American Journal of Pathology, Vol. 147, No. 1, July 1995
Copyright © American Society for Investigative Pathology

Expression of Epithelial Adhesion Proteins and Integrins in Chronic Inflammation

Kirsi Haapasalmi,* Marja Mäkelä,‡ Olli Oksala,‡ Jyrki Heino,§ Kenneth M. Yamada,‖ Veli-Jukka Uitto,* and Hannu Larjava†

From the Departments of Oral Biology and Clinical Dental Sciences,† University of British Columbia, Vancouver, British Columbia, Canada; Institute of Dentistry‡ and Medicity§ Research Laboratory, University of Turku, Finland; and Laboratory of Developmental Biology,‖ National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland*

Epithelial cell behavior in chronic inflammation is poorly characterized. During inflammation of tooth-supporting structures (periodontal disease), increased proliferation of epithelial cells into the inflamed connective tissue stroma is commonly seen. In some areas ulceration and degeneration take place. We studied alterations in the expression of adhesion molecules and integrins during chronic periodontal inflammation. In inflamed tissue, laminin-1 and type IV collagen were still present in the basement membrane and surrounding blood vessels, but they were also found extravascularly in inflamed connective tissue stroma. Type VII collagen and laminin-5 (also known as kalinin, epiligrin, or nicein) were poorly preserved in the basement membrane zone, but both were found in unusual streak-like distributions in the subepithelial connective tissue stroma in inflamed tissue. Both fibronectin and tenascin were substantially decreased in chronically inflamed connective tissue, showing only punctate staining at the basement membrane zone. Integrins of the β1 family showed two distinct staining patterns in epithelial cells during chronic inflammation; focal losses of β1 integrins (α2β1 and α3β1) were found in most areas, while in other areas the entire pocket epithelium was found to be strongly positive for β1 integrins. No members of the αv integrin family were found in any epithelia studied. Expression of the α6β4 integrin was high in basal cells of healthy tissue, but weak in epithelium associated with chronic inflammation. Chronic inflammation therefore involves alterations in both adhesion proteins and integrins expressed by epithelial cells. Basement membrane components found at abnormal sites in stroma in chronic inflammation might serve as new adhesive ligands for various cell types in inflamed stroma. (Am J Pathol 1995, 147:193–206)

During the pathogenesis of periodontal disease, the junctional epithelium between the tooth surface and gingival connective tissue moves apically and becomes transformed into periodontal pocket epithelium.[1] These epithelial cells proliferate and form epithelial ridges in inflamed connective tissue. As a result, the relative volume of gingival tissue occupied by epithelial cells increases compared to healthy tissue. This dynamic process of epithelial cell movement involves adhesion to and migration along an extracellular connective tissue matrix that is altered due to chronic inflammation. Basement membrane also shows alterations, with discontinuities and other morphological changes in chronic inflammation.[2–6] The adhesion and migration mechanisms of epithelial cells in chronically inflamed tissues are, so far, poorly characterized. It is likely, however, that the expression of specific cell surface adhesion receptors is altered as a result of changing functional demands and the effects of bioactive substances present in inflammation.[7]

Integrins are cell surface glycoproteins that function as cell-to-cell and cell-to-extracellular matrix adhesion receptors (for reviews, see refs. 8 to 10). Through binding to matrix proteins integrins mediate information transfer from the extracellular milieau to

Supported by grants from MRC Canada, the British Columbia Health Foundation, the Finnish Cultural Foundation, the Finnish Dental Society, and the Sigrid Juselius Foundation.

Accepted for publication March 28, 1995.

Address reprint requests to Dr. Hannu Larjava, University of British Columbia, Faculty of Dentistry, 2199 Wesbrook Mall, Vancouver, BC V6T 1Z3 Canada.

the cytoplasm and nucleus, leading to alterations in cell functions and ultimately in cell behavior.[11,12] Integrins are composed of a larger α subunit and a smaller β subunit. The α subunit provides information for ligand binding, and the β subunit provides a link to the cytoskeleton. Integrins of epithelial cells mediate cell adhesion to different types of collagens, laminins, and fibronectins.[13,14] Epithelial cell (keratinocyte) integrins also appear to mediate cell-to-cell interactions.[15] *In vivo*, integrins are expressed by basal keratinocytes but are absent from differentiating upper cell layers.[16–20] Loss of functional integrins from the cell surface precedes epithelial differentiation.[21] These findings suggest that integrins have an important role in keratinocyte proliferation and differentiation. The following integrins and their ligands have been reported in cultured keratinocytes of skin and oral mucosa: α5β1 (fibronectin),[22,23] α2β1 (collagens),[23,24] and α3β1 (fibronectin, collagen, and laminin-5).[24–27] In addition, the α6β4 integrin is a structural component of hemidesmosomes,[28–31] and it is believed to bind a recently discovered laminin isomer, laminin-5.[26,32] Cultured keratinocytes also express αvβ5, which mediates cell adhesion to vitronectin.[24,33] Keratinocytes *in vivo*, however, do not express α5β1 and express only low amounts of αv-integrins. The expression of these receptors is found to be induced in keratinocytes during acute inflammation such as wound healing.[34–36]

In the present paper, we demonstrate alterations in the expression of β1 and β4 integrins and basement membrane zone proteins in chronic periodontal inflammation.

Materials and Methods

Materials

Chronically inflamed periodontal tissue specimens were obtained from 22 subjects during extraction of periodontally involved teeth with a clinically hopeless prognosis. Specimens were rinsed in physiological saline, placed on a cork disk in a defined orientation, embedded in Tissue-Tek (Miles Inc., Elkhart, IN), and immediately frozen in liquid nitrogen. Samples were stored at −70°C until used. Specimens were sectioned (6 μm) in a cryostat. Adjacent sections corresponding to those used for immunohistological studies were stained with hematoxylin and eosin for histopathological and morphological analyses. Gomori's periodic acid methenamine silver method[37] was used to histochemically stain the basement membrane zone.

Immunohistochemical Studies

Frozen sections (6 μm) were fixed with −20°C acetone for 5 minutes and stored at −70°C until used. Immunolocalization of integrins was performed as described previously.[34] Briefly, sections were incubated with primary antibodies diluted in phosphate buffered saline (PBS) containing 1 mg/ml bovine serum albumin (BSA) for 60 minutes, after which sections were rinsed four times in 60 minutes with the PBS/BSA solution. After rinsing, sections were incubated with affinity-purified rhodamine-conjugated secondary antibodies (dilution 1:50; Boehringer-Mannheim Biochemicals, Indianapolis, IN) for 60 minutes. Sections were rinsed and mounted using cyanoacrylate adhesive (Chemoco). Samples were examined using a Zeiss Axioskop 20 fluorescence microscope, and photographed using an MC 80 Zeiss microscope camera. Antibodies against the following integrin subunits and their ligands were used (sources for antibodies are given in Table 1; see refs. 38 to 47): α2, α3, α5, α6, αV, β1, β4, β5, β6, fibronectin, type IV and VII collagens, tenascin, laminin-1, and laminin-5 (kalinin, epiligrin). Control stainings were performed omitting the primary antibody or with nonimmune mouse serum. No specific staining was obtained with either of these control staining procedures (not shown).

Results

Microscopic Morphology of Inflamed Periodontal Tissue

Chronically inflamed tissue sections were obtained from patients suffering from advanced adult peri-

Table 1. *Antibodies Used to Recognize Different Integrins and their Ligands in this Study*

Integrin/Ligand	Antibody	Reference/Source
α2	Mab 117	AMAC, Inc. (Westbrook, ME)
α3	Mab J143	38
α5	Mab BIIG2	39
α6	MAb GoH3	40
αv	Mab P3G8	41
β1	Antiserum, 3847	42
β4	Mab 345–11A	43
β5	Mab P1F6	44
β6	Mab E7P6	44
Type IV collagen	Mab 68–124–1	ICN Biomedicals (Costa Mesa, CA)
Type VII collagen	Mab 1345	Chemicon (Temecula, CA)
Laminin-1	Serum	45
Laminin-5 (*Kalinin*)	MAb GB3	46
Fibronectin	antiserum	47
Tenascin	Mab 1927	Chemicon

odontal disease. Specimens containing both relatively healthy and chronically inflamed tissue were investigated. Comparisons were always made between these two areas (Figure 1, A and B) in the same section. Using hematoxylin and eosin staining, a heavy infiltrate of inflammatory cells was seen in close prox-

