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Expression of fibronectin-binding integrins in gingival epithelium in drug-induced gingival overgrowth

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Background and Objective: Gingival overgrowth is a side-effect of nifedipine and cyclosporin medications. Integrins are transmembrane glycoproteins that mediate cell adhesion, regulate cell proliferation and participate in the regulation of tissue fibrosis. The aim of this study was to investigate whether expression of epithelial cell integrins is linked to the development of drug-induced gingival overgrowth.

Material and Methods: Human gingival biopsies of patients taking nifedipine, cyclosporin, or a combination of both medications, were used. Expression of the α5β1, αvβ1 and αvβ6 integrins, and of cellular extra domain A of fibronectin, was localized in frozen sections using immunohistochemistry.

Results: The activated conformation of the β1, α5β1 and αvβ6 integrins were more frequently expressed in distinct locations in the oral epithelium in the combined drug group. Cellular extra domain A of fibronectin, a ligand for both α5β1 and αvβ6 integrins, was expressed within the connective tissue of all groups. It was also expressed around the basal keratinocytes of the control, nifedipine and cyclosporin-induced gingival overgrowth groups, but not in the combined medication group. No relationship between the presence of inflammation and integrin expression was found.

Conclusion: The results indicate that expression of certain integrins is up-regulated in the epithelium of drug-induced gingival overgrowth where they could participate in controlling the formation of elongated rete ridges and tissue fibrosis.

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Nifedipine is a calcium-channel blocker that is used in the treatment of angina and hypertension. The incidence of gingival overgrowth in patients taking nifedipine is ≈6–20% (1,2). Cyclosporin A is an immunosuppressant used to prevent graft rejection in organ transplantation, and it also has therapeutic uses in immunoregulatory disorders (3). The occurrence of cyclosporin-induced overgrowth is ≈30% (2). Combined nifedipine + cyclosporin A therapy produces more overgrowth than if patients are taking either medication alone (2).

The histological features of nifedipine and/or cyclosporin A-induced gingival overgrowth tissue have been described in humans and show similarities at the light microscopic level (4–6). Gingival biopsies show acanthosis of oral epithelium, evident as elongated rete ridges, hyper- and/or parakeratosis. The surface density of epithelial ridges in cyclosporin A-induced gingival overgrowth is markedly elevated when compared with control biopsies. The width of stratum spinosum, and the number of cells in the middle section of the epithelial lining, is greater than in normal gingiva. The mitotic activity of basal keratinocytes is also increased in drug-induced overgrowth (7). Fibrosis in lamina propria with an increased amount of collagen and fibroblasts,

some increase in vascularity and perivascular inflammation have been described (5,6). Increased noncollagenous matrix has been described in cyclosporin A-induced gingival overgrowth (6).

Integrins are cell-surface glycoproteins that mediate cell adhesion, control cell proliferation and regulate gene expression (8). During wound healing and inflammatory disease (such as psoriasis), integrin expression is altered in the epithelial keratinocytes (9). These new integrins are used for cell adhesion and migration, and probably also regulate cell growth, the inflammatory reaction and extracellular matrix production of connective tissue cells (9). Fibronectin-binding integrins are particularly interesting in this context (10). Keratinocytes of oral gingival epithelium do not normally express fibronectin-binding integrins. Expression of α5β1, αvβ1 and αvβ6 integrins, all of which are able to bind fibronectin, is induced during wound healing (11,12). Integrins αvβ1 and αvβ6 have also been demonstrated to bind and activate latent transforming growth factor-β and they regulate fibrosis in experimental models (13). The goal of the present study was to determine whether expression and distribution of keratinocyte integrins is altered in drug-induced gingival overgrowth tissue compared with normal gingiva.

