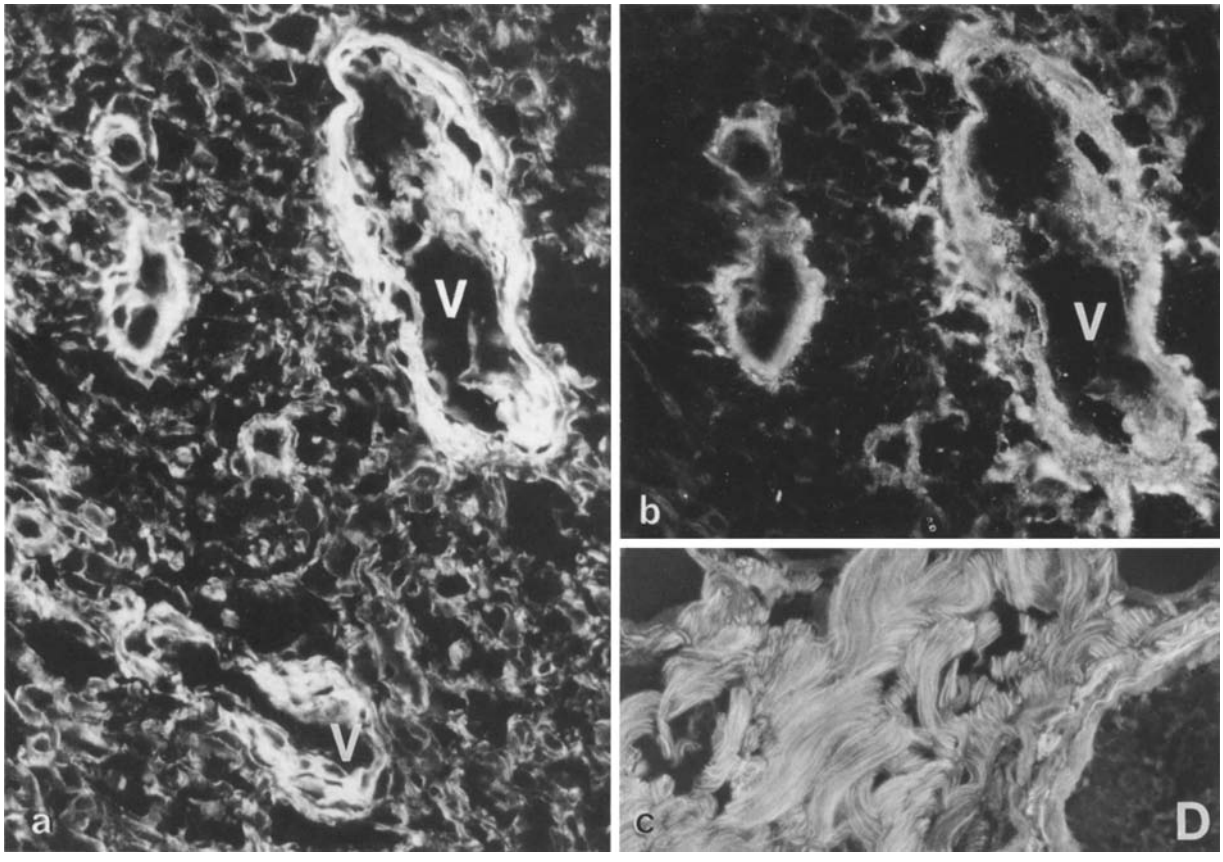

Fig. 6a. NC1: Numerous blood vessels (*V*) in KS with a multilamellar fragmented BM. ×830. **b** Undulin (double staining to Fig. 6a): Amorphous fluorescence in the media and adventitia of blood vessels (*V*) and a nearly negative KS tumour stroma. ×830. **c** Undulin, normal palatal mucosa. Parallely arranged undulating fibers in the interstitium and around a salivary gland duct (*D*). ×830

procollagens type I, III, collagen type VI and undulin, whereas collagen type V was limited to the intimal layer. The broad rim of BM fluorescence in these vessels (Fig. 3a) is caused by pericellular staining of medial smooth muscle cells and by the subendothelial BM itself. In contrast, in KS itself, larger vessels (Fig. 3e) were often surrounded by a multilamellar, often disrupted, BM contrasting to the faint staining around vascular channels in the tumour (Fig. 4a). In addition, the medial layer of larger vessels occasionally appeared to be negative for BM proteins indicating that normal vessels might be pathologically changed by KS tumour cells, because on H & E-stained sections these vessels had all characteristics of arteries and veins. Further evidence for alteration of pre-existing blood vessels in KS was obtained, since in a few arteries and veins the layer of type V collagen, which is normally located in the intima of vessels (Becker et al. 1986a; Schuppan et al. 1986a), was partly degraded and pushed towards the adventitia, probably by KS cells (Fig. 3f). The loss of intimal type V collagen from nearly all larger vessels in the tumour might be a peculiar phenomenon in KS, since it was not observed in other oral diseases, i.e. oral lichen planus, oral squamous cell carcinomas or periodontal diseases (own unpublished results).

Preliminary studies of normal oral mucosa of HIV infected patients revealed a loss of intimal type V collagen in 8 out of 10 cases, which did not reveal advanced stages of ARC or AIDS. Further studies are necessary to decide whether the loss of intimal type V collagen may be a predictor for the development of KS.

Our findings support the immunohistochemical observations of Bendelac et al. (1985) in classical KS and of Kramer et al. (1985) in AIDS-associated KS, that tumour cells lining the vascular spaces maintain one of the major features of endothelium in secreting BM material. Using electron microscopy Leu and Odermatt (1985) found interruptions of BM in KS, which we also observed immunohistochemically (Fig. 1a, 2a). The partly intense,

but diffuse interstitial deposits of BM proteins might be produced either by actively proliferating endothelial cells or even more likely by the spindle cells, as was previously suggested by Bendelac et al. (1985) in non-AIDS KS.

The intense fluorescence for procollagens type I, III and collagen type VI in KS tumour stroma contrasted to the fragmentation of these proteins in the adjacent connective tissue of the tumour (Table 1). These observations and the selective absence of collagen type V (which is normally present in oral mucosa) and undulin led to the suggestion that the matrix proteins in the KS tumour were synthesized de novo by KS tumour cells. This theory is supported by the findings of Sage et al. (1981) that bovine capillary endothelial cells in vitro secreted large amounts of collagens type I and III (but not type V collagen). Further evidence for this theory was obtained by our own results, since the de novo formation of capillaries in pyogenic granulomas and during wound healing of human alveolar bone (as visualized by BM fluorescence and H & E staining) was accompanied by a strong fluorescence for procollagens type I, III and collagen type VI beside the BM but not for collagen type V and undulin (Becker et al. 1987).

The intense degradation of extracellular proteins in the adjacent connective tissue of KS may be explained by the presence of numerous macrophages, which were demonstrated in electron microscopic studies (Leu and Odermatt 1985). It is well known that macrophages contain proteolytic enzymes and that they are able to induce the production of proteolytic enzymes by fibroblasts (Vaes 1985).

In summary, these findings could be interpreted as destruction of the interstitial connective tissue matrix and the preferential dissolution of undulin, which is highly susceptible to proteolytic degradation (Schuppan et al. 1987), during invasion of tumour cells into the connective tissue stroma of the oral mucosa. The intense fluorescence for procollagens type I and III and collagen type VI in the tumour stroma proper is most probably due to their de novo formation by KS tumour cells.

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