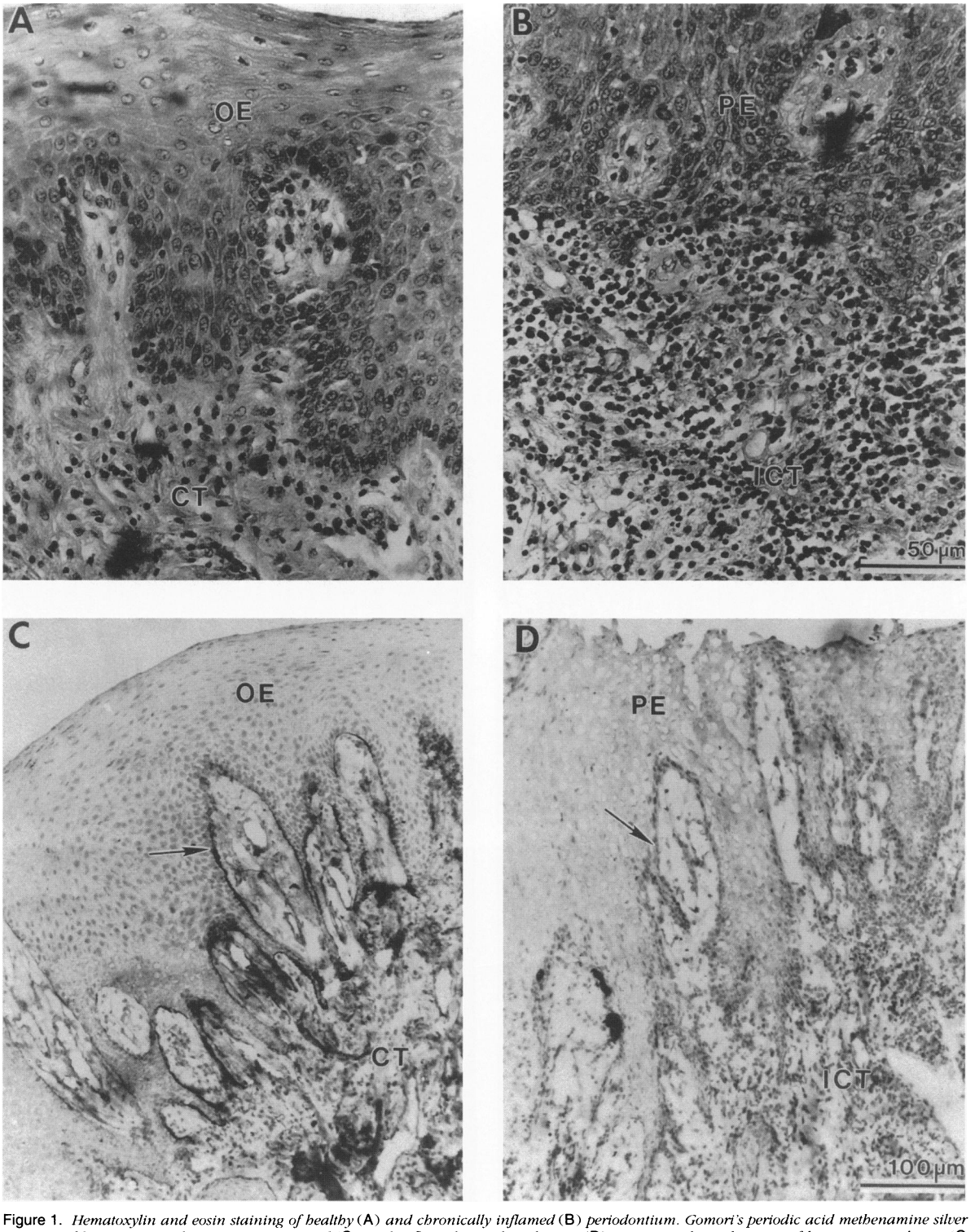

Figure 1. *Hematoxylin and eosin staining of healthy* (A) *and chronically inflamed* (B) *periodontium. Gomori's periodic acid methenamine silver staining of basement membrane zone in normal* (C) *and inflamed periodontal tissue* (D). *Arrows indicate location of basement membrane* (C *and* D). *OE, oral epithelium; CT, connective tissue; PE, periodontal epithelium in chronic inflammation; ICT, inflamed connective tissue. Bar 50 µm,* A, B; *100 µm,* C, D.

imity to the epithelium of inflamed area. Cellular infiltrates were mainly composed of chronic inflammatory cells, ie, lymphocytes and plasma cells (Figure 1B). In chronically inflamed sites, nonkeratinized epithelium varied in thickness from several cell layers to long epithelial extensions into the inflamed connective tissue.

We then used Gomori's periodic acid methenamine silver method[37] to examine the basement membrane underlining the epithelium. This staining visualizes aldehydes generated by periodate treatment, and it can be used to evaluate the integrity of basement membrane in tissue sections. In relatively healthy areas of oral epithelium, basement membrane staining was continuous, separating the oral epithelium from the underlying stroma (Figure 1C). In contrast, in inflamed periodontal tissues only faint, discontinuous staining was observed (Figure 1D).

Localization of Laminin-1 and Type IV Collagen

We then examined the putative adhesion molecules for epithelial cells, laminin-1 (classical laminin) and type IV collagen using immunolocalization techniques. In healthy oral epithelium, both laminin-1 and type IV collagen were found in epithelial and vascular basement membranes (Figure 2, A and B). In chronically inflamed tissue, both laminin-1 and type IV collagen were still present at the epithelial basement membrane zone, although in somewhat reduced amounts (Figure 2). Numerous blood vessels surrounded by laminin-1 and type IV collagen were present in the inflamed connective tissue (Figure 2, C and D). In addition, anomalous localization of punctate extravascular laminin and to a lesser extent type IV collagen was

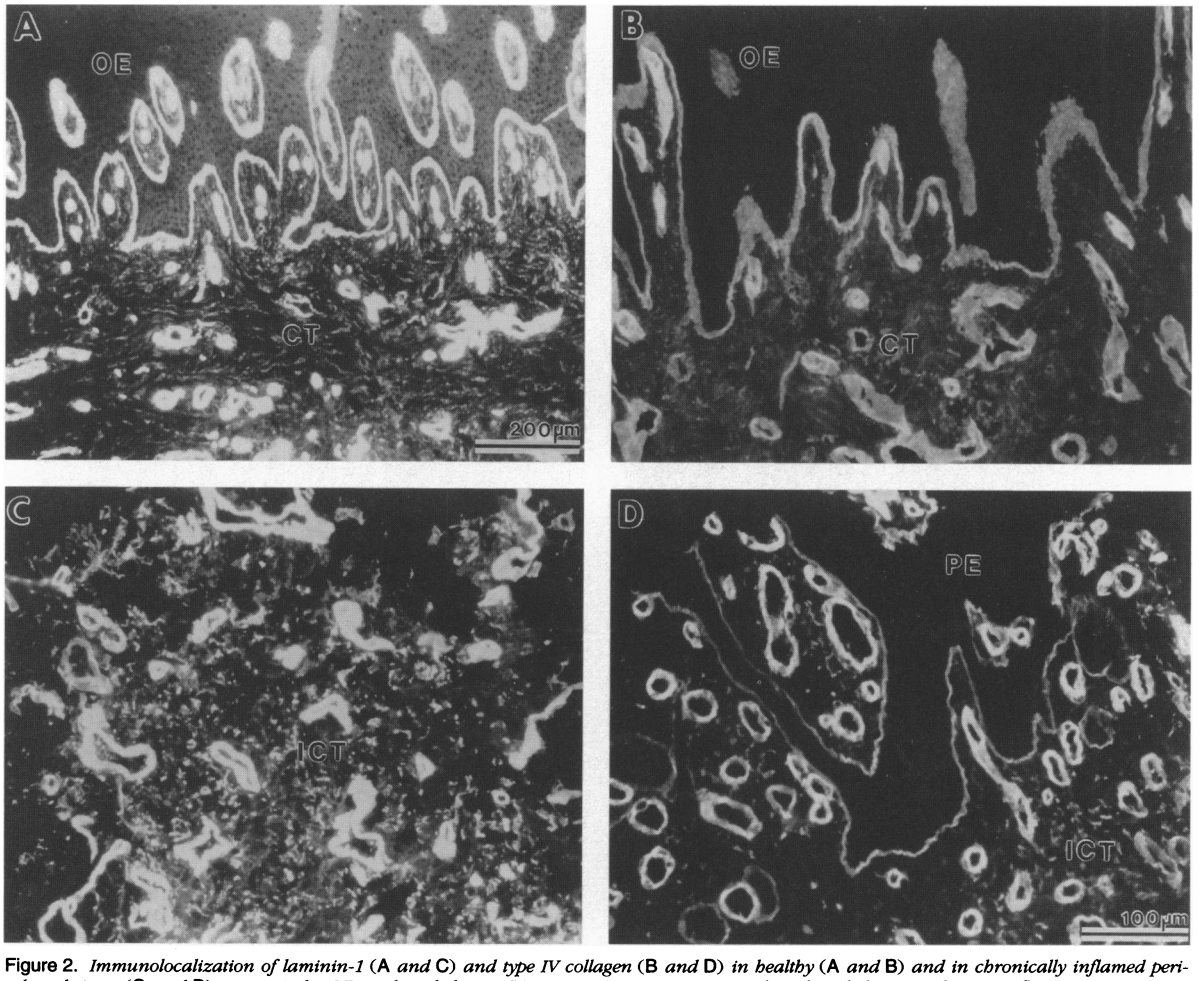

Figure 2. *Immunolocalization of laminin-1* (**A** *and* **C**) *and type IV collagen* (**B** *and* **D**) *in healthy* (**A** *and* **B**) *and in chronically inflamed periodontal tissue* (**C** *and* **D**), *respectively. OE, oral epithelium; CT, connective tissue; PE, periodontal epithelium in chronic inflammation; ICT, inflamed connective tissue. Bar 200 µm,* **A**; *100 µm,* **B** *to* **D**.