Material and methods

Human gingival tissue biopsies were collected from 19 control patients (no medication), from 12 nifedipine-treated cardiac outpatients and from 22 immunosuppressant-treated organ transplant patients (11 patients taking cyclosporin and 11 patients taking both cyclosporin and nifedipine). The cardiac outpatients were referred by their physician or dentist for periodontal surgery as a result of nifedipine-induced gingival overgrowth. The immunosuppressant-treated organ transplant patients were treated according to the treatment protocol followed by organ transplant patients in the Oulu University Hospital, Finland. The immunosuppressive treatment of the transplant patients consisted of triple medication (cyclosporin A + methylprednisolone + azathioprine) in 14 patients and double medication (cyclosporin + methylprednisolone or cyclosporin + azathioprine) in five patients. All patients had moderate to severe gingival overgrowth, which extended between the middle third and coronal two-thirds of the clinical crown (14). The mean whole-blood cyclosporin A concentration was determined according to values measured during the six months before gingival biopsy. The blood tests were standardized as morning values before cyclosporin A medication. All procedures were approved by the Ethical Committee of Human Experimentation of the University of Oulu, Finland, and the patients gave their informed consent to participate in this investigation. Patients were placed into four groups according to medication: (i) immunosuppression; (ii) combined drug (immunosuppression + nifedipine) (iii); nifedipine alone; and (iv) control group. Patient characteristics are illustrated in Table 1. Gingival biopsies were taken during the required gingivectomy procedures. The biopsies were rinsed in physiological saline, embedded in Tissuetek (Miles Inc., Elkhart, IN, USA) and immediately frozen in liquid nitrogen. The tissue samples were stored at −70°C until the frozen sections were prepared. Serial cryostat sections (5 μm) were prepared at −25°C from each specimen in order to obtain cross-sections of the oral and sulcular epithelium and connective tissue. The sections were collected on 3-aminopropyltriethoxysilane-coated (Fluka Chemie, Buchs, Switzerland) glass slides and stored at −70°C until used.

Histological analysis

One section of each specimen was stained with hematoxylin and eosin to allow analyses of tissue morphology (degree of acanthosis and thickness of the epithelium) and degree of inflammation. The criterion for acanthosis was based on the definition that acanthosis is the abnormal thickening of the spinous layer for a particular location of the epithelium. Severe acanthosis was defined as elongation, thickening, blunting and confluence of the rete pegs (15). The histological evaluations were performed using a Zeiss Axiolab E microscope (Carl Zeiss, Jena, Germany).

Immunofluorescence stainings

The frozen sections were used for immunolocalization of integrins and their putative ligands. Immunofluorescence stainings were performed as

Table 1. Patient characteristics

	Immunosuppression $n = 11$	Immunosuppression and nifedipine $n = 11$	Nifedipine $n = 12$	Control $n = 19$
Gender (men/women)	7/4	9/2	8/4	9/10
Age (yr)	44 ± 2	36 ± 3	42 ± 4	47 ± 2
Duration of medication (mo)				
Nifedipine	–	29.8 ± 4.9[a]	18.4 ± 3.7	–
Immunosuppression	29.2 ± 7.5	29.2 ± 4.5	–	–
Daily oral dose of medication (mg)				
Nifedipine	–	40.0 ± 5.2	54.2 ± 4.3	–
Cyclosporin A	158 ± 24	225 ± 18[b]	–	–
Methylprednisolone	4.7 ± 1.4	5.0 ± 1.3	–	–
Azathioprine	42.1 ± 10.1	52.3 ± 8.6	–	–
Mean whole blood concentration of cyclosporin A (μg/ml)	132 ± 12	127 ± 16	–	–

[a] $p = 0.079$ (Mann–Whitney U-test).
[b] $p = 0.012$ (Mann–Whitney U-test).
Values represent means ± standard error of the mean, except in relation to gender.

previously described (11). Sections were incubated with phosphate-buffered saline (PBS), containing 1 mg/ml bovine serum albumin (BSA), at room temperature for 60 min. Primary antibodies to α5 (mAb 1986; Chemicon, Temecula, CA, USA) (16), αv (mAb L230; School of Dentistry, University of Oulu, Oulu, Finland) (17), β1 (National Institutes of Health, Bethesda, MD, USA) (18), active β1 (mAb 2079Z; Chemicon) (19) and β6 (mAb E7P6; Chemicon) (20) integrins, and to the extra domain A of fibronectin (MAS521; Harlan Sera-Laboratory Limited, Loughborough, UK) (21), were diluted in PBS/BSA, added to the sections and incubated at 4°C for a minimum of 12 h. The sections were then washed twice for 5 min with PBS/BSA and then incubated with appropriate Alexa 546-conjugated affinity-purified secondary antibodies (dilution 1 : 100; Molecular Probes, Inc., Eugene, OR, USA) at room temperature for 60 min. Sections were then washed twice with PBS/BSA for 5 min, rinsed with distilled water, air dried and mounted using cyanoacrylate adhesive (Borden Co., Willowdale, ON, Canada). The sections were then examined using a Zeiss Axioskop 20 fluorescence microscope and photographed using an MC 80 Zeiss microscope camera (Carl Zeiss). Control stainings for nonspecific antibody binding were performed using nonimmune serum instead of the primary antibody (Chemicon) or by omitting the primary antibody incubation step. All control specimens stained were negative.