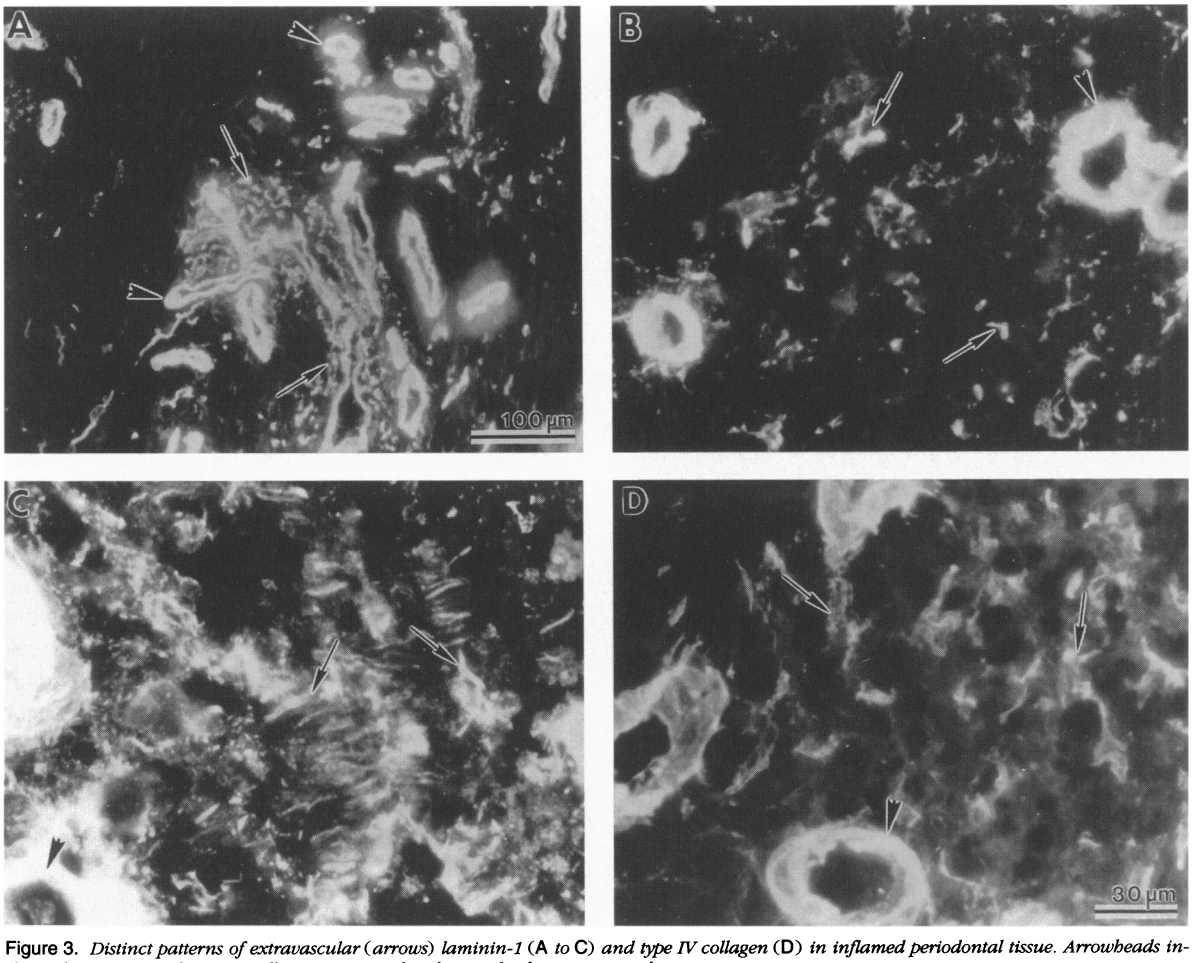

Figure 3. *Distinct patterns of extravascular (arrows) laminin-1 (A to C) and type IV collagen (D) in inflamed periodontal tissue. Arrowheads indicate laminin-1 and type IV collagen associated with vascular basement membrane.*

found to be present in the inflamed connective tissue stroma. Visually, the total amount of basement membrane components therefore often appeared to be increased in the inflamed area due to the deposition of new vascular and extravascular basement membrane material. Using higher magnification, we were able to demonstrate several different patterns of extravascular laminin-1 accumulation (Figure 3, A to C). Clearly, laminin-1 was often present surrounding blood vessels (Figure 3A), but it was also found in punctate (Figure 3B) and streak-like (Figure 3C) distributions. Type IV collagen was also present in the stroma in a punctate pattern (Figure 3D). Staining appeared to be specific, because only faint diffuse background staining was obtained with control antibodies (mouse nonimmune IgG or rabbit serum), or by omitting the first antibody (conjugate only) (not shown).

Localization of Components of Epithelial Anchoring System

Epithelium is anchored to the underlying connective tissue via hemidesmosome structures in which anchoring filaments and anchoring fibers composed of laminin-5 and type VII collagen, respectively, serve as linking elements.[32,48] Since the $\alpha 6\beta 4$ integrin is believed to use some of these proteins as adhesive ligands, we compared their distributions in healthy and chronically inflamed tissues. Based on immunolocalization, type VII collagen was relatively well preserved at the basement membrane zone in chronic inflammation (Figure 4). With higher magnification there was, however, a visible reduction of the intensity of staining in areas of chronic inflammation (compare Figure 5, C and D). In 5 out of 11 of the samples, type VII collagen was also found in unusual streak-like

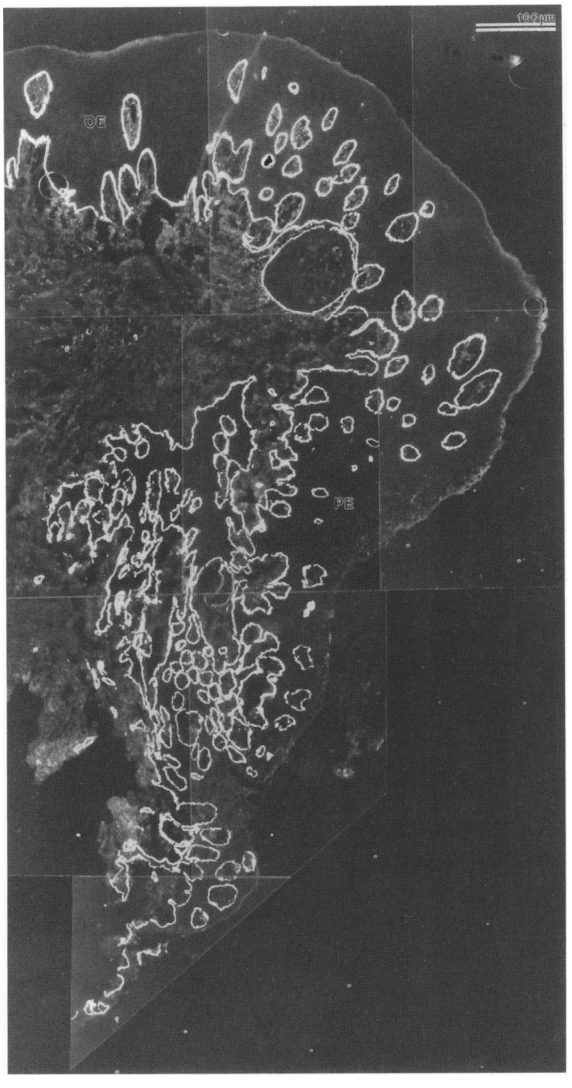

Figure 4. *Immunostaining of type VII collagen associated with oral epithelium (OE) and with periodontal epithelium in chronic inflammation (PE).*

distributions in the subepithelial connective tissue stroma of inflamed tissue (compare Figure 5B with Figures 5D and 9F). In 8 out of 11 samples the intensity of laminin-5 staining was generally reduced at the basement membrane zone in chronic inflammation, but in three samples it seemed to be relatively well preserved (Figure 5, E and F). In 4 out of 11 samples laminin-5 was found in similar streak-like patterns as type VII collagen in the connective tissue stroma of inflamed tissue (see Figure 9, E and F).