Statistical analysis

Before statistical analysis, normalities of the data distributions were tested using the Shapiro–Wilks procedure. Significances of differences in patient characteristics between the groups were determined with the Kruskall–Wallis test, and significances of differences between pairs of results were tested with the Mann–Whitney *U*-test. Significances of differences in distributions of integrins between the groups were determined with one-way analysis of variance (ANOVA) and Dunnett's post-test. Values of $p < 0.05$ were considered to be statistically significant. The calculations were made using the SPSS® for Windows statistical package.

Results

Dose and duration of drug usage

The four patient groups were similar with respect to the average age and gender distribution. Eleven of the transplant recipients were taking nifedipine. The duration of nifedipine medication was longer in the immunosuppression and nifedipine group than in the nifedipine-only group, but the mean oral dose of nifedipine was similar in both groups. Among transplant recipients, no intergroup differences were found with respect to the duration of cyclosporin A medication, the mean whole-blood cyclosporin A concentration, or the mean oral doses of methylprednisolone and azathioprine medication. The mean oral dose of cyclosporin A was significantly higher in the immunosuppression + nifedipine group when compared with the immunosuppression-only group ($p < 0.012$) (Table 1).

Histological analysis

Rete ridge length and epithelial thickness was calculated from microscopic fields of all the specimens. Both the epithelial thickness and elongation of rete ridges were increased in the drug-induced specimens compared with controls. There were, however, no differences between the different groups of medication (Table 2). The degree of acanthosis ranged from mild to moderate for the control specimens, whereas the drug-induced specimens had a degree of acanthosis ranging from moderate to severe (data not shown). There was no clear difference in the degree of inflammation adjacent to the oral epithelium between the control and medication groups (data not shown).

Expression of α5β1, αvβ6 and αvβ1 integrins

The integrins were documented as being present or absent in both localized or generalized areas of the oral epithelium of the papillary gingiva of the control and drug-induced overgrowth groups. In addition, integrin expression in the basal and/or suprabasal cell layers was recorded. The α5β1 integrin was expressed in the basal keratinocytes in 31% of the control specimens (Table 3, Fig. 1). The expression frequency was not significantly different in the nifedipine or immunosuppression groups. However, basal keratinocytes in the combined medication group demonstrated expression of α5β1 integrin in almost all specimens (91%; $p < 0.01$). There was no expression of this integrin in the suprabasal cell layers, except in one specimen of the combined drug group. The α5β1 integrin was similarly expressed in the connective tissue and endothelial cells in all specimens.

All β1 integrins (keratinocyte α2β1, α3β1, α5β1 and αvβ1) were then localized using a specific antibody to the β1 integrin subunit. Integrins can be present in cells in inactive or active (ligand-binding) conformations. Therefore, we also used another β1

Table 2. Rete ridge length, epithelial thickness and acanthosis of the samples

Type f tissue	Average length of rete ridge (μm)	Average epithelial thickness (μm)	Degree of acanthosis
Control	26 ± 11.53	21 ± 14.65	Mild to moderate
Cyclosporin	49 ± 20.71*	29 ± 12.21	Moderate to severe
Nifedipine	40 ± 10.22*	32 ± 18.64*	Moderate to severe
Cyclosporin + nifedipine	40.5 ± 14.1*	28 ± 14.21	Moderate to severe

*$p < 0.05$, significant at a level of 95% confidence interval.