Localization of Fibronectin and Tenascin

Fibronectin and tenascin are also putative ligands for epithelial cell adhesion receptors. Fibronectin was present throughout the connective tissue adjacent to healthy oral epithelium, but it was clearly diminished in inflamed stroma (Figure 6, A and B). A fragmented and punctate staining pattern of fibronectin surrounded the inflammatory infiltrate. Tenascin was enriched in the subepithelial zone of healthy oral epithelium (Figure 6C). In chronically inflamed tissue, tenascin was almost completely absent (Figure 6D), and positive staining for tenascin was only seen at the basement membrane zone.

Distribution of β1 and α6β4 Integrins in Epithelial Cells

Staining for β1 integrins was found around the periphery of basal keratinocytes of healthy oral epithelium (Figure 7A) as we have reported earlier.[34] In chronic inflammation, two distinct patterns were seen. Most often, a focal loss of epithelial β1 integrins was seen in inflamed areas (Figure 7D). Most of the samples, however, also included areas where the full thickness of epithelium (Figure 7G) or the extending tip of it (Figure 7H) was found to be positive for β1 integrins. In both cases, cells in the inflamed stroma showed bright staining for β1 integrins (see Figure 7H). Immunolocalization of α2β1 and α3β1 integrins by specific antibodies confirmed the variable staining pattern of β1 integrins observed with β1 specific antibodies (Figure 8). Epithelial cells showed a loss of integrin expression in some areas of the section, while upregulation was observed in others. During inflammatory stimulation, the staining pattern also changed from localization around basal cells to a pattern encompassing cells in all cell layers (Figure 8, G to I). The transition from a nearly normal staining pattern to that of strongly induced expression was often remarkably abrupt sharp (e.g., see Figure 8G). A continuous staining pattern was observed for α6 and β4 integrins at the basal aspect of basal keratinocytes in noninflamed areas (Figure 7, B and C). Focal losses of both α6 and β4 integrin subunits were common in epithelial cells at inflamed sites (Figure 7, E and F). Double staining for β1 integrins and type VII collagen permitted better visualization of the alterations, since the loss of integrin staining sometimes made it difficult to distinguish epithelial cells from other cells in the stroma (Figure 7, H and I). It was noted that in areas of relatively high β1 integrin expression in epithelium, also type VII collagen often became localized in streaks in the subepithelial connective tissue (Figure 7, H and I). This view was supported by findings from serial parallel sections that were stained for β1, α6, β4, type IV collagen, kalinin (laminin-5), and type VII collagen (Figure 9, A to F).

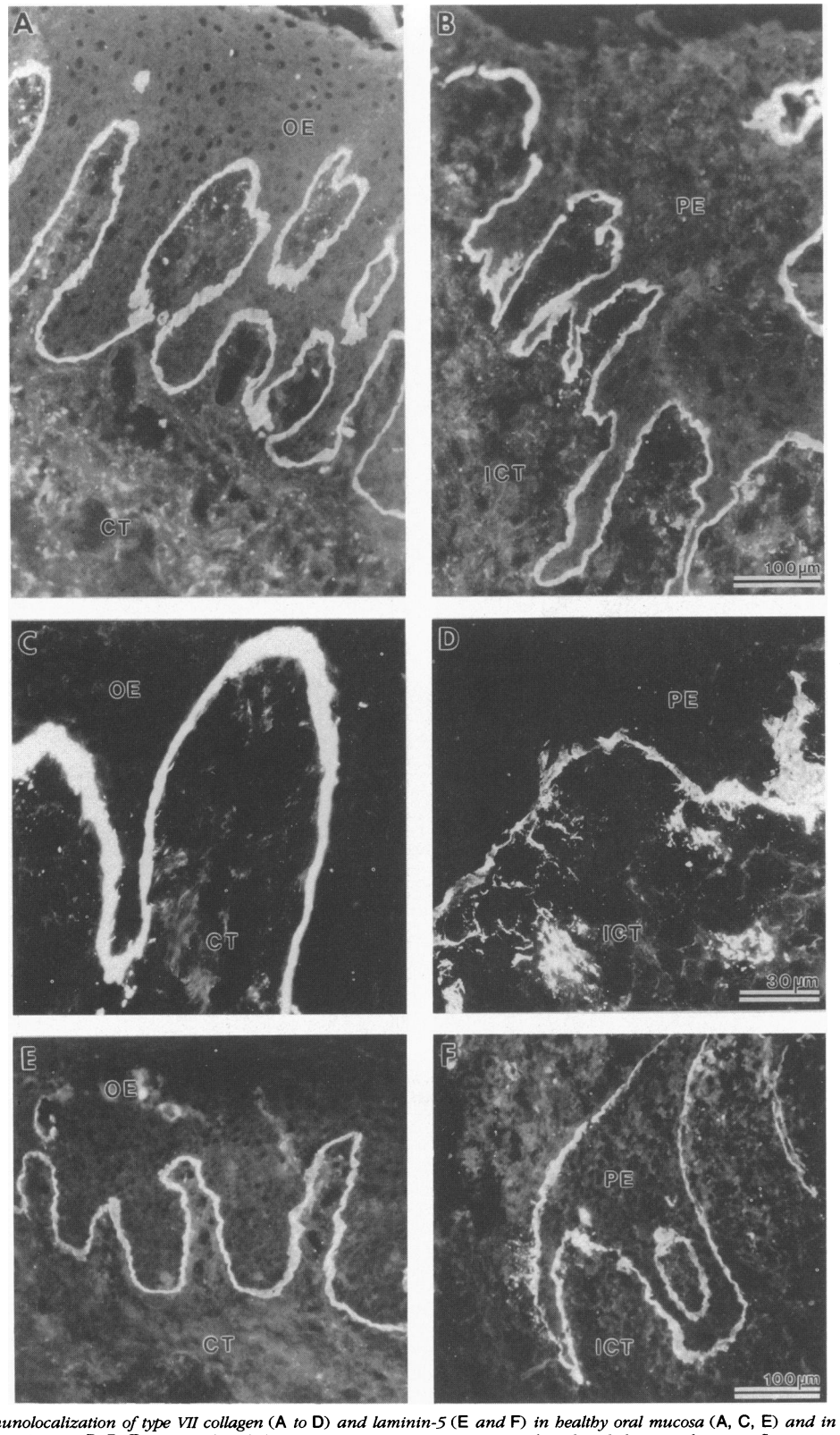

Figure 5. *Immunolocalization of type VII collagen* (**A** *to* **D**) *and laminin-5* (**E** *and* **F**) *in healthy oral mucosa* (**A, C, E**) *and in chronically inflamed periodontal tissue* (**B, D, F**). *OE, oral epithelium; CT, connective tissue; PE, periodontal epithelium in chronic inflammation; ICT, inflamed connective tissue. Bar 100 µm,* **A, B, E, F**; *bar 30 µm,* **C, D**.