Table 3. Expression of α5, β1, and β1 active integrin in oral epithelium of gingival overgrowth tissue

	α5		β1		β1 active	
Group	Basal	Suprabasal	Basal	Suprabasal	Basal	Suprabasal
Control	4/13 (31%)	0/13 (0%)	19/19 (100%)	0/19 (0%)	14/14 (100%)	2/14 (14%)
Nif	2/10 (20%)	0/10 (0%)	11/12 (92%)	2/12 (17%)	9/12 (75%)	1/12 (8%)
IS	2/11 (18%)	0/11 (0%)	11/11 (100%)	1/11 (9%)	9/10 (90%)	0/11 (0%)
IS + Nif	10/11 (91%)**	1/11 (9%)	11/11 (100%)	6/11 (55%)**	11/11 (100%)	6/11 (55%)*

The values represent summative frequencies of either local or generalized expression in each group.
IS, immunosuppression; Nif, nifedipine.
*$p < 0.05$; **$p < 0.01$

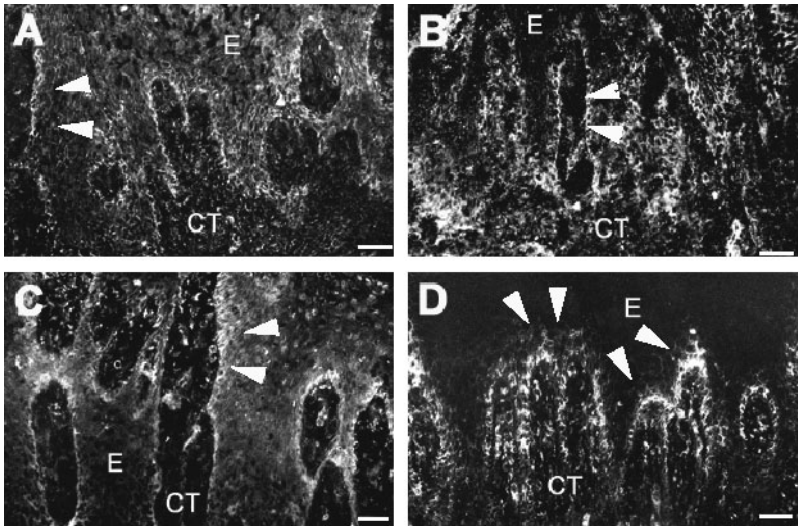

Fig. 1. Distribution of α5β1 integrin in specimens (only positively stained specimens are selected) representing control papillary gingiva (A), nifedipine overgrowth (B), cyclosporin A overgrowth (C) or combined drug overgrowth (D). Arrowheads indicate the areas of strong α5β1 integrin expression in the basal cell layer. Some connective tissue cells are also positive. The expression for this integrin was rare in control and single-drug regimen groups, but frequent in the combination-drug group. CT, connective tissue; E, oral epithelium. Bar, 20 μm.

integrin antibody that specifically recognizes the activated β1 integrin (19). As expected, practically all basal keratinocytes, in all specimens, expressed β1 integrins (Table 3). There was, however, a higher frequency of β1 integrin expression in the suprabasal cell layers in the combined drug group compared with controls ($p < 0.01$). These integrins appeared to be in the active form as they were recognized by the antibody reactive to the active conformation of β1 integrins (Table 3).

The αvβ6 integrin was expressed with high frequency (79–100%) in all groups in the basal cell layer of the oral epithelium (Table 4, Fig. 2). Although basal keratinocytes of the control tissue expressed αvβ6 integrin, its expression was often less generalized and weaker than in the drug-induced gingival-overgrowth groups (Fig. 2). In addition, the αvβ6 integrin was present in the suprabasal layers of the oral epithelium of the combined drug group, while it was practically absent in the suprabasal layers of the other groups ($p < 0.01$).

The αv integrin can combine with the β6, β5 or β1 subunit in keratinocytes (9). No clear staining was obtained in any of the samples using an antibody against the β5 integrin subunit (not shown). Distribution of the αv integrin was often much more extensive than that of the αvβ6 integrin, suggesting that the αv integrin could pair with another β-subunit. The only possible pairing β-subunit that was localized in the αv integrin-expressing cells was the β1-subunit, which is known to be present in

Table 4. Expression of αv, β6 integrin and extra domain A of fibronectin (EDA fibronectin) in oral epithelium of gingival overgrowth tissue.

	αv		β6		EDA-fibronectin	
Group	Basal	Suprabasal	Basal	Suprabasal	Epithelium	CT
Control	15/19 (79%)	1/19 (5%)	11/14 (79%)	0/14 (0%)	6/15 (40%)	13/15 (87%)
Nif	11/12 (92%)	1/12 (8%)	12/12 (100%)	1/12 (8%)	5/12 (42%)	10/12 (83%)
IS	10/11 (91%)	4/11 (36%)	9/10 (90%)	0/10 (0%)	5/11 (45%)	8/11 (72%)
IS + Nif	10/11 (91%)	3/11 (27%)	9/11 (82%)	4/11 (36%)**	0/11 (0%)**	11/11 (100%)

The values represent summative frequencies of either local or generalized expression in each group.
CT, connective tissue; IS, immunosuppression; Nif, nifedipine.
**$p < 0.01$.

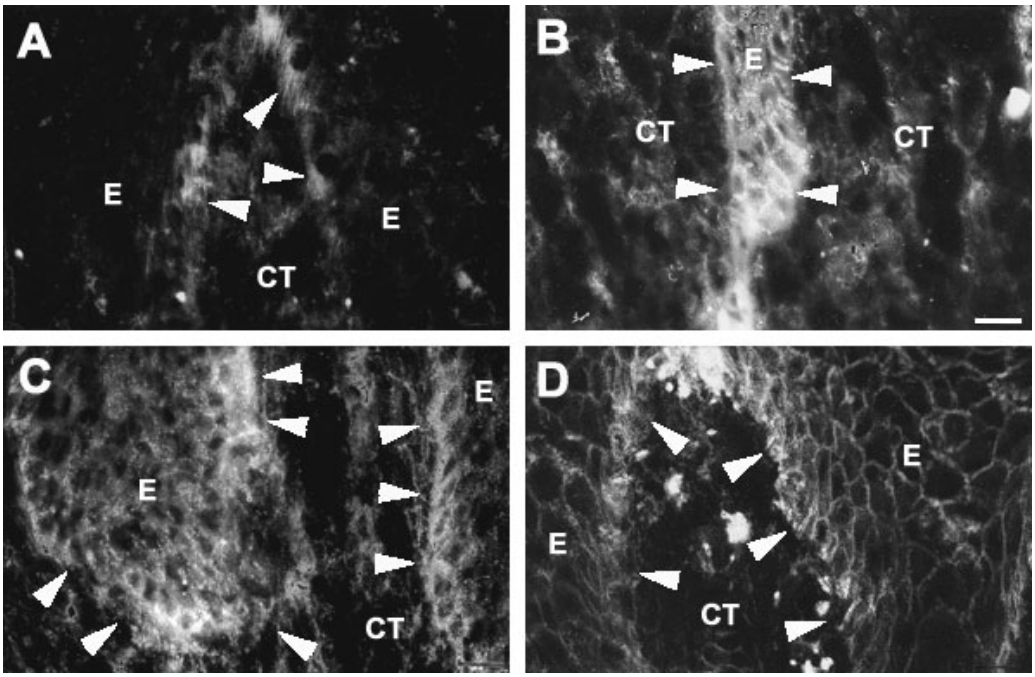

Fig. 2. Localization of αvβ6 integrin in specimens representing control papillary gingiva (A), nifedipine overgrowth (B), cyclosporin A overgrowth (C) or combined drug overgrowth (D). Arrowheads indicate keratinocytes expressing αvβ6 integrin. Note relatively weak expression in the control tissue, and strong basal and suprabasal expression in the combined drug group. CT, connective tissue; E, oral epithelium. Bar, 20 μm.