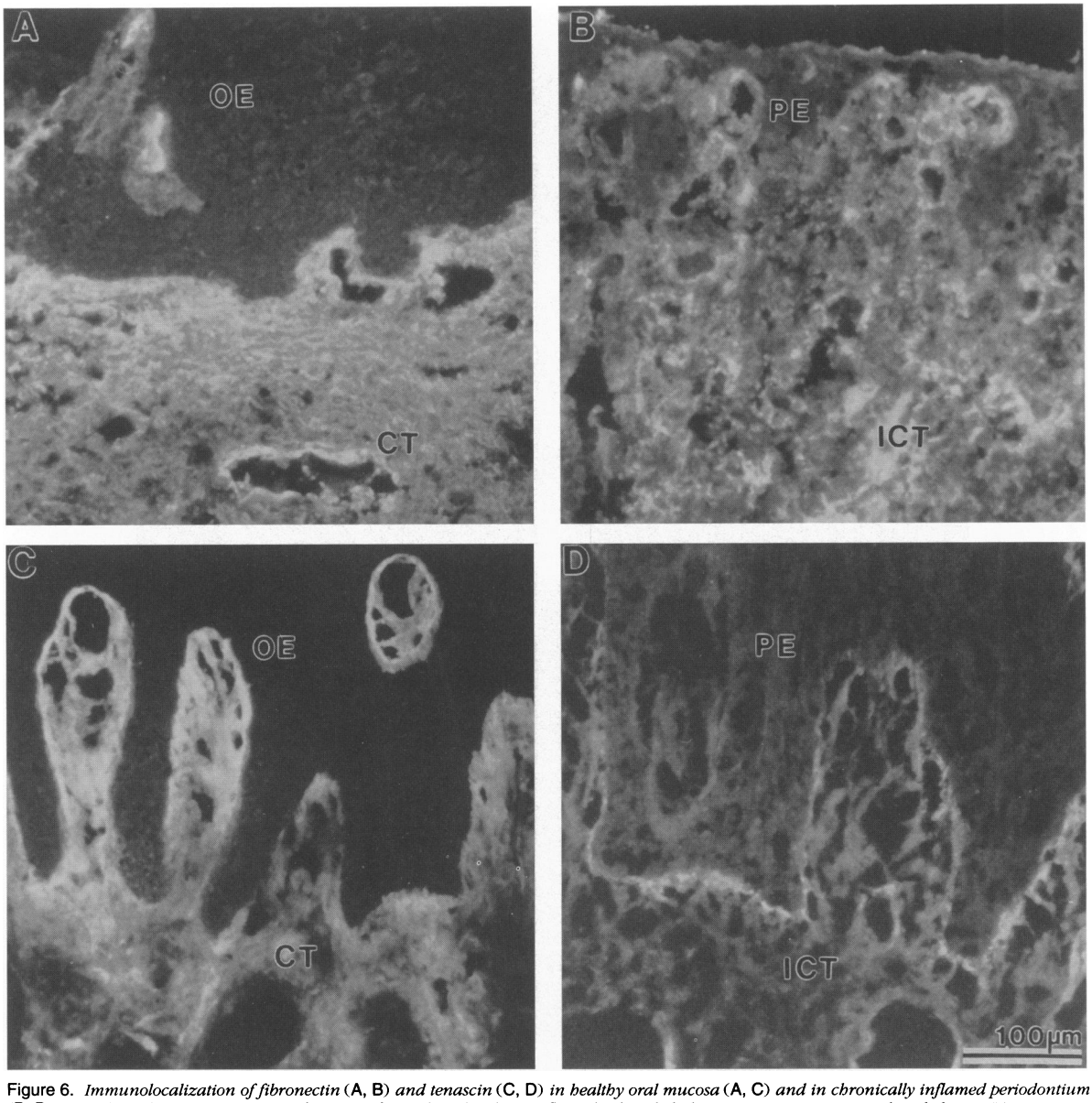

Figure 6. *Immunolocalization of fibronectin* (A, B) *and tenascin* (C, D) *in healthy oral mucosa* (A, C) *and in chronically inflamed periodontium* (B, D). *Note the reduction in intensity of staining for each molecule in inflamed subepithelial connective tissue. OE, oral epithelium, CT, connective tissue; PE, periodontal epithelium in chronic inflammation; ICT, inflamed connective tissue. Bar 100 μm, all figures.*

In areas that showed streak-like distributions of type IV and VII collagen and laminin-5, all cell layers of epithelium were strongly positive for $\beta 1$ integrins. In addition, staining for both $\alpha 6$ and $\beta 4$ integrins was relatively intense.

Members of the αv-integrin family were not detected in epithelia of healthy or inflamed tissue. Attempted immunolocalization with specific antibodies recognizing $\alpha v\beta 5$ and $\alpha v\beta 6$ integrins gave negative results in all samples (data not shown).

Discussion

Epithelial cell adhesion, migration and proliferation have been studied in acute inflammatory conditions such as wound healing (reviewed in ref. 49). However, epithelial cell behavior in chronic inflammatory conditions is much less characterized. Periodontal disease is characterized by chronic inflammation and slowly progressive tissue loss and provides, therefore, an interesting model to study epithelial cell

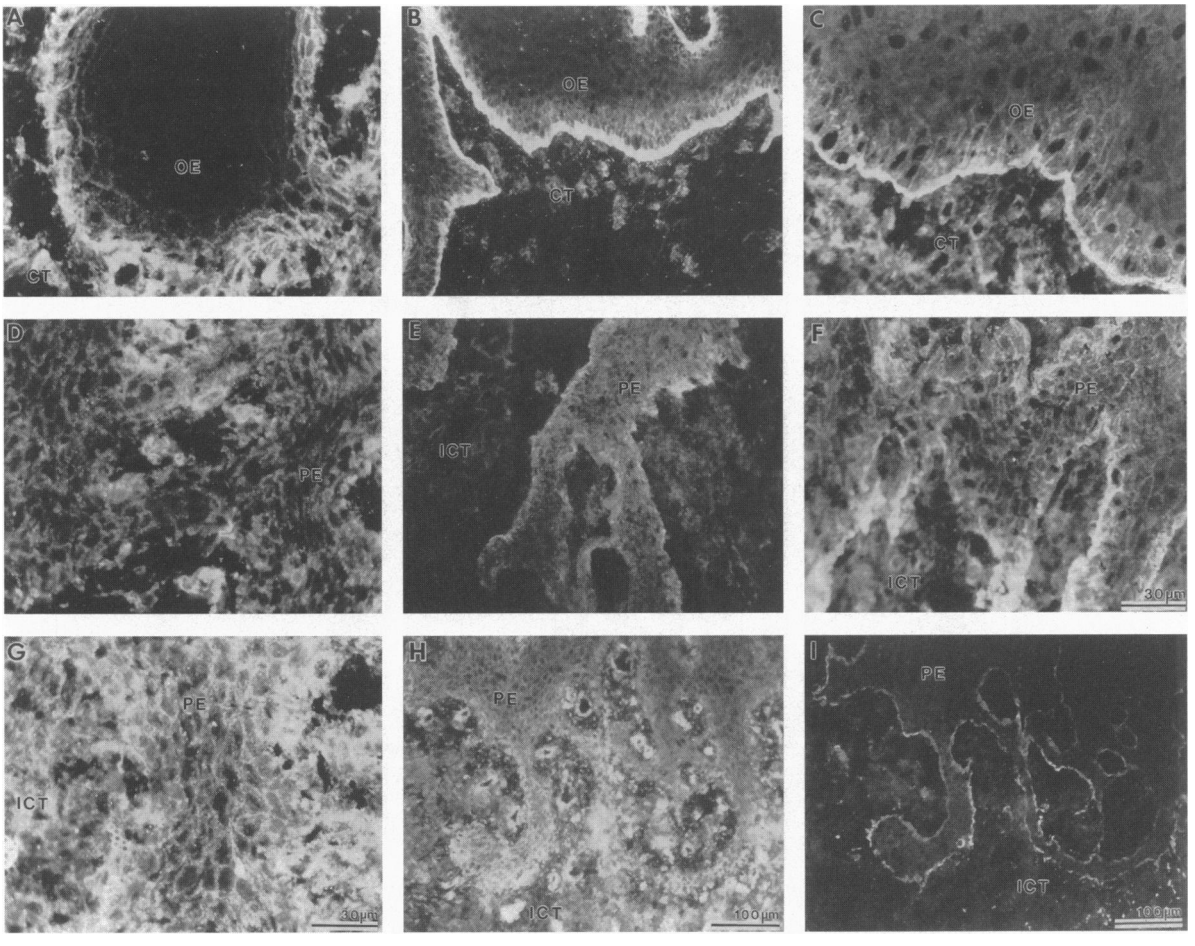

Figure 7. *Immunolocalization of β1-integrin* (A, D, G), *α6-integrin* (B, E), *β4-integrin* (C, F) *in healthy oral epithelium* (A *to* C) *and in chronically inflamed periodontal tissue* (D *to* G). *Double-immunostaining of β1-integrin* (H) *and type VII-collagen* (I) *in inflamed periodontal tissue. OE, oral epithelium; CT, connective tissue; PE, periodontal epithelium in chronic inflammation; ICT, inflamed connective tissue. Bar 30 µm,* A, C, D, F, G; *bar 100 µm,* B, E, H, I.

behavior during chronic inflammation. The epithelium undergoes alterations that lead to ulceration, degeneration, and also increased proliferation and formation of epithelial rete ridges into inflamed connective tissue stroma. Our study demonstrates that major alterations take place in the expression of epithelial adhesion molecules and their receptors in the chronic inflammation of periodontal disease.