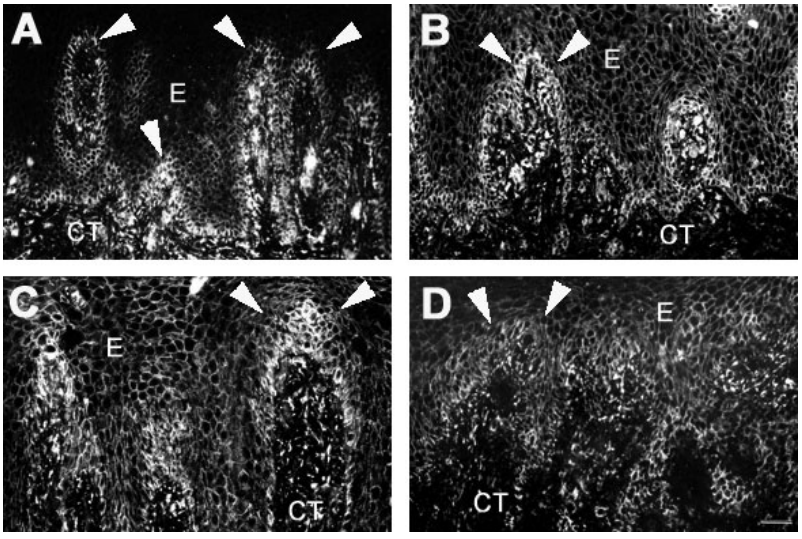

Fig. 3. Distribution of αv integrins in specimens representing control papillary gingiva (A), nifedipine overgrowth (B), cyclosporin A overgrowth (C) or combined drug overgrowth (D). Arrowheads indicate the generalized expression of αv integrins in basal keratinocytes of the oral epithelium. Some connective tissue cells are also positive. Note the suprabasal expression of αv integrin in (B–D). CT, connective tissue; E, oral epithelium. Bar, 20 μm.

keratinocytes (22). Generalized expression of the αv integrin in the basal keratinocytes was seen in most samples (79–92%; Table 4, Fig. 3), regardless of the medication. However, there was a tendency for a higher frequency of αv integrin expression in the suprabasal layers of the epithelium of groups with immunosuppressive medication.

Expression of EDA-fibronectin

Cellular EDA-fibronectin was expressed with a high frequency (40–45%) around the basal keratinocytes of the oral epithelium in all the groups except the combined drug group (0%, $p < 0.01$; Table 4, Fig. 4). Typically, EDA-fibronectin decorated the entire cell periphery, but was also often present at the basement membrane zone (Fig. 4). To rule out basement membrane defects in these specimens, we stained them with antibodies to type IV collagen and laminin-5 and found that these components of the basement membrane were present in all specimens (data not shown). The connective tissue distribution of EDA-fibronectin was similar in all groups (72–100%; Table 4).

Discussion

The pathogenesis of drug-induced gingival overgrowth is multifactorial. Genetic predisposition, pharmacokinetic variables and the presence of dental plaque may all contribute to the changes seen in the gingival tissue.

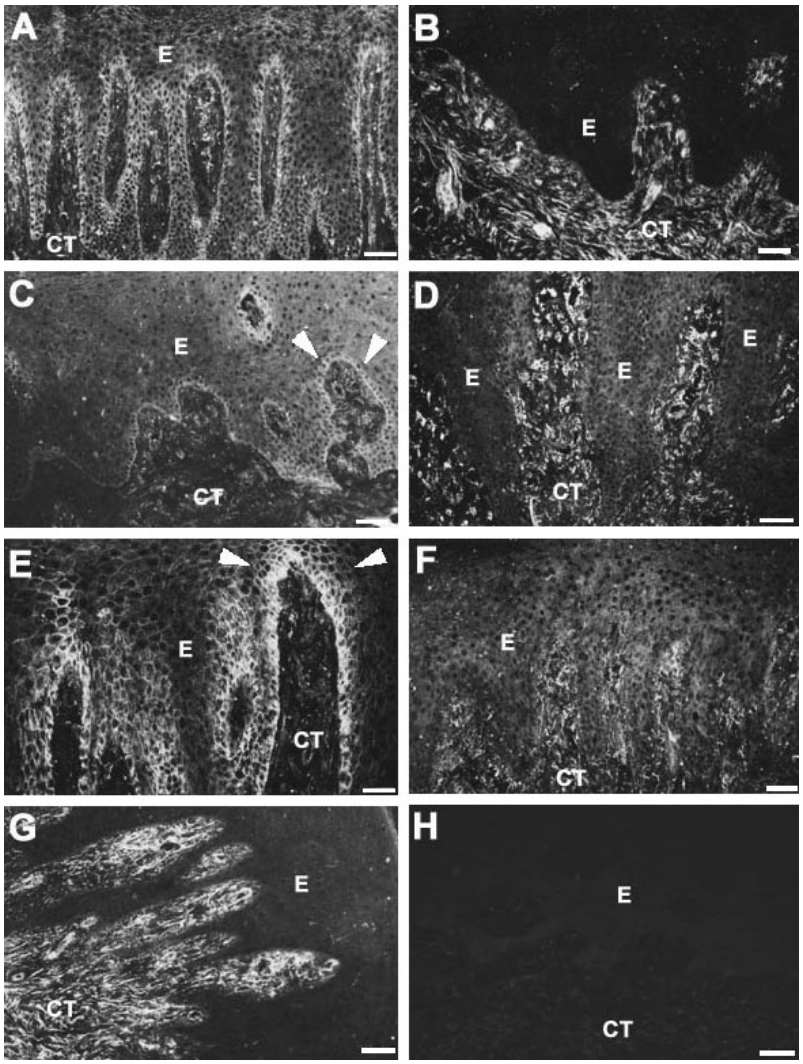

Fig. 4. Localization of extra domain A of fibronectin (EDA-fibronectin) in specimens representing control papillary gingiva (A,B), nifedipine overgrowth (C,D), cyclosporin A overgrowth (E,F) or combined drug overgrowth (G). Panels A, C and E represent specimens in which EDA-fibronectin was expressed in basal keratinocytes. Panels B, D, F and G represent specimens in which EDA-fibronectin was expressed in connective tissue only. Panel H represents a specimen immunostained with nonimmune serum. Arrowheads indicate keratinocytes expressing EDA-fibronectin in basal keratinocytes. CT, connective tissue; E, oral epithelium. Bar, 20 μm.

Many previous studies have concentrated on the alterations in gingival connective tissue homeostasis in drug-induced overgrowth. Cyclosporin appears to have a direct effect on fibroblasts, which may contribute to the pathogenesis of overgrowth, including inhibition of matrix metalliproteinase-1 and tissue inhibition of metalloproteinase production, inhibition of collagen phagocytosis, and increased production of transforming growth factor-β and connective tissue growth factor (23–27). Less attention has been focused on the role of epithelial cells in the pathogenesis of overgrowth. Histological changes in drug-induced gingival overgrowth tissue include acanthosis, which is evident as elongated rete ridges, hyperkeratosis and an increase in epithelial width, also confirmed in the present study (28,29). Epithelial cells are a source of multiple growth factors and cytokines, and have recently been demonstrated to participate actively in connective tissue regulation through mechanisms involving epithelial cell integrins, as discussed below. Contrary to our hypothesis, increased integrin expression (particularly that of αvβ6 integrin) was not associated with gingival overgrowth, except in the combined medication group. Overall high-expression levels of integrins in normal gingival papilla may explain why such differences were not found (see below). It is also likely that multiple mechanisms may initiate signaling and activation pathways that converge to regulate the expression of genes essential to gingival overgrowth.

The present study demonstrated that some integrins were expressed with greater frequency or altered distribution (suprabasal expression) in the gingival overgrowth of the combined drug group when compared with the control group. One possible explanation for the up-regulation of certain integrins may be related to the altered expression of cytokines as a result of the influence of the medication, or because of the direct influence of the medication on the epithelium. The α5β1 integrin expression in the basal cell layer was greater in the combined drug group when compared with the other drug-induced overgrowth groups and the control group. This integrin is expressed by wound keratinocytes, but not by normal resting keratinocytes (11). The expression of the α5β1 integrin by the control group is a new finding and deserves further discussion. The α5β1 integrin has been previously reported as being absent from oral epithelium in normal gingiva (11) and from oral epithelium in periodontitis patients (30). In these studies, the tissue was obtained mainly from marginal gingiva and did not contain interdental papilla tissue or sulcular/junctional epithelium. There are no previous studies available which address the expression of the α5β1 integrin in the interdental papilla or sulcular/junctional epithelium. Therefore, the presence of the α5β1 integrin may be a reflection of the unique phenotype of the gingival epithelium in the interdental papilla region. The increased expression of the α5β1 integrin in the combined drug group resembles that