General structural alterations of the basement membrane zone in inflammatory periodontal lesions have been previously well characterized. Several studies describe a diffuse appearance,[2] localized discontinuities,[3] or degradation[4,5] of the basement membrane facing inflamed connective tissue. Our study showed major alterations of individual adhesion molecules at the basement membrane zone. Despite extensive loss of subepithelial collagen, most of the matrix components associated with the basement membrane zone were partially retained in chronic inflammation. Generally, all components of the basement membrane zone including type IV collagen and laminin demonstrated reduced staining intensity compared with normal noninflamed tissue. These proteins were, however, better preserved than stromal proteins fibronectin and tenascin, quantities of which were found to be markedly reduced in inflamed areas. *In vitro* experiments have shown that all of the above-mentioned proteins are readily cleaved by matrix metalloproteinases,[50] which are present in high amounts in inflamed tissues.[51] Fragments of fibronectin have been demonstrated directly in fluids collected from sites of chronic inflammation.[52–54] Our results suggest, however, that proteins of the basement membrane may be woven into a meshwork in which protein epitopes persist although proteins are partially degraded. Focal loss of type IV collagen, laminin-1 and laminin-5 was observed in some samples, similar to data obtained in a previous study of type IV collagen

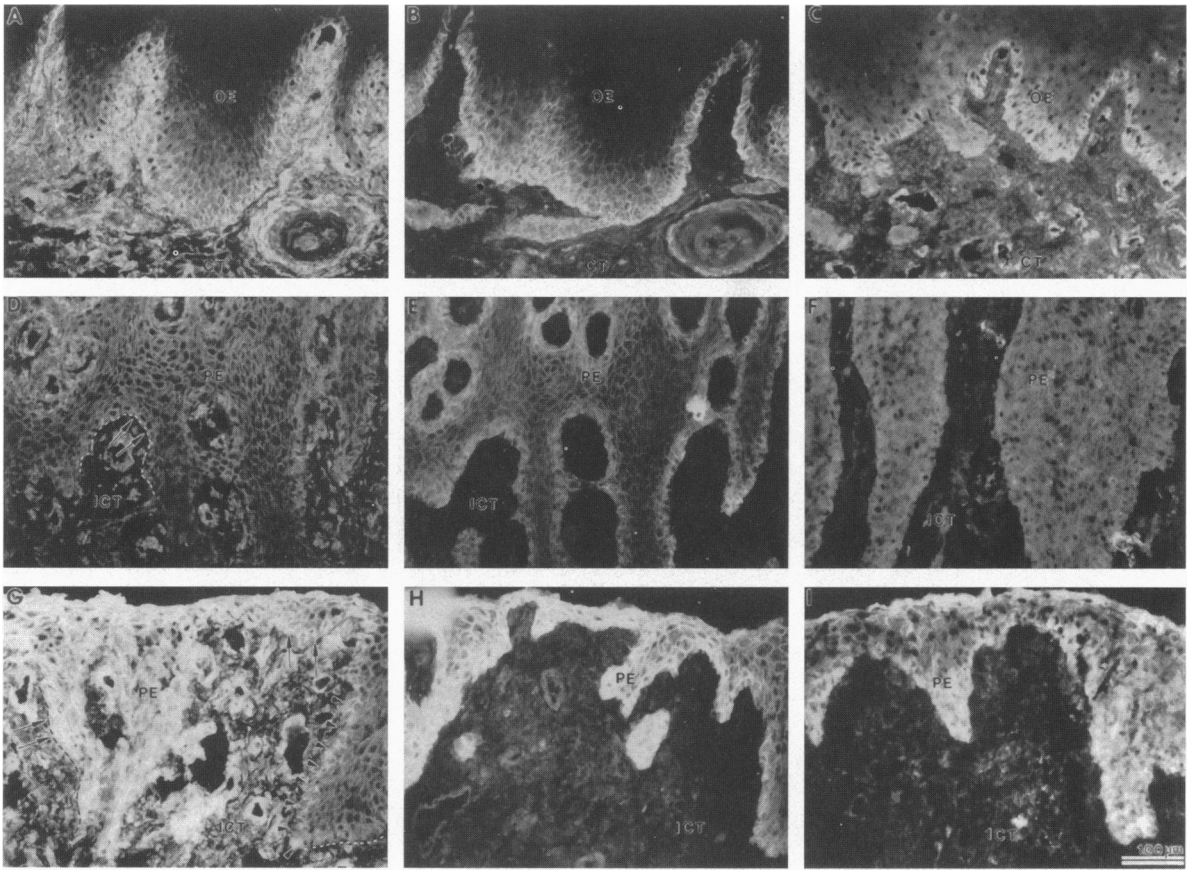

Figure 8. *Immunolocalization of β1* (**A, D, G**), *α3* (**B, E, H**) *and α2* (**C, F, I**) *integrins in healthy oral epithelium* (**A** *to* **C**), *and in periodontal epithelium in chronic inflammation* (**D** *to* **I**). *In inflammation, some areas of periodontal epithelium show focal loss of these integrins* (**D** *to* **F**), *whereas in others the full thickness of inflamed periodontal epithelium is strongly positive for β1, α3, and α2 integrins* (**G** *to* **I**). *Arrows mark epithelial-connective tissue boundary* (*dashed black line,* **G**). *Arrowheads* (**G**) *mark β1 staining that is comparable to normal oral epithelium, and dashed white line indicates area of epithelium that demonstrates a loss of β1 staining. OE, oral epithelium; CT, connective tissue; PE, periodontal epithelium in chronic inflammation; ICT, inflamed connective tissue. Bar 100 μm.*

immunolocalization in inflamed periodontal tissue.[6] In many specimens, however, type VII collagen and to a lesser extent laminin-5 were found in unusual locations in inflamed stroma. It is believed that type VII collagen is synthesized by keratinocytes, although stimulus from fibroblasts seems to be needed for optimal synthesis.[55,56] It is also possible, however, that fibroblasts in chronic inflammation could contribute to collagen VII accumulation, since TGF-β is able to induce type VII collagen expression in fibroblasts.[57]

Chronic lesions of periodontal disease contain large numbers of newly formed capillaries surrounded by basement membrane, as demonstrated in our samples stained with antibodies against laminin-1 and type IV collagen. These basement membrane components associated with new vessels were not found to be degraded at sites of inflammation. In fact, significant amounts of extravascular laminin-1 and to a lesser extent type IV collagen were found in the inflammatory sites. This atypical localization seems to be rather specific to chronic inflammation of periodontal disease and is not found during acute inflammation of wound healing[34] or during chronic inflammation of rheumatoid arthritis,[58] where type IV collagen and laminin are found exclusively in the basement membrane area. Electron microscopic studies have earlier demonstrated the presence of extra layers of basement membrane-like material surrounding the capillaries of inflamed gingival lesions.[59] It is not known which cells deposit this material, but sprouting endothelial cells are candidates for this activity. In addition granulation tissue fibroblasts may be responsible, because they have been found to synthesize basement membrane components *in vivo*.[60] Our findings point to the conclusion that during chronic periodontal inflammation, the basement membrane zone proteins type VII collagen and laminin partly replace the connective tissue stroma. These proteins may then serve as adhesive

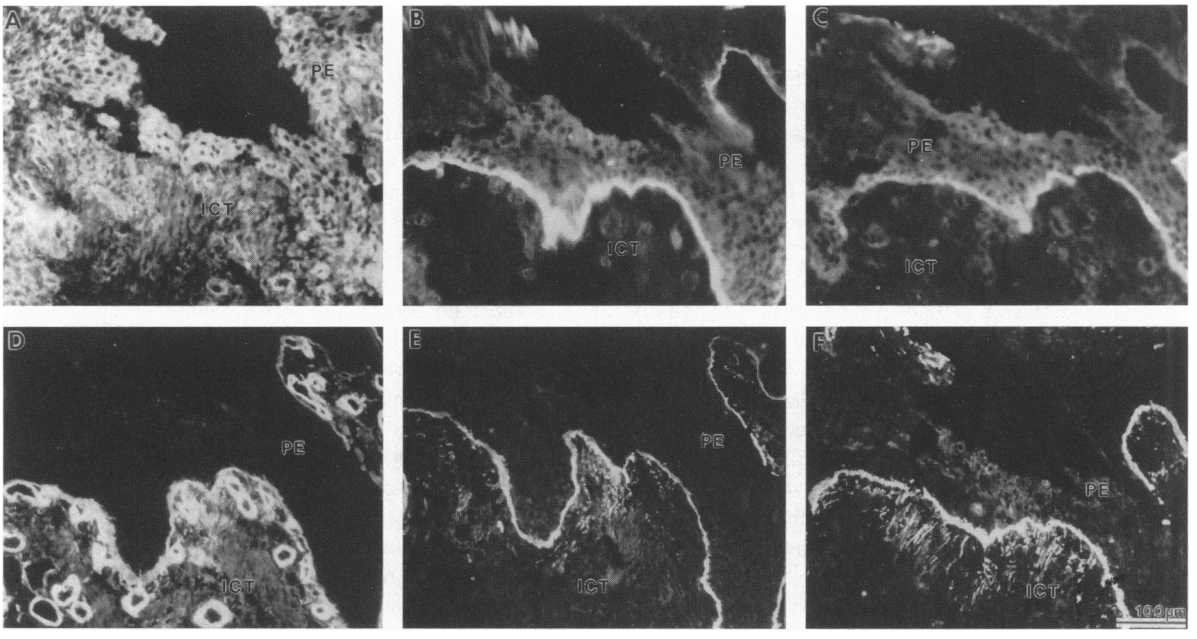

Figure 9. *Immunolocalization of β1* (**A**), *α6* (**B**), *β4* (**C**), *type IV* (**D**), *laminin-5* (**E**), *and type VII* (**F**) *in parallel serial sections of chronically inflamed periodontal tissue. Note the strong expression of β1 integrin* (**A**) *in epithelium and also the streak-like appearance of laminin-5 and type VII collagen* (**E, F**) *in connective tissue. PE, periodontal epithelium in chronic inflammation; ICT, inflamed connective tissue. Bar 100 µm.*

ligands for epithelial cells, granulation tissue fibroblasts and inflammatory cells.