seen in wound healing. In wounds, however, α5β1 integrin-expressing keratinocytes are contacting wound bed matrix and not basement membrane (11). Therefore, we localized basement membrane proteins type IV collagen and laminin-5, together with cellular EDA-fibronectin, in the specimens. Both type IV collagen and laminin-5 were present in all specimens (data not shown), indicating that there were no obvious defects in the basement membrane zone that could explain the up-regulation of α5β1 integrin. Interestingly, the distribution of cellular EDA-fibronectin around the basal keratinocytes of many specimens of the control, nifedipine- and cyclosporin-induced overgrowth groups was also observed. Localization of EDA-fibronectin around basal keratinocytes is atypical and therefore these findings were confirmed by repeated stainings. Previous studies have identified plasma fibronectin in psoriasis lesions from skin where it was localized around the basal cells of the epidermis in the uninvolved perilesional skin (31,32). This pattern of staining was also found around the keratinocytes from intralesional areas of psoriasis. Induced expression of the α5β1 integrin in the nonlesional psoriatic epidermis has been observed (31). Initially it was believed that plasma fibronectin leaks into the epidermis from the circulation, resulting in an up-regulation of α5β1 integrin by the keratinocytes (31). Later studies have shown, however, that cellular EDA-fibronectin, synthesized *in situ* by the proliferating keratinocytes, was present at the dermal–epidermal junction in uninvolved psoriasis specimens, but was not present in the control samples (33,34). In our study, the combined drug group showed up-regulation of the α5β1 integrin, with no evidence of fibronectin around the basal keratinocytes, ruling out the role of fibronectin in this event. Therefore, it remains unknown as to why fibronectin is expressed in the basal keratinocytes in those specific groups, and why α5β1 integrin is up-regulated only in the combined drug group.

In our study, the αvβ6 integrin is strongly expressed by wound keratinocytes, but not by unwounded tissue or by healthy or inflamed human periodontal tissue samples (30,35,36). This integrin is, however, expressed by epithelial cells in oral leukoplakia, dysplasia and by hyperkeratotic, hyperplastic and atypic tissue (37). In this study, the αvβ6 integrin was seen with similar frequency in all groups, including the control group. Thus, the finding in this study, showing expression of αvβ6 integrin in normal gingiva, contradicts previous findings showing no expression in the normal marginal gingiva (30,35). Explanations for this finding may include a difference in tissue location because gingival tissue from interdental papilla was analyzed for the first time in the present study. The combined drug group demonstrated stonger staining intensity and higher staining frequency of αvβ6 integrin in suprabasal epithelial cells. In addition to binding fibronectin and tenascin, αvβ6 integrin has been shown to bind latent transforming growth factor-β (13). Interestingly, αvβ6 integrin can activate endogenous latent transforming growth factor-β, and this mechanism may regulate pulmonary inflammation and fibrosis in mice (13). Transforming growth factor-β has been shown to be present in overgrowth tissue where it may regulate connective tissue production (24,25,38). Our results suggest that αvβ6 integrin expression, as such, may not be associated with gingival overgrowth (as it was also expressed in the control tissue) but its up-regulation might contribute to the pathogenesis of overgrowth in the combined drug group. Clearly, these observations must be supported with functional studies before any conclusions can be drawn about the role of this pathway in gingival overgrowth.

Suprabasal expression of β1 integrins was observed more frequently in the combined drug group than in the others. In general, suprabasal expression of integrins has been shown to occur because of keratinocyte hyperproliferation or abnormal terminal differentiation, but not as a result of inflammation (39). The suprabasal expression of integrins in the combined drug group may be a function of perturbed keratinocyte differentiation or hyperproliferation in drug-induced gingival overgrowth (7). Altered integrin expression may actually regulate the hyperproliferative stage, as evidenced by experiments with transgenic mice that expressed suprabasal integrins (40). Relatively large individual variation in suprabasal integrin expression among the study groups suggests that either the overgrowth tissue undergoes continuous dynamic changes at differentiation, or multiple mechanisms contribute to the pathogenesis of drug-induced gingival overgrowth.

In summary, the integrin, α5β1, was found to be up-regulated in the combined drug-induced gingival overgrowth samples that also expressed more suprabasal β1 integrins and αvβ6 integrin. This pattern of expression shares features with both wound healing and psoriasis (32,35).

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