We have shown earlier that β1 integrins localize mainly at lateral borders of basal cells in normal mucosa and skin[16,19,34] and that these integrins could mediate cell-cell adhesion of keratinocytes.[15,61] The hemidesmosomal integrin α6β4 is exclusively localized at the basal aspect of basal cells in normal epithelia. Both of these integrin types are found in an altered distribution surrounding suprabasal cells during wound healing[34,62] and in psoriatic epidermis.[62,63] In the present study, focal loss of integrins was a common finding in epithelium of chronically inflamed periodontal lesions. This loss may result from specific downregulation of integrin expression, since the cells in the same area seemed to deposit normal amounts of basement membrane components. There were, however, also areas in which the expression of both α2β1 and α3β1 integrins appeared to be paradoxically markedly upregulated. Integrin expression is clearly upregulated during wound healing.[34–36,64] Stimulated expression of β1 integrins by keratinocytes has been found in many inflammatory dermal lesions such as psoriasis,[63] lichen,[65,66] and cutaneous lymphoma.[66] In contrast, integrin expression has been reported to be relatively unchanged in dystrophic or systemic diseases such as epidermolysis bullosa[67] and systemic sclerosis.[68] Periodontal lesions are known to contain areas with variable disease involvement as judged by varying gingival surface topography and tissue histology.[69] Cytokines are known to exert potent cell-specific effects on integrin expression.[70–72] It is therefore likely that integrin expression is regulated locally in pocket epithelial cells by combinations of inflammatory cytokines.

In summary, periodontal inflammation involves alterations in both cell adhesion ligands and receptors. Basement membrane zone components are found in an extravascular stromal location providing a new adhesive environment for epithelial, endothelial, and granulation tissue fibroblasts as well as inflammatory cells. Expression of integrins in inflamed tissue is variable, but the major feature is loss of integrins from the epithelial cell surface.

Acknowledgments

During this work, Dr. Kirsi Haapasalmi was the recipient of the Henry M. Thornton/SCADA Fellowship award.

References

1. Page RC, Schroeder HE: Structure and pathogenesis. Periodontal Diseases. Edited by S Schluger, R Yuodelis, RC Page, and RH Johnson. Lea & Febiger, Philadelphia, 1990, pp 183–220

2. Thilander H: Epithelial changes in gingivitis. J Periodont Res 1968, 3:303–312
3. Freedman HL, Listgarten MA, Taichman NS: Electron microscopic features of chronically inflamed human gingiva. J Periodont Res 1968, 3:313–319
4. Takarada H, Cattoni M, Sugimoto A, Rose GG: Ultrastructural studies of human gingiva. II. The lower part of the pocket epithelium in chronic periodontitis. J Periodont Res 1974, 45:155–169
5. Takarada H, Cattoni M, Sugimoto A, Rose GG: Ultrastructural studies of human gingiva. III. Changes of the basal lamina in chronic periodontitis. J Periodont Res 1974, 45:288–302
6. Peng TK, Nisengard RJ, and Levine MJ: The alteration in gingival basement membrane antigens in chronic periodontitis. J Periodontol 1986, 57:20–24
7. Uitto V-J, Larjava H: Extracellular matrix molecules and their receptors: an overview with special emphasis on periodontal tissues. Crit Rev Oral Biol Med 1991, 2:323–354
8. Albelda SM, Buck CA: Integrins and other cell adhesion molecules. FASEB J 1990, 4:2868–2880.
9. Ruoslahti E: Integrins. J Clin Invest 1991, 87:1–5.
10. Hynes RO: Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992, 69:11–25.
11. Schwartz MA: Transmembrane signalling by integrins. Trends Cell Biol 1992, 2:304–308
12. Giancotti FG, Mainiero F: Integrin-mediated adhesion and signalling in tumorigenesis. Biochim Biophys Acta 1994, 1198:47–64
13. Larjava H: Expression of β1 integrins in normal human keratinocytes. Am J Med Sci 1991, 301:63–68
14. Watt FM, Jones PH: Expression and function of the keratinocyte integrins. Development, Suppl 1993, 185–192
15. Larjava H, Peltonen J, Akiyama SK, Yamada SS, Gralnick HR, Uitto J, Yamada KM: Novel function for β1 integrins in keratinocyte cell-cell interactions. J Cell Biol 1990, 110:803–815
16. Peltonen J, Larjava H, Jaakkola S, Gralnick H, Akiyama SK, Yamada SS, Yamada KM, Uitto J: Localization of integrin receptors for fibronectin, collagen, and laminin in human skin: variable expression in basal and squamous cell carcinomas. J Clin Invest 1989, 84:1916–1923
17. Konter U, Kellner I, Klein E, Kaufmann R, Mielke V, Sterry W: Adhesion molecule mapping in normal human skin. Arch Dermatol Res 1989, 281:454–462
18. Hertle MD, Adams JC, Watt FM: Integrin expression during human epidermal development *in vivo* and *in vitro*. Development 1991, 112:193–206
19. Larjava H, Zhou C, Larjava I, Rahemtulla F: Immunolocalization of β1 integrins in human gingival epithelium and cultured keratinocytes. Scand J Dent Res 1992, 100:266–273
20. Jones J, Sugiyama M, Watt FM, Speight PM: Integrin expression in normal, hyperplastic, and malignant oral epithelium. J Pathol 1993, 169:235–243
21. Hodivala KJ, Watt FM: Evidence that cadherins play a role in the downregulation of integrin expression that occurs during keratinocyte terminal differentiation. J Cell Biol 1994, 124:589–600
22. Adams JC, Watt FM: Changes in keratinocytes adhesion during terminal differentiation: reduction in fibronectin binding precedes $\alpha5\beta1$ integrin loss from the cell surface. Cell 1990, 63:425–435
23. Carter WG, Wayner EA, Bouchard TS, Kaur P: The role of integrins $\alpha2\beta1$ and $\alpha3\beta1$ in cell-cell and cell-substrate adhesion of human epidermal cells. J Cell Biol 1990, 110:1387–1404
24. Adams JC, Watt FM: Expression of $\beta1$, $\beta3$, $\beta4$, and $\beta5$ integrins by human epidermal keratinocytes and non-differentiating keratinocytes. J Cell Biol 1991, 115: 829–841
25. Carter WG, Kaur P, Gil SG, Gahr PJ, Wayner EA: Distinct functions for integrins $\alpha3\beta1$ in focal adhesions and $\alpha6\beta4$/bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. J Cell Biol 1990, 111:3141–3154
26. Carter WG, Ryan MC, Gahr PJ: Epiligrin, a new cell adhesion ligand for integrin $\alpha3\beta1$ in epithelial basement membranes. Cell 1991, 65:599–610
27. Larjava H, Lyons JG, Salo T, Mäkelä M, Koivisto L, Birkedal-Hansen H, Akiyama SK, Yamada KM, Heino J: Anti-integrin antibodies induce type IV collagenase expression in keratinocytes. J Cell Physiol 1993, 157: 190–200
28. Jones JCR, Kurpakus MA, Cooper HM, Quaranta V: A function for the integrin $\alpha6\beta4$ in the hemidesmosome. Cell Regul 1991, 2:427–438
29. Kurpakus MA, Quaranta V, Jones JCR: Surface relocation of $\alpha6\beta4$ integrins and assembly of hemidesmosomes in an *in vitro* model of wound healing. J Cell Biol 1991, 115:1737–175030.
30. Sonnenberg A, Calafat J, Janssen H, Daams H, van der Raaij-Hemler LMH, Falcioni R, Kennel SJ, Aplin JD, Baker J, Loizidou M, Garrod, D: Integrin $\alpha6\beta4$ complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J Cell Biol 1991, 113:907–917
31. Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK: $\alpha6\beta4$ integrin heterodimer is a component of hemidesmosomes. Proc Natl Acad Sci USA 1990, 87: 8970–8974
32. Rousselle P, Lunstrum GP, Keene DR, Burgeson RE: Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J Cell Biol 1991, 114:567–576
33. Marchisio PC, Bondanza S, Cremona O, Cancedda R, De Luca M: Polarized expression of integrin receptors ($\alpha6\beta4$, $\alpha2\beta1$, $\alpha3\beta1$, and $\alpha V\beta5$) and their relationship with the cytoskeleton and basement membrane matrix in cultured human keratinocytes. J Cell Biol 1991, 112: 761–773
34. Larjava H, Salo T, Haapasalmi K, Kramer RH, Heino J: Expression of integrins and basement membrane

components by wound keratinocytes. J Clin Invest 1993, 92:1425–1435
35. Cavani A, Zambruno G, Marconi A, Manca V, Marchetti M, Giannetti A: Distinctive integrin expression in the newly forming epidermis during wound healing in humans. J Invest Dermatol 1993, 101:600–604
36. Juhasz I, Murphy GF, Yan HC, Herlyn M, Albelda SM: Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing *in vivo*. Am J Pathol 1993, 43:1458–1469
37. Culling CFA: Handbook of Histopathological Techniques, ed. 2. Butterworths, London 1963, pp 230–231
38. Kantor RRS, Mattes MJ, Lloyd KO, Old LJ, Albino AP: Biochemical analysis of two cell surface glycoprotein complexes, very common antigen 1 and very common antigen 2. J Biol Chem 1987, 262:15158–15165
39. Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH: Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. J Cell Biol 1989, 109:877–889
40. Sonnenberg A, Hogervorst F, Osterop A, Veltman FEM: Identification and characterization of a novel antigen complex on mouse mammary tumor cells using a monoclonal antibody against platelet glycoprotein IIc. J Biol Chem 1988, 263:14030–14038
41. Wayner EA, Orlando RA, Cheresh DA: Integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ contribute to cell attachment to vitronectin but differentially distribute on the cell surface. J Cell Biol 1991, 113:919–929
42. Roberts CJ, Birkenmeier TM, McQuillan JJ, Akiyama SK, Yamada SS, Chen W-T, Yamada KM, McDonald JA: Transforming growth factor β stimulates the expression of fibronectin and of both subunits of the human fibronectin receptor by cultured human lung fibroblasts. J Biol Chem 1988, 263:4586–4592
43. Kennel SJ, Foote LJ, Falcioni R, Sonnenberg A, Stringer CD, Crouse C, Hemler ME: Analysis of the tumor-associated antigen TSP-180. J Biol Chem 1989, 264:15515–15521
44. Weinacker A, Chen A, Agrez M, Cone RI, Nishimura S, Wayner E, Pytela R, Sheppard D: Role of integrin $\alpha v\beta 6$ in cell attachment to fibronectin: heterologous expression of intact and secreted forms of the receptor. J Biol Chem 1994, 269:6940–6948
45. Risteli L, Timpl R: Isolation and characterization of pepsin fragments of laminin from human placental and renal basement membranes. Biochem J 1981, 193:749–755
46. Verrando P, Hsi BL, Yeh C-J, Pisani A, Serieys N, Ortonne J-P: Monoclonal antibody GB3, a new probe for the study of human basement membranes and hemidesmosomes. Exp Cell Res 1987, 170:116–128
47. Chen W-T, Hasegawa E, Hasegawa T, Weinstock C, Yamada KM: Development of cell surface linkage complexes in cultured fibroblasts. J Cell Biol 1985, 100:1103–1114
48. Keene DR, Sakai LY, Lundstrum GP, Morris NP, Burgeson RE: Type VII collagen forms an extended network of anchoring fibrils. J Cell Biol 1987, 104:611–616
49. Stenn KS, Malhotra R: Epithelialization. Wound Healing: Biochemical and Clinical Aspects. Edited by IK Cohe, RF Diegelmann, WJ Lindblad. WB Saunders Co., Philadelphia, 1992, pp 115–127
50. Birkedal-Hansen H: Role of matrix metalloproteinases in human periodontal diseases. J Periodontol 1993, 64:474–484
51. Mäkelä M, Salo T, Uitto V-J, Larjava H: Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relationship to periodontal status. J Dent Res 1994, 73:1397–1406
52. Carsons S, Lavietes BB, Diamond HS, Kinney SG: The immunoreactivity, ligand, and cell binding characteristics of rheumatoid synovial fluid fibronectin. Arthritis Rheum 1985, 28:601–612
53. Talonpoika J, Heino J, Larjava H, Häkkinen L, Paunio K: Gingival crevicular fluid fibronectin degradation in periodontal health and disease. Scand J Dent Res 1989, 97:415–421
54. Wysocki AB, Grinnell F: Fibronectin profiles in normal and chronic wound fluid. Lab Invest 1990, 63:825–831
55. Regauer S, Seiler GR, Barrandon Y, Easley KW, Compton CC: Epithelial origin of cutaneous anchoring fibrils. J Cell Biol 1990, 111:2109–2115
56. König A, Bruckner-Tuderman L: Epithelial-mesenchymal interactions enhance expression of collagen VII *in vitro*. J Invest Dermatol 1991, 96:803–808
57. König A, Bruckner-Tuderman L: Transforming growth factor-β stimulates collagen VII expression by cutaneous cells *in vitro*. J Cell Biol 1992, 117:679–685
58. Nikkari L, Aho H, Yli-Jama T, Larjava H, Jalkanen M, Heino J: Expression of integrin family of cell adhesion receptors in rheumatoid synovium. $\alpha 6$ integrin subunit in normal and hyperplastic synovial lining cell layer. Am J Pathol 1993, 142:1019–1027
59. Gavin JB: Ultrastructural features of chronic marginal gingivitis. J Periodont Res 1970, 5:19–29
60. Betz P, Nerlich A, Wilske J, Tübel J, Wiest I, Penning R, Eisenmenger W: Time-dependent pericellular expression of collagen type IV, laminin, and heparan sulfate proteoglycan in myofibroblasts. Int J Legal Med 1992, 105:169–172
61. Carter WG, Wayner EA, Bouchard TS, Kaur P: The role of $\alpha 2\beta 1$ and $\alpha 3\beta 1$ in cell-cell and cell-substrate adhesion of human epidermal cells. J Cell Biol 1990, 110: 1387–1404
62. Hertle MD, Kubler M-D, Leigh IM, Watt FM: Aberrant integrin expression during epidermal wound healing and in psoriatic epidermis. J Clin Invest 1992, 89: 1892–1901
63. Pellegrini G, De Luca M, Orecchia G, Balzac F, Cremona O, Savoia P, Cancedda R, Marchisio PC: Expression, topography, and function of integrin receptors are severely altered in keratinocytes from involved

and uninvolved psoriatic skin. J Clin Invest 1992, 89: 1783–1795
64. Clark RAF: Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. J Invest Dermatol 1990, 96:128S-134S
65. Konter U, Kellner I, Hoffmeister B, Sterry W: Induction and upregulation of adhesion receptors in oral and dermal lichen planus. J Oral Pathol Med 1990, 19: 459–463
66. Ralfkiaer E, Thomsen K, Vejlsgaard GL: Expression of a cell adhesion protein (VLA β) in normal and diseased skin. Br J Dermatol 1991, 124:527–532
67. Nazzaro V, Berti E, Cerri A, Brusasco A, Cavalli R, Caputo R: Expression of integrins in junctional and dystrophic epidermolysis bullosa. J Invest Dermatol 1990, 95:60–64
68. Sollberg S, Peltonen J, Uitto J, Jimenez SA: Elevated expression of β1 and β2 integrins, intercellular adhesion molecule 1, and endothelial leukocyte adhesion molecule 1 in the skin of patients with systemic sclerosis of recent onset. Arthritis Rheum 1992, 35: 290–298
69. Saglie R, Carranza FA Jr, Newman MG, Pattison GA: Scanning electron microscopy of the gingival wall of deep periodontal pockets in humans. J Periodont Res 1982, 17:284–293
70. Heino J: Integrin-type extracellular matrix receptors in cancer and inflammation. Ann Med 1993, 25:335–342
71. Heino J, Ignotz RA, Hemler ME, Crouse C, Massagué J: Regulation of cell adhesion receptors by transforming growth factor-β. Concomitant regulation of integrins that share a common β1 subunit. J Biol Chem 1989, 262:380–388
72. Milam SB, Magnuson VL, Steffensen B, Chen D, Klebe RJ: IL-1 β and prostaglandins regulate integrin mRNA expression. J Cell Physiol 1991, 149:173–183